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Research Brief

A *Toxocara cati* eggs concentration method from cats' faeces, for experimental and diagnostic purposes

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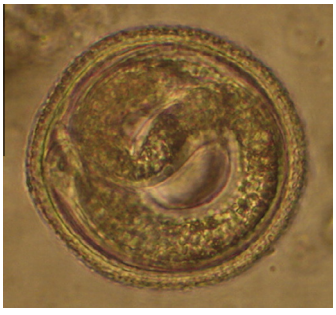
HIGHLIGHTS

- A novel method for *Toxocara* spp. eggs concentration from faeces was performed.
- The concentration method and McMaster egg counting technique were compared.
- High number of eggs were recovered with the concentration method.
- A good quality inoculum was obtained for experimental and diagnostic purposes.

GRAPHICAL ABSTRACT



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ABSTRACT

Toxocariosis is a zoonotic parasite infection worldwide distributed, now considered a neglected disease associated to poverty. For experimental infection in animals and to develop the diagnosis in humans it is necessary to obtain large number of *Toxocara* spp. larval eggs. *Toxocara cati* eggs recovered percentage from faeces of infected cats was determined employing a novel egg concentration method. The McMaster egg counting technique and the concentration method were applied on 20 positive cats' sample faeces obtained from naturally infected cats. The mean percentage of eggs recovered by the concentration method was 24.37% higher than the count obtained by McMaster egg counting technique. The main advantage of this method is that it can be obtained a small final volume with a high number of recovered eggs and a good quality inoculum for experimental and diagnostic purposes.

1. Introduction

Toxocariosis is a highly prevalent parasitic infection that affects cats and dogs worldwide (Overgaauw, 1997a, 1997b). Adult parasites produce eggs that are eliminated through the faeces to the environment, which become a source of infection for humans and paratenic hosts like mammals, birds and invertebrates (Schantz and Glickman, 1981; Dubinsky et al., 1995; Taira et al., 2004). *Toxocara* spp. larval eggs developed in the environment and once they are ingested by a suitable host, can migrate and cause damage through their tissues (Sprent, 1952; Overgaauw, 1997a, 1997b). In human beings, *Toxocara* spp. is the aethiological agent of toxocariosis (Lee et al., 2010; Rubinsky Elenfant et al., 2010). To understand human disease and to study the ability of some animal species to become suitable paratenic hosts, experimental infections have been conducted on the migratory behaviour and infectivity of *Toxocara canis* and *Toxocara cati* larvae in tissues (Sommerfelt et al., 2006; Cardillo et al., 2009; Santos et al., 2009; Zibaei et al., 2010; Taira et al., 2012, 2013). The human infection is demonstrated by detecting IgG antibodies against excretory-secretory (ES) larval antigens in human sera (Ponce-Macotela

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et al., 2011; Maizels, 2013; Santillán et al., 2013; Zibaei et al., 2013). For experimental infection and to obtain ES antigens for diagnostic purpose, it is necessary to collect large number of larvae (Ponce-Macotela et al., 2011).

Toxocara spp. larvae were usually obtained by culturing eggs isolated from the uterus of female worms, collected from naturally infected cats and dogs (De Savigny, 1975; Fan et al., 2003; Rodríguez-Caballero et al., 2007; Liao et al., 2008; Zibaei et al., 2007, 2010). Obtaining adult worms is not easy (Ponce-Macotela et al., 2011), especially from cats. In some reports, researchers needed to euthanatize young dogs and cats so as to get adult worms (Oryan et al., 2009). It has been shown that eggs isolated from the female parasites do not developed uniformly, they took long time and in most cases they never developed in culture because of the presence of both fertilized and unfertilized eggs (Ponce-Macotela et al., 2011).

The present study aimed to develop a new method for the recovery and concentration of *T. cati* eggs from sample faeces of naturally infected cats and to provide an alternative tool to get good quality inoculums for experimental and diagnostic purposes.

2. Materials and methods

2.1. Samples

The experiment was carried out on 20 sample faeces obtained from naturally infected cats. To ascertain the utility of the Concentration technique and have a reference of the number of eggs present per gram in faeces, it was compared the number of eggs obtained using the McMaster egg counting technique with Raynaud's modification (Raynau, 1970) with the number of eggs per gram using the Concentration method. Both techniques were performed on faecal subsamples weighting 2 g .taken from each sample, previously homogenized.

2.2. Concentration method

One of the subsamples was processed by Benbrook's flotation technique with saturated sugar solution (Dolcetti, 1947). Two ml of supernatant containing eggs were aspirated from the surface with a Pasteur pipette (tube 1) and placed in a 10 ml centrifuge tube (tube 2). In the first tube, the volume extracted was replaced by 2 ml of 5% formalin to wash and remove the eggs attached to the tube walls. The second 2 ml were extracted and placed in the centrifuge tube (tube 2), and 6 ml of 5% formalin were added. The eggs in the tube (tube 2) were left to sediment overnight. The supernatant was poured off, leaving 1 ml of sediment. The sediment was filtered on filter meshes of decreasing number (230, 177 and 105 μm) and washes were performed with 5% formalin. The filtrate was centrifuged at 1500 rpm for 3 min; afterward, the supernatant was poured off. This washing procedure with 5% formalin and centrifugation was performed twice. One ml of final sediment was homogenized, 3 aliquots of 20 μl were taken and eggs were counted. Mean number of eggs were calculated when differences between the aliquots were under 20%, otherwise the procedure was repeated.

2.3. Eggs development

Sediment containing *Toxocara's* eggs was placed in Eppendorf tubes with the addition of 2.5% formalin/ringer. Tubes were kept under shaker, at 25 °C and 9.8% humidity during 3 weeks, according to Zibaei et al. (2007) report. They were monitored periodically and oxygenated.

2.4. Statistic analysis

Normality distribution of data was tested by Shapiro–Wilk test ($P < 0.05$). Paired T test was used to assess significance of statistical differences ($P < 0.05$) between the results obtained by both methods. All results were presented with reference to 1 g of faeces.

3. Results

Normally distributed data were obtained in both methods. Mean number of detected eggs obtained in Concentration method was 1635.5 eggs/gr (95% IC: 804.6–2466.4), 24.37% higher than those recovered by McMaster egg counting technique which was 1315 eggs/gr (95% IC: 708.9–1921.1); this differences resulted statistically significative ($t = 2.03$; $p = 0.028$). The maximum recovered number of detected eggs was 6536.7 eggs/gr for the concentration method and 4000 eggs/gr for the McMaster egg counting technique. Eggs fully embryonated within 3 weeks.

4. Discussion

In the present study it was showed a simple alternative method to obtain *Toxocara* spp. eggs from faeces, in view of the difficulty to obtain parasite females. Kochanowski et al., 2013, reported that the calculation of parasite eggs from the whole McMaster chamber provides the most accurate estimation of the number of eggs per unit of mass's faeces. According to our results, it was shown that with this concentration method it could be obtained a good quality inoculum which means an inoculum with high number of infective larval eggs developed uniformly in a small and clean volume and in a short time (3 weeks).

Kochanowski et al., 2013, reported significant differences in the accuracy and precision of a method in relation to the type of parasite eggs. They suggested that the lower detection of *T. canis* eggs in the McMaster chamber was caused by the specific shell structure of these eggs with strong adhesive properties (Overgaau and van Knapen, 2008), which could result in a higher loss of parasite eggs during their handling. Like Kochanowski et al., 2013 report, to determine the accuracy and precision of the egg concentration method on faeces, several repetitions of the technique could be done on samples enriched with different known numbers of eggs, to establish the highest limit of detection, sensitivity and the lowest coefficients of variation.

With the concentration method proposed could be obtained an adequate eggs recovery percentage from faeces and even a good quality inoculum for experimental and diagnostic purposes.

References

Cardillo, N., Rosa, A., Ribicich, M., López, C., Sommerfelt, I., 2009. Experimental infection with *Toxocara cati* in BALB/c mice, migratory behaviour and pathological changes. Zoonoses Public Health 56, 198–205.

de Savigny, D.H., 1975. In vitro maintenance of *Toxocara canis* larvae and a simple method for the production of *Toxocara* ES antigen for use in serodiagnostic tests for visceral larva migrans. J. Parasitol. 61, 781–782.

Dolcetti, M., 1947. Modificación al método de Benbrook mediante la centrifugación doble con solución de azúcar para el examen microscópico de las materias fecales de los animales domésticos. Rev. C. E. Med. 16 (24), 29–35.

Dubinsky, P., Havasiova-Reiterova, K., Petko, B., Hovorka, I., Tomasovicova, O., 1995. Role of small mammals in the epidemiology of toxocariasis. Parasitology 110, 187–193.

Fan, C.K., Lin, Y.H., Du, W.Y., Su, K.E., 2003. Infectivity and pathogenicity of 14-month-cultured embryonated eggs of *Toxocara canis* in mice. Vet. Parasitol. 113, 145–155.

Kochanowski, M., Dabrowska, J., Karamon, J., Cencek, T., Osiński, Z., 2013. Analysis of the accuracy and precision of the McMaster method in detection of the eggs of *Toxocara* and *Trichuris* species (Nematoda) in dog faeces. Folia Parasitol. (Praha) 60, 264–272.

Lee, A.C.Y., Schantz, P.M., Kazacos, K.R., Montgomery, S.P., Bowman, D.D., 2010. Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. Trends Parasitol. 26 (4), 155–161.

Liao, C.W., Cho, W.L., Kao, T.C., Su, K.E., Lin, Y.H., Fan, C.K., 2008. Blood-brain barrier impairment with enhanced SP, NK-1R, GFAP and claudin-5 expressions in experimental cerebral toxocariasis. Parasite Immunol. 30, 525–534.

Maizels, R.M., 2013. *Toxocara canis*: molecular basis of immune recognition and evasion. Vet. Parasitol. 193, 365–374.

Oryan, A., Sadjjani, S.M., Azizi, S., 2009. The effects of benzimidazoles on the larval stage of *Toxocara cati* in experimentally infected chickens. Trop. Biomed. 26, 30–39.

Overgaau, P.A.M., van Knapen, f., 2008. Toxocarosis, an important zoonosis. Eur. J. Comp. Anim. Pract. 18, 259–266.

Overgaauw, P.A.M., 1997a. Aspects of *Toxocara* epidemiology: toxocarosis in dogs and cats. Crit. Rev. Microbiol. 23, 233–251.

Overgaauw, P.A.M., 1997b. Aspects of *Toxocara* epidemiology: human toxocarosis. Crit. Rev. Microbiol. 23, 215–331.

Ponce-Macotela, M., Rodríguez-Caballero, A., Peralta-Abarca, G.E., Martínez-Gordillo, M.N., 2011. A simplified method for hatching and isolating *Toxocara canis* larvae to facilitate excretory-secretory antigen collection in vitro. Vet. Parasitol. 175, 382–385.

Raynau, D.J.P., 1970. Etude de l'efficacité d'une technique de co- proscopie quantitative pour le diagnostic de routine et le con- trole des infestations parasitaires des bovins, ovins, equines et porcins. Ann. Parasitol. (Paris) 45, 321–342.

Rodríguez-Caballero, A., Luna-Ochoa, R.I., Ponce-Macotela, M., Peralata-Abarca, G.E., Martínez-Gordillo, M.N., 2007. A simple and inexpensive in vitro method for retrieving fertilized *Toxocara canis* eggs. Parasitol. Res. 101 (3), 829–832.

Rubinsky Elenfant, G., Hirata, C.E., Yamamoto, J.H., Ferreira, M.U., 2010. Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. Ann. Trop. Med. Parasitol. 104 (1), 3–23.

Santillán, G., Bastin, V., Céspedes, G., Monkiewicz, A., 2013. Evaluación de un antígeno purificado para el diagnóstico de toxocariosis. Rev. Argent. Microbiol. 45, 80–85.

Santos, S.V., Lescano, S.Z., Castro, J.M., Chieffi, P.P., 2009. Larval recovery of *Toxocara cati* in experimentally infected *Rattus norvegicus* and analysis of the rat as potential reservoir for this ascarid. Mem. Inst. Oswaldo Cruz 104, 933–934.

Schantz, P.M., Glickman, L., 1981. Roundworms in dogs and cats veterinary and public health consideration. Compend. Contin. Educ. 3, 773–784.

Sommerfelt, I.E., Santillán, G., Mira, G., Ribicich, M., Betti, A., De Torres, R., 2006. *Toxocara canis* infections in a pig model: immunological, haematological and blood biochemistry responses. J. Helminthol. 80, 73–77.

Sprent, J.F.A., 1952. On the migratory behavior of the larvae of various *Ascaris* species in white mice. I. Distribution of larvae in tissues. J. Infect. Dis. 90, 165–176.

Taira, K., Saeed, I., Permin, A., Kapel, C.M., 2004. Zoonotic risk of *Toxocara canis* infection through consumption of pig or poultry viscera. Vet. Parasitol. 121, 115–124.

Taira, K., Saitoh, Y., Okada, N., Sugiyama, H., Kapel, C.M.O., 2012. Tolerance to low temperatures of *Toxocara cati* larvae in chicken muscle tissue. Vet. Parasitol. 189, 383–386.

Taira, K., Yanagida, T., Akazawa, N., Saitoh, Y., 2013. High infectivity of *Toxocara cati* larvae from muscles of experimentally infected rats. Vet. Parasitol. 196, 397–400.

Zibaei, M., Sadjjadi, S.M., JahadiHosseini, S.H., Sarkari, B., 2007. A Method for Accelerating the Maturation of *Toxocara cati* eggs. Iran. J. Parasitol. 2, 39–42.

Zibaei, M., Sadjjadi, S.M., Uga, S., 2010. Experimental *Toxocara cati* infection in gerbils and rats. Korean J. Parasitol. 48, 331–333.

Zibaei, M., Firoozeh, F., Bahrami, P., Sadjjadi, S.M., 2013. Investigation of anti-*Toxocara* antibodies in epileptic patients and comparison of two methods: ELISA and Western blotting. Epilepsy Res. Treat. 2013, 156815.