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Research paper

Flotation and adherence characteristics of *Toxocara canis* and *T. cati* and a reliable method for recovering *Toxocara* eggs from soil

Annika Kleine, Elisabeth Janecek, Patrick Waindok, Christina Strube*

Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine Hannover, Buenteweg 17, 30559 Hannover, Germany

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ABSTRACT

Toxocara canis and *T. cati* are worldwide distributed intestinal nematodes of canids and felids and pose a threat to public health due to possible clinical manifestations in humans. Different methods for detection of *Toxocara* eggs in soil have been described, but conducted studies deal with egg recovery rates of *T. canis* or "*Toxocara* spp." only whereas *T. cati* egg recovery has not been taken into consideration. Thus, flotation properties in sodium chloride solution and adherence characteristics to different substrates possibly coming into contact with *Toxocara* eggs before or during purification from soil were evaluated for both, *T. canis* and *T. cati* eggs. No significant difference was observed in flotation characteristics, but comparison of adherence properties revealed significantly less adherence of *T. cati* eggs on almost all evaluated substrates ("sand", side sealed bags, glass beaker, centrifuge tube) and different washing solutions (tap water, Tween® 80, Triton™ X-100). Mean adhesion rates of *T. cati* eggs ranged from 15.9% to 68.9%, those of *T. canis* eggs from 28.3% to 83.9%. While adherence of *T. cati* eggs on any substrate was significantly reduced when rinsing with Tween® 80 solution, no effect on *T. canis* eggs could be observed. Generally, *Toxocara* eggs adhere better on plastic than on glass. Evaluation of a method including only non-hazardous substances for purification of *Toxocara* eggs from soil resulted in a statistically significant higher recovery rate of *T. canis* (42.6% recovered eggs) compared to *T. cati* eggs (30.9% recovered eggs). As these percentages are above average for described methods to recover *Toxocara* eggs from soil, the presented method is considered reliable for prevalence studies.

1. Introduction

Toxocara canis and *T. cati* are worldwide distributed intestinal helminth parasites of dogs and cats with high zoonotic potential. Eggs are passed in the environment with faeces of infected carnivores and develop into the infective third larval stage within 3–6 weeks depending on environmental conditions (Overgaauw, 1997). After ingestion of those embryonated eggs by humans, larvae hatch in the intestine and migrate into a variety of organs, where they persist without further development into the adult stage (Sprent, 1952; Beaver, 1969). Depending on the affected organs, multiple and partially severe symptoms may be observed (Magnaval et al., 2001). Children are especially at risk as their hygienic behaviour is not fully developed and eggs may easily be ingested while playing on contaminated places. Consequently, accumulation of embryonated eggs within the environment, especially on public places, poses a high infection risk. Thus, methods for detection and recovery of *Toxocara* eggs from soil were described or improved, respectively, and several studies assessed *Toxocara* spp. contamination rates of public parks and playgrounds to estimate the infection risk for humans (Dada and Lindquist, 1979; Dunsmore et al., 1984; Horn et al., 1990a; Ruiz De Ybáñez et al., 2000; Paquet-Durand et al., 2007; Borecka and Gawor, 2008; Kirchheimer and Jacobs, 2008; Macuhova et al., 2010; Blaszkowska et al., 2011; Manini et al., 2012; Mohd Zain et al., 2014; Thomas and Jeyathilakan, 2014; Blaszkowska et al., 2015). However, only few studies provide data on flotation and adherence characteristics of *Toxocara* eggs, which might influence egg recovery. Thus, knowledge of these characteristics is of particular importance for accurate estimation of contamination rates. Additionally, almost all studies are merely dealing with recovery of *T. canis* eggs as most attention is paid to this species as zoonotic agent, whereas *T. cati*-infections in humans are underestimated (Fisher, 2003). The assumption that *T. canis* is the causative agent of human toxocarosis is strongly based on larval migration patterns and pathology in the paratenic host, where *T. canis* larvae show a strong affinity to the brain and infection presents more severe than infection with *T. cati* (Burren, 1971; Prokopic and Figallová, 1982; Havasiová-Reiterová et al., 1995;

* Corresponding author.
E-mail address: christina.strube@tiho-hannover.de (C. Strube).

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Santos et al., 2009; Janecek et al., 2014). Nevertheless, human cases linked to *T. cati*-infection have been described (Fukae et al., 2012). Additionally, the infection risk is considered to be high as most playgrounds are presumably contaminated with *T. cati* eggs based on defecation habits of cats whereas parks are likely to be contaminated with *T. canis* eggs by dogs being taken for a walk (Shimizu, 1993; Jansen et al., 1993; Uga et al., 1996). However, dogs can also excrete *T. cati* eggs after coprophagy of cat faeces and subsequent intestinal passage. Fahrion et al. (2011) determined 68.5% of *Toxocara* egg isolates originating from dogs as *T. canis* and 31.5% as *T. cati*. Thus, dogs can contribute to *T. cati* infection risk in parks. Therefore, evaluation of flotation and adherence characteristics as well as egg recovery rates of *T. cati* eggs is as important as for *T. canis* eggs. Thus, the current study aimed to provide a comparative description of flotation and adherence properties on different substrates which may be used during purification from soil. Additionally, a reliable and reproducible, non-hazardous method for recovery of *T. canis* and *T. cati* eggs from soil was evaluated.

2. Material and methods

2.1. *Preparation of* Toxocara egg solution

T. canis and *T. cati* eggs were purified from faeces of experimentally infected dogs and cats. Animal experiments were permitted by the ethics commission of the German Lower Saxony State Office for Consumer Protection and Food Safety under reference number 33.9-42502-05-01A038. Maximum one day old faeces were mixed with tap water to obtain a viscous consistency and subsequently strained through sieves with different mesh sizes (2000 μm, 125 μm and 36 μm) to separate larger faecal components from eggs. The content of the 36 μm sieve was rinsed with tap water into a 1 l beaker. After one hour of sedimentation, the supernatant was discarded and the sediment thoroughly mixed with 600 ml saturated NaCl solution (specific gravity 1.2). After one hour of flotation, eggs were collected by passing the supernatant through a 36 μm sieve and washing the content of the sieve with tap water. Eggs were then rinsed into a flask and stored in tap water at 4 °C until use.

2.2. *Flotation characteristics of* T. canis *and* T. cati *eggs*

About 200 *T. canis* or *T. cati* eggs in a volume of 100–105 μl tap water were transferred to 50 ml centrifuge tubes and 50 ml saturated NaCl solution were added. Tubes were inverted three times and cover slips were placed carefully on the surface of the solution. Samples were centrifuged at 300 × *g* for 30 min. Subsequently, cover slips were removed with forceps and placed on glass slides to microscopically determine the number of floated eggs. A total of 130 repetitions for each *Toxocara* sp. were performed.

2.3. *Adherence characteristics of* T. canis *and* T. catie *ggs*

To characterize adherence properties of *T. canis* and *T. cati* eggs, about 100 eggs of each species were applied to different substrates which were chosen based on potential usage and associated prospective contact to *Toxocara* eggs during the process of soil sampling, transport to the laboratory and egg recovery via flotation. As substrates, sandblasted slides (Science Services GmbH, Munich, Germany) were used to resemble the rough surface of sand grains. Side sealed bags (Pelle Vakuumverpackung, Spelle, Germany) were used as potential bags for transport and storage of larger volumes of sand. From these, rectangles were cut out and fixed on glass slides. The adherence properties of *Toxocara* eggs to glass beakers used for flotation were evaluated by using regular glass slides (Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany). Adherence to tubes during a potential centrifugation step to enhance flotation was analysed using pieces obtained from 50 ml polypropylene centrifuge tubes (nerbe plus GmbH, Winsen, Germany).

Respective slides were dried for 24 h at 37 °C after addition and determination of the exact number of *Toxocara* eggs. Afterwards, slides were placed in a staining rack which was inserted into a plastic box containing either 300 ml of pure tap water or tap water supplemented with 0.2% Tween® 80 (Carl Roth GmbH, Karlsruhe, Germany) or 0.2% Triton™ X-100 (Sigma Chemical Co., St. Louis, USA). The plastic box was then perforated with a scorching screw in order to allow the respective solution to flow out. Adherent *Toxocara* eggs on different substrates were counted microscopically.

2.4. *Recovery of* Toxocara *eggs from soil*

To recover *Toxocara* eggs from soil, a method using solely non-hazardous substances (Tween® 80 and NaCl) during all processing steps was evaluated (depicted graphically in Fig. 1). Sand was freshly obtained from a dredging lake and incubated at 100 °C for one week to eradicate living organisms. Recovery rates for sensitivity determination were evaluated by portioning 250 g sand into side sealed bags followed by addition of 1, 5, 10, 25, 50, 75, 100, 150 and 200 eggs of *T. canis* or *T. cati*, respectively. To imitate a natural distribution of eggs within soil, samples were rotated several times and incubated at room temperature overnight. Samples were subsequently treated with 250 ml of saturated NaCl solution with addition of 0.5 ml (0.2%) Tween® 80 (Carl Roth GmbH, Karlsruhe, Germany). Incubation of samples for 10 min at 25 °C in a shaking water bath following 20 min on a shaking table at room temperature allowed even distribution of NaCl/Tween® 80 solution between sand grains. Afterwards, samples were transferred to a 1 l beaker and saturated NaCl solution was added to a total volume of 750 ml. After thorough stirring, eggs were allowed to float one hour with subsequent draining of the supernatant through a 25 μm sieve. The content of the sieve was transferred with tap water into a 50 ml centrifuge tube and centrifuged at 3000 × *g* for 10 min. The supernatant was discarded and recovered eggs were counted by microscopic examination the sediment. A total of 10 repetitions for each concentration level and *Toxocara* sp. were performed.

2.5. *Statistical analysis*

Statistical analysis was carried out using GraphPad Prism 6 (version 6.03, GraphPad Software Inc., La Jolla, USA). Recovery rates and flotation characteristics of *T. canis* vs. *T. cati* as well as comparison of adherence rates of *T. canis* vs. *T. cati* eggs on different substrates by using different solutions were compared applying pairwise comparison with a *t*-test. If data lacked a Gaussian distribution, Mann-Whitney test was applied. To statistically analyse adherence characteristics of each *Toxocara* species, a one way analysis of variance (ANOVA) with subsequent Tukey's test was conducted comparing the respective solutions on different substrates. Statistical tests were performed at the level of alpha = 0.05.

3. Results

3.1. *Flotation characteristics of* T. canis *and* T. cati *eggs*

Microscopic determination of *Toxocara* egg numbers after flotation with saturated NaCl solution resulted in a mean percentage of 30.1% floated *T. canis* eggs and 28.4% *T. cati* eggs. No significant difference was observed between both species. Data are graphically displayed in Fig. 2.