Evaluation of the initial and chronic phases of toxocariasis after consumption of liver treated by freezing or…

Article *in* Parasitology Research · March 2013

DOI: 10.1007/s00436-013-3376-5 · Source: PubMed

8 authors, including:

Gisele Ferreira Dutra
Universidade Católica de Pelotas (UCPel)
12 PUBLICATIONS **29** CITATIONS
SEE PROFILE

Luciana Farias da Costa de Avila
Universidade Federal do Rio Grande (FURG)
14 PUBLICATIONS **42** CITATIONS
SEE PROFILE

Vanusa Pousada da Hora
Universidade Federal do Rio Grande (FURG)
63 PUBLICATIONS **171** CITATIONS
SEE PROFILE

Carlos James Scaini
Universidade Federal do Rio Grande (FURG)
37 PUBLICATIONS **253** CITATIONS
SEE PROFILE

Parasitol Res (2013) 112:2171–2175
DOI 10.1007/s00436-013-3376-5

ORIGINAL PAPER

Evaluation of the initial and chronic phases of toxocariasis after consumption of liver treated by freezing or cooling

Gisele Ferreira Dutra · Nitza Souto França Pinto · Luciana Farias da Costa de Avila · Paula de Lima Telmo · Vanusa Pousada da Hora · Lourdes Helena Rodrigues Martins · Maria Elisabeth Aires Berne · Carlos James Scaini

Received: 20 January 2013 / Accepted: 28 February 2013 / Published online: 15 March 2013

Abstract Human toxocariasis is a neglected parasitic zoonosis of worldwide distribution. The consumption of raw or undercooked meat and offal from paratenic hosts of the *Toxocara canis* nematode can cause infection in humans, but there have been a lack of studies examining specific prophylactic measures to combat this mode of transmission. The aim of this study was to evaluate the establishment of infection by *T. canis* larvae at the initial and chronic phases of visceral toxocariasis after the consumption of mouse liver subjected to cold treatment. This study was divided into two stages using groups (G) of five donor mice inoculated with 2,000 eggs of *T. canis*. Two days post-inoculation, the livers of donor mice in G1 and G2 were kept at −20 °C and between 0 and 4 °C, respectively, for 10 days. In the first stage of the study, the livers of mice from G1, G2, and G3 (control) were subjected to a tissue digestion technique and found to be positive for infection. In the second stage, which evaluated infection in mice that had consumed livers from donor mice, receiver mice of G4 and G7 were fed with livers of donor mice from G1 (freezing), receiver mice of G5 and G8 were fed with livers of donor mice from G2 (cooling), and receiver mice of G6 and G9 with livers from G3 (control). Then, the tissue digestion technique was performed for recovering larvae from organs and carcasses of mice, at 2 days (G4, G5, and G6) and 60 days after liver consumption (G7, G8, and G9). It was observed that freezing inhibited the viability of 100 % of the larvae, while cooling promoted 87.7 and 95.7 % reductions in the intensity of infection at 2 and 60 days after liver consumption, respectively. Under the studied conditions, cold treatment shows great potential to help control this parasitosis, both in the initial and chronic phases of toxocariasis.

G. F. Dutra (✉) · V. P. da Hora · C. J. Scaini
Post-Graduate Program in Health Sciences, Parasitology Laboratory, Universidade Federal do Rio Grande (FURG), Academic Area of the University Hospital–FURG, Rua General Osório, S/N, CEP 96.200-190, Rio Grande, RS, Brazil
e-mail: gisele_fd@yahoo.com.br

N. S. F. Pinto · L. H. R. Martins
Parasitology Laboratory, FURG, Rio Grande, Brazil

L. F. da Costa de Avila · M. E. A. Berne
Department of Microbiology and Parasitology, UFPEL, Pelotas, Brazil

P. de Lima Telmo
Center for Technological Development, Universidade Federal de Pelotas (UFPEL), Pelotas, Brazil

Introduction

Human toxocariasis presents a worldwide distribution, but its prevalence and impact on public health are underestimated mainly due to difficulties with clinical and laboratory diagnoses (Smith et al. 2009). The mode of infection associated with this parasitosis occurs by the accidental ingestion of embryonated eggs of the nematode *Toxocara canis*, the etiologic agent most frequently associated to the disease, or *Toxocara cati*, intestinal parasites of dogs and cats, respectively (Azizi et al. 2007). There are also reports of human infections caused by *Baylisascaris procyonis*, the raccoon roundworm (Park et al. 2000; Perlman et al. 2010). However, the ingestion of raw or undercooked meat and offal from mammalian and bird species that act as paratenic hosts of *T. canis* can also trigger infection in humans, and this practice is present in several cultures (Hoffmeister et al. 2007). Clinical manifestations associated with this type of transmission are varied, and headache, fatigue, hives, respiratory distress, and severe brain involvement with hemiparesis can be observed (España et al. 1993; Morimatsu et al. 2006; Hoffmeister et al. 2007).

In recent decades, cases of human toxocariasis associated with the consumption of raw or undercooked liver from chickens and ducks (Morimatsu et al. 2006; Hoffmeister et al. 2007) and sheep and cattle (Salem and Schantz 1992; Yoshikawa et al. 2008) have been reported. An association between the consumption of uncooked beef and this parasitic infection in patients with blood eosinophilia of unknown cause was also reported (Choi et al. 2008), and the occurrence of infection by *Toxoplasma gondii*, a parasite also transmitted by the consumption of uncooked meat, doubles the risk of infection by *T. canis* (Jones et al. 2008).

The importance of this type of transmission has been demonstrated in experimental studies with mice, pigs, and poultry (Pahari and Sasmal 1990; Tüzer et al. 2002; Taira et al. 2004), which have revealed that the intensity of infection may be higher with the consumption of liver from parasitized animals rather than the direct inoculation of *T. canis* eggs (Tüzer et al. 2002). Given this context, it is important to conduct studies examining specific prophylactic measures against this mode of infection.

The treatment of meat and offal by freezing may represent an important tool for the prevention of human toxocariasis because this procedure is used as a key control measure for parasitic zoonoses such as toxoplasmosis and taeniasis (Almeida et al. 2003; El-Nawawi et al. 2008). However, this procedure was not efficient to decrease the survival of larvae of *Trichinella* spp. (Theodoropoulos et al. 2000) and *B. procyonis* eggs (Shafir et al. 2011).

With regard to visceral toxocariasis, cooling treatment was shown to have potential in reducing the intensity of infection after the consumption of viscera of birds and pigs that were experimentally inoculated (Taira et al. 2004), while freezing treatment showed conflicting results (Sprent 1953). Thus, it has become important to broaden our knowledge of the potential use of cold treatment for the control of toxocariasis and evaluate the effects of this treatment at different stages of the disease. Given this context, this study aimed to evaluate the establishment of infection by *T. canis* at the initial and chronic phases of visceral toxocariasis in mice that consumed liver that had undergone treatment by freezing or cooling.

Materials and methods

This study was approved by the Research Ethics Committee of the Universidade Federal do Rio Grande, and all of the experiments were performed according to Brazilian legislation on animal care. Groups of BALB/c female mice aged between 6 and 7 weeks were used. The animals were kept in an acclimatized environment at 22 °C (±2 °C) with a bright–dark cycle of 12 h, and food and water were available ad libitum.

T. canis eggs were first collected directly from the uterine tubes of adult female parasites obtained following treatment of young dogs with pyrantel pamoate (15 mg/kg). Afterwards, the eggs were incubated in a 2 % formalin solution at 28 °C for 30 days (Avila et al. 2012a). The donor mice were inoculated with 2,000 embryonated *T. canis* eggs by a stomach tube (Taira et al. 2004).

In both stages of this study, groups of five donor mice were used. The livers of donor mice in group 1 (G1) and group 2 (G2) were individually placed in Petri plates and frozen at −20 °C or cooled between 0 and 4 °C, respectively, for 10 days. The minimum and maximum temperatures were monitored (Incoterm® thermometer). The livers of the mice in group 3 (G3) were not subjected to cold treatment (control group). The temperatures used were based on national and international regulations that establish temperatures below −18 and 7 °C for the freezing and refrigeration of products of animal origin, respectively (Brasil 1999; European Community 2004).

In the first stage of the study, five donor mice from G1 (freezing), G2 (cooling), and G3 (control) were euthanized 2 days after the inoculation of embryonated *T. canis* eggs. After 10 days of freezing or cooling, the livers were subjected to a tissue digestion technique using 1 % hydrochloric acid solution and 1 % pepsin for 12 h under constant agitation at 37 °C. On the same day, the livers of the control group were also subjected to the tissue digestion technique (Xi and Jin 1998). Observation and analysis of the morphology of larvae were performed by optical microscopy (400 times increase).

In the second stage, groups of five receiver mice were used to assess the establishment of infection during the initial and chronic phases of toxocariasis. The receiver mice of G4, G5, and G6 were fed with livers of donor mice from G1 (freezing), G2 (cooling), and G3 (control), respectively. Each liver was offered in a cage containing only one receiver mouse, and it was removed from the cage only after total consumption of the viscera. Two days after the consumption of the liver, the receiver mice were euthanized. The liver, lungs, brain, kidneys, eyes, heart, and skeletal muscle (carcass) of each receiver mouse were subjected to tissue digestion and larvae observation (Xi and Jin 1998). The receiver mice of G7, G8, and G9 were also fed with livers of donor mice from G1, G2, and G3, respectively. At 60 days after liver consumption (Pahari and Sasmal 1990), the receiver mice were euthanized using the same methodology described in the experiment with G4, G5, and G6.

The number of larvae recovered from the mice was subjected to mathematical transformation represented by: log no. of larvae+1. Then, variance analysis was performed, and the means were compared using the Tukey test with a significance level of 5 %. To the number of larvae recovered from the receiver mice, calculating the sample size was performed and a statistical power of 95 % was found.

Results

The livers of donor mice subjected to tissue digestion were positive for *T. canis* larvae, and there was no significant difference among the three groups (Table 1). However, the larvae recovered from livers that were frozen showed morphological changes with signs of degeneration in internal organs and rupture of the cuticle, whereas the larvae recovered from the cooled livers and those obtained from the control group were shown to be morphologically intact.

When the consumption of livers from donor mice by receiver mice was studied, it was observed that only freezing treatment inhibited the viability of *T. canis* larvae. Cooling treatment caused a reduction in the intensity of infection by 87.7 % in G5 mice examined 2 days post-liver consumption and 95.7 % in G8 mice examined 60 days after viscera consumption when compared with the control groups G6 and G9, respectively (Table 2).

When the cooling results were examined, it was observed that for the mice in G5, which were examined 2 days after consuming cooled liver, the mean recovery was 4.2 larvae of *T. canis* (±3.11), and in the G6 mice (control), the mean recovery was 34 larvae (±10.98); these were significantly different (p=0.0002). However, among the G8 mice, which were examined 60 days post-consumption of cooled liver, only two mice were positive, and one larva recovered from each animal, whereas among the G9 (control) mice, four mice were positive and 9.2 larvae (±10.83) were recovered on average (p=0.06).

When the initial phase of toxocariasis (2 days after liver consumption) was studied, *T. canis* larvae were recovered from skeletal muscle (carcass) and all examined organs, except the eyes, for both the test groups and the control group. When chronic visceral toxocariasis (60 days after liver consumption) was studied, it was observed that in the control group, most larvae (91.3 %) migrated to the brain. For the group that consumed cooled liver, only two mice were positive, and the detected larvae were located exclusively in the brain.

Discussion

The reported cases of human toxocariasis associated with the consumption of raw or undercooked meat and liver from different species of mammals and birds, as well as experimental studies in mice, pigs, and poultry, have demonstrated the importance of this type of parasite transmission. Moreover, the ease of movement of people across countries and continents also favors the introduction of habits such as the consumption of "foie gras," "steak tartar," and raw kebab, which are foods considered exotic to some cultures and customary to others.

The absence of infection by *T. canis* larvae in mice consuming donor mouse livers treated by freezing (G4, G7) was expected because, in the first stage of this study, the larvae recovered from livers subjected to this treatment showed significant morphological changes, allowing us to confirm that they had lost their viability. The formation of particles during the freezing process can promote the disruption of cell membranes and lead to the loss of pathogen viability (Boziaris and Adams 2001), which must have happened with the *T. canis* larvae.

The effectiveness of freezing observed in this study confirms the importance of this type of procedure as a prophylactic measure against this parasite via the same mechanism as has been indicated to decrease the survival of *Taenia solium* (Sotelo et al. 1986), *Taenia saginata* (Almeida et al. 2003), and *T. gondii* cysts (Dubey 1988; Hill et al. 2006; El-Nawawi et al. 2008). However, these results do not agree with those obtained by Sprent (1953), who recovered *T. canis* larvae from the carcasses of mice kept at −20 °C for 4 weeks. The author stated that the larvae presented motility after defrosting the carcasses, but the infection viability of these larvae was not evaluated. In other studies, freezing at −18 °C for 4 weeks was not efficient to decrease the survival of *Trichinella* spp. larvae in experimentally infected sheep (Theodoropoulos et al. 2000) or *B. procyonis* eggs, frozen at −15 °C for 6 months (Shafir et al. 2011). It is important to mention that there have been reported cases of larva migrans in humans caused by *B. procyonis*, as an 11-month-old boy, from California (Park et al. 2000) and in another one, 12 years old, from New York (Perlman et al. 2010). Azizi et al. (2007) suggested risk of toxocariasis by consumption of offal contaminated with *T. cati* larvae, since larval migration occurred in chickens which are paratenic hosts. However, it is possible that the contribution of the *T. cati* as a causative agent of toxocariasis might be underestimated due to the inability of many diagnostic

Table 1 Recovery of *T. canis* larvae from the livers of donor mice subjected to freezing or cooling at 2 days post-inoculation of 2,000 eggs. Five mice per group

Group	Liver preservation	Mean number of larvae	Standard deviation	Amplitude	Total larvae	Positive mice
G1	−20 °C/10 days	24.40 a	15.08	2–94	122	05
G2	0–4 °C/10 days	35.20 a	36.87	2–44	176	05
G3	Control	31.40 a	26.73	3–65	157	05

The same letters indicate no significant difference (p>0.05)

Table 2 Number of *T. canis* larvae recovered from organs and skeletal muscle of receiver mice after the consumption of livers from donor mice inoculated with 2,000 eggs. Five mice per group

Donor mice			Receiver mice	
Groups	Treatment of liver	Groups	Total number of larvae recovered	Positive mice
2 days post-consumption of liver				
G1	−20 °C/10 days	G4	0	0
G2	0–4 °C/10 days	G5	21	05
G3	Control	G6	170	05
60 days post-consumption of liver				
G1	−20 °C/10 days	G7	0	0
G2	0–4 °C/10 days	G8	02	02
G3	Control	G9	46	04

methods currently used to differentiate between exposure to *T. canis* or *T. cati* (Rubinsky-Elefant 2010). In the case of larva migrans caused by *B. procyonis*, Western blotting technique can be an effective tool for the differential diagnosis of infections caused by this parasite or *Toxocara* species, when used in conjunction with enzyme-linked immunosorbent assay (Dangoudoubiyam and Kazacos 2009). Another difficulty is the similarity between larvae of *T. canis* and *Ascaridia* sp., a common nematode of chickens, since both can parasitize the liver of these birds. Although there is a consensus that the small intestine is the normal habitat of these larvae (Borji and Razmyar 2012), histopathological effects, particularly hemorrhagic lesions, can be observed in the liver, lungs, and intestines and may be linked to the migration of the larvae during the tissue phase of the life cycle (Adang et al. 2010). Thus, it can be inferred that the consumption of meat and offal contaminated with different *Toxocara* species or other parasites may also cause infection.

The liver cooling treatment promoted a reduction in the intensity of infection in mice (G5 and G8) who consumed cooled liver compared to control groups (G6 and G9), but the treatment did not disable the infection. The reduction of 87.7 and 95.7 % in mice examined 2 days (p=0.0002) and 60 days (p=0.0572) after liver consumption, respectively, is an important result. These data are higher than those obtained by Taira et al. (2004), who observed a reduction of 32.7 and 44.8 % in the recovery of *T. canis* larvae in pigs fed with poultry and pork offal with toxocariasis, respectively, that were chilled to 4 °C for 7 days. The marked reduction in the intensity of infection obtained in the present study may be explained by the fact that the effectiveness of cold treatment appears to be related not only to temperature but also to shelf life (El-Nawawi et al. 2008) and to the mass of the viscera being evaluated. In this study, mouse liver refrigerated for 10 days was used, whereas in the work of Taira et al. (2004), a mixture of offal from pigs and poultry that was cooled for a shorter period of time was used.

With regard to the distribution of larvae in receiver mice (G5 and G6) 2 days after consuming cooled liver and liver untreated by cold (control), the distribution of larvae in several organs (liver, lungs, brain, kidneys, and heart), which particularly characterizes the initial stage of toxocariasis, was observed. In mice that consumed cooled liver that were examined 60 days post-consumption (G8), an observed reduction in the intensity of infection of 95.7 % was observed, but although only two mice were positive, the larvae detected were located in the brain. It is important to highlight that infection with a reduced number of larvae in people, even asymptomatic, can trigger migration of the larvae to the eyes or brain, and it is important to administer treatment in these cases (Magnaval et al. 2000). In the control group, the migration of most larvae (91.3 %) to the brain was observed, which characterizes chronic infection by *T. canis* in mice (Dunsmore et al. 1983).

The distributions of larvae observed in mice in the control groups (G6 and G9) and the groups examined 2 days (G5) and 60 days (G8) after the consumption of cooled liver were similar to that observed in studies in which mice were directly inoculated with *T. canis* eggs (Avila et al. 2012b), which demonstrates the importance of the liver as a vehicle as well as the relevance of such zoonotic transmission. These results are in agreement with those obtained in studies with other paratenic host species, such as chickens and pigs (Taira et al. 2004). Furthermore, Taira et al. (2003) reinforced the hypothesis zoonotic transmission of *T. canis* especially due to consumption of *in natura* chicken liver, since the authors found accumulation of larvae in this organ.

However, it is necessary to consider that the number of recovered larvae in receiver mice can vary because infection in these animals was based on the consumption of viscera, that is, donor mouse liver following 2 days of infection by *T. canis*.

Under the studied conditions, only the freezing treatment prevented infection by *T. canis* larvae, but the cooling treatment promoted a significant reduction in the intensity of infection, both in the initial and chronic phases of toxocariasis. Thus, cold treatment may represent an important tool to prevent or reduce the risk of infection by the

consumption of raw or undercooked foods of paratenic hosts, such as chickens, cattle, sheep, and pigs.

Acknowledgments Thanks to the Post-Graduate Program in Public Health for support and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for the scholarship grant for studies. The experiments were carried out following the Federal Government legislation on animal care.

References

Adang KL, Abdu PA, Ajanusi JO, Oniye SJ, Ezealor AU (2010) Histopathology of *Ascaridia galli* infection on the liver, lungs, intestines, heart and kidneys of experimentally infected domestic pigeons (*C. l. domestica*) in Zaria, Nigeria. Pac J Sci Technol 11:511–515

Almeida LP, Reis DO, Moreira MD, Oliveira MEF (2003) Viabilidade do *Cysticercus bovis* em câmara frigorífica à temperatura de −30 °C. Rev Hig Alim 17:97–100

Avila LFC, Conceição FR, Telmo PL, Dutra GF, Santos DG Dl, Martins LHR, Berne MEA, Silva PEA, Scaini CJ (2012a) *Saccharomyces boulardii* reduces infection intensity of mice with toxocariasis. Vet Parasitol 187:337–340

Avila LFC, Fonseca JSV, Dutra GF, Telmo PL, Silva AMWA, Berne MEA, Silva PEA, Conceição FR, Scaini CJ (2012b) Evaluation of the immunosuppressive effect of cyclophosphamide and dexamethasone in mice with visceral toxocariasis. Parasitol Res 110:443–447

Azizi S, Oryan A, Sadjjadi SM, Zibael M (2007) Histopathologic changes and larval recovery of *Toxocara cati* in experimentally infected chickens. Parasitol Res 102:47–52

Borji H, Razmyar J (2012) Aberrant migration of *Ascaridia galli* in a myna (*Acridotheres tristis*). Sci Parasitol 13:129–131

Boziaris IS, Adams MR (2001) Temperature shock, injury and transient sensitivity to nisin in Gram negatives. J Appl Microbiol 91:715–724

Brasil (1999) Portaria CVS 6, de 10/03/1999. Dispõe sobre o regulamento técnico, sobre os parâmetros e critérios para o controle higiênico-sanitário em estabelecimento de alimentos. Diário Oficial do Estado de São Paulo União. Disponível em <http://www.cvs.saude.sp.gov.br>. Acesso em: 05 de maio de 2012

Choi D, Lim JH, Choi DC, Paik SW, Kim SH, Huh S (2008) Toxocariasis and ingestion of raw cow liver in patients with eosinophilia. Korean J Parasitol 46:139–143

Dangoudoubiyam S, Kazacos KR (2009) Differentiation of larva migrans caused by *Baylisascaris procyonis* and *Toxocara* species by Western blotting. Clin Vaccine Immunol 16:1563–1568

Dubey JP (1988) Long-term persistence of *Toxoplasma gondii* in tissues of pigs inoculated with *T. gondii* oocysts and effect of freezing on viability of tissue cysts in pork. Am J Vet Res 49:910–913

Dunsmore JD, Thompson RC, Bates IA (1983) The accumulation of *Toxocara canis* larvae in the brains of mice. Int J Parasitol 13:517–521

El-Nawawi FA, Tawfik MA, Shaapan RM (2008) Methods for inactivation of *Toxoplasma gondii* cysts in meat and tissues of experimentally infected sheep. Foodborne Pathog Dis 5:687–690

España A, Serna MJ, Rubio M, Redondo P, Quintanilla E (1993) Secondary urticaria due to toxocariasis: possibly caused by ingesting raw cattle meat? J Investig Allergol Clin Immunol 3:51–52

European Community (2004) Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:139:0055:0205:EN:PDF. Accessed 4 Nov 2010

Hill DE, Benedetto SM, Coss C, McCrary JL, Fournet VM, Dubey JP (2006) Effects of time and temperature on the viability of *Toxoplasma gondii* tissue cysts in enhanced pork loin. J Food Prot 69:1961–1965

Hoffmeister B, Glaeser S, Flick H, Pornschlegel S, Suttorp N, Bergmann F (2007) Cerebral toxocariasis after consumption of raw duck liver. AmJTrop Med Hyg 76:600–602

Jones JL, Kruszon-Moran D, Won K, Wilson M, Schantz PM (2008) *Toxoplasma gondii* and *Toxocara* spp. co-infection. AmJTrop Med Hyg 78:35–39

Magnaval JF, Dorchies P, Morassin B (2000) Actualités de la toxocarose humaine. Pyrexie 4:111–115

Morimatsu Y, Akao N, Akiyoshi H, Kawazu T, Okabe Y, Aizawa H (2006) A familial case of visceral larva migrans after ingestion of raw chicken livers: appearance of specific antibody in bronchoalveolar lavage fluid of the patients. AmJTrop Med Hyg 75:303–306

Pahari TK, Sasmal NK (1990) Experimental infection of mice with *Toxocara canis* larvae obtained from Japanese quails. Int J Parasitol 20:263–264

Park SY, Glaser C, Murray WJ, Kazacos KR, Rowley HA, Fredrick DR, Bass N (2000) Raccoon roundworm (*Baylisascaris procyonis*) encephalitis: case report and field investigation. Pediatrics 106:E56

Perlman JE, Kazacos KR, Imperato GH, Desai RU, Schulman SK, Edwards J, Pontrelli LR, Machado FS, Tanowitz HB, Saffra NA (2010) *Baylisascaris procyonis* neural larva migrans in an infant in New York City. J Neuroparasitology 1:1–5

Rubinsky-Elefant G, Hirata CE, Yamamoto JH, Ferreira MU (2010) Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. Ann Trop Med Parasitol 104:3–23

Salem G, Schantz P (1992) Toxocaral visceral larva migrans after ingestion of raw lamb liver. Clin Infect Dis 15:743–744

Shafir SC, Sorvillo FJ, Sorvillo T, Eberhard ML (2011) Viability of *Baylisascaris procyonis* eggs. Emerg Infect Dis 17:1293–1295

Smith H, Holland C, Taylor M, Magnaval JF, Schantz P, Maizels RM (2009) How common is human toxocariasis? Towards standardising our knowledge. Trends Parasitol 25:182–188

Sotelo J, Rosas N, Palencia G (1986) Freezing of infested pork muscle kills cysticerci. J Am Med Assoc 256:893–894

Sprent JFA (1953) On the migratory behaviour of the larvae of various *Ascaris* species in white mice. II. Longevity of encapsulated larvae and their resistance to freezing and putrefaction. J Infect Dis 92:114–117

Taira K, Permin A, Kapel CMO (2003) Establishment and migration pattern of *Toxocara canis* larvae in chickens. Parasitol Res 90:521–523

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

Theodoropoulos G, Kapel CM, Webster P, Saravanos L, Zaki J, Koutsotolis K (2000) Infectivity, predilection sites, and freeze tolerance of *Trichinella* spp. in experimentally infected sheep. Parasitol Res 86:401–405

Tüzer E, Toparlak M, Gargili A, Gülanber A, Keles V, Efil I, Ulutas Esatgil M (2002) Visceral larva migrans in mice caused by eating *Toxocara canis*-infected chick livers. Turk J Vet Anim Sci 26:293–297

Xi WG, Jin LZ (1998) A novel method for the recovery of *Toxocara canis* in mice. J Helminthol 72:183–184

Yoshikawa M, Nishiofuku M, Moriya K, Ouji Y, Ishizaka S, Kasahara K, Mikasa K, Hirai T, Mizuno Y, Ogawa S, Nakamura T, Maruyama H, Akao N (2008) A familial case of visceral toxocariasis due to consumption of raw bovine liver. Parasitol Int 57:525–529