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Effects of temperature and humidity on the development of eggs of *Toxocara canis* under laboratory conditions

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Abstract

The influence of temperature and humidity on the survival and development of *Toxocara canis* eggs in an *in vitro* model system was investigated. Two soil samples were inoculated with *T. canis* eggs and maintained at 3% and 50% humidity and temperatures of 19–24°C. Nine soil samples were inoculated with *T. canis* eggs of which three samples were kept at 4°C with humidities at 3%, 15%, and 30%; three were maintained at 21°C and three more were incubated at 34°C, and at the same three humidity levels. Samples were monitored every 7 days for a total of 2 months, for the presence and development of eggs. With increasing temperature, the number of eggs undergoing development increased ($P < 0.01$); the number of deformed eggs decreased, the number of infective eggs increased ($P < 0.01$), and egg maturation was accelerated. A decrease in the survival of infective eggs occurred at 34°C. An increase in humidity produced a rise in the number of developed eggs at all three temperatures ($P < 0.01$). This study suggests that elevated temperatures accelerated the development as well as the degradation of eggs of *T. canis*, whereas the range in humidity was directly correlated with egg development.

Introduction

The contamination of soil by helminths is affected by moisture levels, climatic conditions, poor disposal of excrement, and poor sanitation habits of residents in the community (Kroeger *et al.*, 1992; Gamboa *et al.*, 1998). These latter two factors enhance the spread of geohelminths eggs, which need to spend about three weeks in the soil to become infective (Glickman *et al.*, 1981). The contamination of public places with infective stages of parasites creates a risk of infection, especially for children due to their play habits (Minvielle *et al.*, 1993; Gamboa *et al.*, 2000; Córdoba *et al.*, 2002).

The introduction of large numbers of geohelminth eggs into the topsoil through animal defecation and/or fertilizers can have negative effects on the homeostatic balance of the ground (Lysek & Nigenda, 1989; Schulz & Kroeger, 1992). Environmental factors such as temperature, moisture and the nature of the soil itself can influence *T. canis* development as well as survival of the eggs.

Toxocara canis eggs have a long life span in the soil and their tough outer shells enhance their resistance to environmental fluxes and waste-water treatment. (Wharton, 1980; Black *et al.*, 1982; Lloyd, 1993). Prior research on the development of *T. canis* eggs has shown that changes in soil conditions result in a varying risk of toxocariasis in public recreation areas where dogs are allowed to defecate (Nunes *et al.*, 1994; Sommerfelt *et al.*, 1994).

The development of second stage larvae (L2) within eggs requires an optimum temperature, an adequate source of oxygen and sufficient humidity levels in the immediate environment. Since both solar irradiation and desiccation rapidly destroy the eggs of *Toxocara* sp., these eggs would be expected to survive poorly in a sandy or drained environment during warm and sunny summers (Lapage, 1974). Temperature controls the speed of the developmental process, but as moisture in the soil prevents egg desiccation, no development will occur below threshold levels of moisture (Stromberg, 1997).

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Gaspard *et al.* (1997) in France reported that temperature and humidity were the environmental parameters which most greatly influenced the survival of helminth eggs in soil. They observed that eggs kept at 4°C survived for more than 2 years, and those eggs maintained at wilting point were less viable than those maintained under field conditions.

The aim of the present study was to determine the influence of temperature and humidity on the survival and development of *T. canis* eggs in an *in vitro* model system.

Materials and methods

This work involved two experimental phases. Phase 1 was a preliminary study, in which the effect of humidity on the development and survival of eggs of *T. canis* at room temperature (19–24°C) was evaluated. Phase 2 considered the simultaneous effect of temperature and humidity.

Phase 1

Two 20 g soil samples (diameter 10 cm, depth 2 cm) were autoclaved at 121°C for 30 min to eliminate any possible larvae or eggs before inoculating with *T. canis*. In the first sample, the soil was flooded by controlled irrigation with sterilized water to produce a final volume of 0.5 ml of water per g of earth (50% (v/w) moisture). In the second sample, 0.03 ml of water per g of soil (3% (v/w) moisture), were added. Humidity was monitored by the continuous measurement of weight and controlled irrigation was necessary. The two soil samples were maintained at laboratory temperatures (between 19 and 24°C).

Toxocara canis eggs were extracted from mature female worms using Oshima's (1961) technique. Eggs were washed with sterilized water by successive centrifugations, counted and scored according to their stage of maturity using light microscopy. All eggs used developed with only a single cell and of these, 530 ± 10 were placed into a 2 cm^2 area of each soil sample, and the samples separated by cardboard.

On days 7, 14, 21, 28, 35, 42, 49 and 56 after inoculation, two samples of 0.1 g of soil were taken from each sample for observation using light microscopy. A maximum of 40 eggs per sample were counted.

Phase 2

Eggs were extracted from gravid *T. canis* females as in phase 1. Each of nine 100 g soil samples were seeded with 1000 ± 50 *T. canis* eggs and maintained under different conditions of temperature and moisture to evaluate egg development and survival.

The soil samples were maintained under constant temperatures of 4°C, 21°C and 34°C and at 3%, 15% and 30% humidities (H), throughout the experiment using daily weight control and constant irrigation.

Two grams of soil were removed weekly from each sample for analysis for a period of 8 weeks. The flotation technique of Kazacos (1983) was used to concentrate the eggs. After 24 h the samples were analysed for the presence of *T. canis* eggs.

Eggs were classified as: (i) deformed eggs, with an internal vacuole formation or rupture of the egg shell; (ii) immature eggs, without development to another phase after the inoculation; (iii) maturing eggs, without L2; and (iv) infective eggs, with L2 present.

The number of eggs in each category was counted and data were statistically analysed using the Chi-square test, at a confidence level of $P < 0.01$.

Results

Phase 1

Tables 1 and 2 show the results obtained in both soil samples of phase 1. In the first soil sample (humidity, 50% (v/w)), 320 *T. canis* eggs were observed (60.3% of the eggs seeded). Of the total stages recorded in this incubation, the cumulative scores up to day 56 after inoculation were as follows: 11.8% were deformed, 21.2% were immature, 39% were maturing, and 28% were infective, (fig. 1). A high percentage of developed eggs were observed from the first week onwards. The difference between developed and undeveloped eggs was statistically significant at day 56 after seeding ($\chi^2 = 34.9$; $P < 0.01$). Infective eggs (with an interior L2) were found from day 7 onwards.

Table 1. Proportion (%) of *Toxocara canis* eggs developing under saturated moisture conditions of 50% (v/w) soil humidity up to 56 days at a temperature range of 19–24°C.

	T. canis eggs			
	Undeveloped		Developed	
Days	No.	%	No.	%
7	6	15	34	85
14	7	18	33	83
21	19	48	21	53
28	23	58	17	43
35	15	38	25	63
42	19	48	21	53
49	10	25	30	75
56	7	18	33	83
Total	106	33.2	214	66.8

Table 2. Proportion (%) of *Toxocara canis* eggs developing under saturated moisture conditions of 3% (v/w) soil humidity up to 56 days at a temperature range of 19–24°C.

	T. canis eggs			
	Undeveloped		Developed	
Days	No.	%	No.	%
7	40	100	–	
14	30	75	10	25
21	26	86.6	4	13.3
28	30	100	–	
35	20	100	–	
42	–		–	
49	–		–	
56	–		–	
Total	146	91.5	14	8.7

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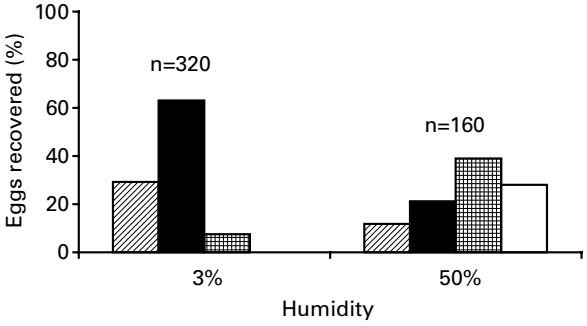

Fig. 1. Frequency (%) of *Toxocara canis* eggs recovered (▨, deformed; ■, immature; ▤, maturing; □, infective), from soil samples maintained at 3% and 50% humidity and at a temperature range of 19–24°C for up to 56 days. n = total number of eggs recovered.

In the second soil sample (humidity at 3% (v/w)), 160 eggs were observed (30.2% of those seeded), of which 29.3% were deformed, 63.1% were immature, and 7.5% exhibited detectable development (maturing eggs). Eggs with an L2 were not observed. By day 21 after seeding, it was not possible to comply with the prescribed experimental sampling count (40 eggs), as there were fewer than 40 eggs present in the 0.1 g of soil sample. From day 42 onwards no eggs were found. The difference between undeveloped versus developed eggs was statistically significant ($\chi^2 = 22.65$; $P < 0.01$).

Phase 2

The mean number of *T. canis* eggs recovered from the nine experimental soil samples was 207 per sample. The total number of eggs present in the samples at 4, 21 and 34°C and at the whole range of humidity was 765, 786 and 276, respectively at day 56 after seeding (table 3). Table 4 shows the comparison between undeveloped (deformed and immature) and developed (maturing and infective) eggs. A rise in temperature produced a progressive increase in the number of developed (especially infective) eggs ($\chi^2 = 622$, $P < 0.01$); and a decrease in the number of undeveloped ($\chi^2 = 409.8$, $P < 0.01$) and deformed eggs ($\chi^2 = 63.17$, $P < 0.01$). In soil samples maintained at 4°C, 3% and 15% H, no infective eggs were found, whereas at 21°C, 51% of eggs were infective. In the three samples incubated at 34°C, 46.7% of eggs were infective (fig. 2).

In the three samples kept at 4°C, the frequency of undeveloped eggs was greater than that of developed eggs (tables 3 and 4). In these samples, the frequency of maturing eggs was low, with a high proportion of deformed and immature eggs (fig. 2a). Maturing eggs were first found on day 14 in samples maintained at 4°C and 3% H, and on day 7 in the other two samples at this temperature (15% and 30% H). Only two infective eggs were observed on day 42 at 4°C and 30% H.

At 21°C the frequency of developed eggs was greater than undeveloped eggs (table 4), with significant differences being obtained in samples maintained at 15% and 30% humidity ($\chi^2 = 12.55$, $P < 0.01$, table 4). The recovery of infective eggs increased progressively as the humidity levels increased ($\chi^2 = 22.62$, $P < 0.01$, fig. 2b), with a greater proportion of maturing and infective eggs being present compared with samples incubated at 4°C (fig. 2a,b). Maturing eggs were only observed from day 14 at 3% H and from day 7 at 15% and 30% H. Infective eggs first appeared at day 21 at 3% H and at day 14 at 15% and 30% H.

At 34°C (fig. 2c) the recovery of eggs was at its lowest, together with a lower frequency of immature and maturing eggs. The number of deformed eggs progressively decreased with increasing humidity ($\chi^2 = 38.26$, $P < 0.01$) whereas the frequency of developed eggs increased ($\chi^2 = 50.6$, $P < 0.01$, fig. 2c), most notably the infective eggs ($\chi^2 = 39.79$, $P < 0.01$). At 15% H, the number of developed and undeveloped eggs was similar (table 4), with the highest proportion of developed and infective eggs (77%) occurring in samples maintained at 30% H.

Table 3. The frequency of developed (D +) and undeveloped (D −) *Toxocara canis* eggs in nine soil samples maintained at 4°C, 21°C and 34°C for up to 56 days at humidity levels of 5%, 15% and 30%.

	Temperature (°C)					
	4		21		34	
Day	D −	D +	D −	D +	D −	D +
7	6	3	41	49	5	8
14	49	13	30	39	6	7
21	115	20	70	182	24	27
28	109	39	46	96	40	24
35	104	4	23	57	10	41
42	109	10	38	5	19	8
49	116	10	8	51	17	14
56	52	6	33	18	12	14
Total	660	105	289	497	133	143

Discussion

Various studies in Argentina have demonstrated that an average of 13% to 68% of public places are contaminated with *T. canis* (Sommerfelt *et al.*, 1992; Minvielle *et al.*, 1993; Gamboa *et al.*, 1998, 2000; Fonrouge *et al.*, 2000; Córdoba *et al.*, 2002). Nunes *et al.* (1994) confirmed that sandy soil does not retain water and that a low humidity is lethal for *Toxocara* eggs. In the present study, soil samples with 3% humidity presented extremely adverse conditions to egg survival and development, and only some eggs were observed in the

Table 4. Frequency (%) of *Toxocara canis* eggs reaching full development in nine soil samples maintained at temperatures of 4°C, 21°C and 34°C for up to 56 days.

	Humidity (%)		
Temperature °C	3	15	30
4	14.9*	11.9*	11.8*
21	53.5 n.s.	64.9*	69.3*
34	25.8*	54.4 n.s.	77*

*Significant $P < 0.01$; n.s., not significant $P > 0.05$.

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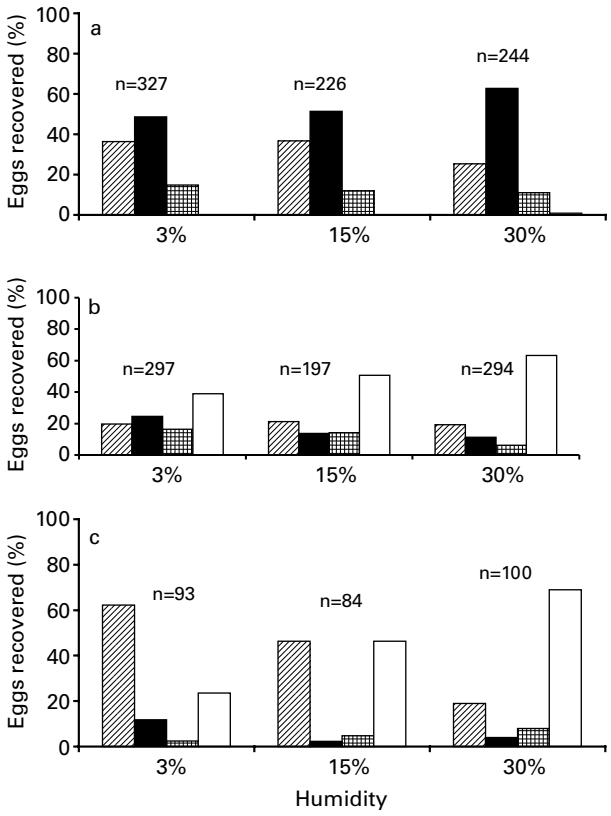

Fig. 2. Frequency (%) of *Toxocara canis* eggs recovered (▨, deformed; ■, immature; ▤, maturing; □, infective), from soil samples maintained at (a) 4°C, (b) 21°C and (c) 34°C for up to 56 days at humidity levels of 3%, 15% and 30%. n = total number of eggs recovered.

early phases of development. In the presence of 50% moisture, 66.8% of eggs developed, and 28% were infective by day 56. It was evident that a high humidity accelerated egg development as infective eggs were observed by day 7. Sommerfelt *et al.* (1992, 1994) in Argentina, O'Lorcain (1994) in Ireland, and Uga *et al.* (1995, 1996, 1997) in Asia, screened soil samples from public parks for enteroparasites. All agreed that soils in the more humid areas were more highly contaminated with *T. canis* eggs, but no specific values for moisture levels in the soils were reported. In the present experiment, a humidity value of 50% allowed the survival and rapid development of *T. canis* eggs.

In studies undertaken by Dubey (1978), Lloyd (1993), Huwer *et al.* (1989), Nakamura *et al.* (1991) and Degregorio *et al.* (1997), *T. canis* eggs produced larvae from 11 to 20 days at 30°C, 14 to 28 days at 25°C, 21 to 42 days at 22–26°C, 54 days at 11–18°C and 90 days at 10°C. In the present study, infective eggs were observed from day 7 at 34°C with humidities at 15 and 30%, from day 14 at 21°C with similar humidity levels, and from day 21 to 49 at 21°C, 3% H; 34°C, 3% H; and 4°C, 30% H.

These results indicate that eggs developed earlier than previously reported. Rapid development of larvae within the eggs occurs upon exposure to higher temperatures and humidities, e.g. at 21°C, 34°C and a humidity of 30%. An increase in soil moisture therefore facilitates the development of *T. canis* eggs and corroborates the findings of Gaspard *et al.* (1997) that an elevation in humidity (under field conditions) enables the continued survival and infectivity of helminth eggs. Furthermore, the lower survival of *T. canis* eggs at low humidity levels, e.g. 3% is also consistent with the findings of Gaspard *et al.* (1997). In contrast, at 4°C an equally low percentage of developed eggs were present at all three humidity levels (fig. 2a), although two eggs were found to be infective at 30% humidity. However, the mean number of deformed eggs at 4°C (fig. 2a) was lower than at 34°C (fig. 2c), which is consistent with the conclusion of O'Donnell *et al.* (1984), that low temperatures help to maintain eggs of *Toxocara* sp. for a longer time without deformation, whereas elevated temperatures accelerate egg degradation. Therefore, a low temperature, although delaying embryonic development, serves to preserve eggs by reducing their degradation.

The number of developed eggs at 4°C was lower than at 21°C and such a difference is attributable to a moderate temperature of 21°C being more favourable for development. This finding is in accordance with those of O'Donnell *et al.* (1984), Degregorio *et al.* (1997), Gaspard *et al.* (1997) and Minvielle *et al.* (1999), where temperature was seen to regulate the viability and infectivity of helminth eggs.

On the other hand, the lowest recovery of *T. canis* eggs occurred at 34°C, probably due to egg degradation at this temperature and resulting in a notably reduced survival rate, as the observed by O'Donnell *et al.* (1984). When the humidity was low (3%) and the temperature high (34°C), a high frequency of deformed eggs occurred (Gaspard *et al.*, 1997).

It can therefore be concluded that an increase in ambient temperature produces an acceleration in development, although when the temperatures are consistently high, eggs of *T. canis* are rapidly degraded. An increase in soil humidity is directly associated with increasing development of eggs.

This research on the effects of temperature and humidity on the development of *T. canis* will provide a basis for future studies on other environmental risk factors for *Toxocara* infections in public recreation areas.

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References

Black, M.I., Scarpino, P.V., O'Donnell, C.J., Meyer, K.B., Jones, J.V. & Kaneshiro, E. (1982) Survival rates of parasite eggs in sludge during aerobic and anaerobic digestion. *Applied and Environmental Microbiology* **44**, 1138–1143.

Córdoba, A., Ciarmela, L., Pezzani, B., Gamboa, M., De Luca, M., Minvielle, M. & Basualdo, J. (2002)

https://doi.org/10.1079/JOH2005287 Published online by Cambridge University Press

Presencia de parásitos intestinales en paseos públicos urbanos en La Plata, Argentina. *Parasitología Latinoamericana* **57** (1–2), 25–29.

Degregorio, O.J., Sommerfelt, I.E., de Cousandier, A.S., López, C.M. & Franco, A.J. (1997) Development of *Toxocara canis* eggs to their infective stage. *Avances en Ciencias Veterinarias* **12**, 101–103.

Dubey, J.P. (1978) Patent *Toxocara canis* infection in ascarid-naive dogs. *Journal of Parasitology* **82**, 383–387.

Fonrouge, R., Guardis, M.V., Radman, N.E. & Archelli, S.M. (2000) Soil contamination with *Toxocara* sp. eggs in squares and public places from the city of La Plata, Buenos Aires, Argentina. *Chilean Bulletin of Parasitology* **55**, 83–85.

Gamboa, M.I., Basualdo Farjat, J.A., Kozubsky, L., Costas, M.E. & Cueto Rúa, E. (1998) Prevalence of intestinal parasitosis within three population groups in La Plata, Buenos Aires, Argentina. *European Journal of Epidemiology* **14**, 55–61.

Gamboa, M.I., Ciarmela, M.L., Sujatovich, V.E., Bautista, L.E., Pezzani, B.C., Minvielle, M.C. & Basualdo, J.A. (2000) Detección de formas parasitarias en paseos públicos del casco urbano de la ciudad de La Plata. III Congreso Argentino de Parasitología, Mar del Plata, Argentina, 429 pp.

Gaspard, P., Ambolet, Y. & Schwartzbrod, J. (1997) Urban sludge reused for agricultural purposes: soil contamination and model development for the parasitological risk assessment. *Bulletin de l'Academie National de Medicin* **181**, 43–57.

Glickman, L., Schantz, P. & Cypess, R. (1981) Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiologic Reviews* **3**, 230–250.

Huwer, M.S., Sanft, S. & Ahmed, J. (1989) Enhancement of neutrophil adherence to *Toxocara canis* larvae by the C3 component of complement and IgG antibodies. *Zeitblatt Bakteriologische Hygiene* **270**, 418–423.

Kazacos, K.R. (1983) Improved method for recovering ascarid and other helminth eggs from soil associated with epizootics and during survey studies. *American Journal of Veterinary Research* **44**, 896–900.

Kroeger, A., Schulz, S., Witte, B., Skewes-Ramm, R. & Etzler, A. (1992) Helminthiasis and cultural change in the Peruvian rainforest. *Journal of Tropical Medicine and Hygiene* **95**, 104–113.

Lapage, G. (1974) Capítulo 5: Clase Nematoda: Orden Ascaridoidea in Parasitología veterinaria. Edit. Continental S.A., pp. 59–84.

Lloyd, S. (1993) *Toxocara canis*: the dog. pp. 11–24 *in* Lewis, J.W. & Maizels, R.D. (*Eds*) *Toxocara and toxocariasis. Clinical, epidemiological and molecular prospectives*. London, Institute of Biology and British Society for Parasitology.

Lysek, H. & Nigenda, G. (1989) Capacidad de autodeshelmintización del suelo. *Salud Pública Mexicana* **31**, 763–771.

Minvielle, M.C., Pezzani, B.C. & Basualdo, J.A. (1993) Frequency of finding helminth eggs in canine stool samples collected in public places from La Plata city, Argentina. *Chilean Bulletin of Parasitology* **48**, 63–67.

Minvielle, M.C., Niedfeld, G., Ciarmela, M.L. & Basualdo, J.A. (1999) Toxocariosis causada por *Toxocara canis*: aspectos clinicoepidemiológicos. *Enfermedades Infecciosas y Microbiología Clínica* **17**, 300–306.

Nakamura, S., Sotoyama, T., Hayasaka, S., Kameyama, Y., Maruyama, S. & Katsube, Y. (1991) Parasitism of *Toxocara canis* larvae in Japanese quails by inoculation of the ascarid eggs. *Journal of Veterinary and Medical Sciences* **53**, 865–872.

Nunes, C.M., Sinhorini, I.L. & Ogassawara, S. (1994) Influence of soil texture in the recovery of *Toxocara canis* eggs by a flotation method. *Veterinary Parasitology* **53**, 269–274.

O'Donnell, C.J., Meyer, K.B., Jones, J.V., Benton, T., Kaneshiro, E.S., Nichols, J.S. & Schaefer, F.W. (1984) Survival of parasite eggs upon storage in sludge. *Applied Environmental Microbiology* **48**, 618–625.

O'Lorcain, P. (1994) Prevalence of *Toxocara canis* ova in public playgrounds in the Dublin area of Ireland. *Journal of Helminthology* **68**, 237–241.

Oshima, T. (1961) Standardization of techniques for infecting mice with *Toxocara canis* and observations on the normal migration routes of the larvae. *Journal of Parasitology* **47**, 652–656.

Schulz, S. & Kroeger, A. (1992) Soil contamination with *Ascaris lumbricoides* eggs as an indicator of environmental hygiene in urban areas of north-east Brazil. *Journal of Tropical Medicine and Hygiene* **95**, 95–103.

Sommerfelt, I.E., Degregorio, O.J., Barrera, M. & Gallo, G. (1992) Presencia de huevos de *Toxocara* sp. en paseos públicos de la ciudad de Buenos Aires, Argentina 1989–1990. *Revista de Medicina Veterinaria* **73**, 70–74.

Sommerfelt, I.E., Degregorio, O.J., Barrera, M., Gallo, G. & Betti, A. (1994) Contaminación ambiental urbana con huevos de endoparásitos de origen animal. *Veterinaria Argentina* **10**, 457–461.

Stromberg, B.E. (1997) Environmental factors influencing transmission. *Veterinary Parasitology* **72**, 247–264.

Uga, S., Ono, K., Kataoka, N., Safriah, A., Tantular, I.S., Dachlan, P. & Ranuh, I.G. (1995) Contamination of soil with parasite eggs in Surabaya, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health* **26**, 730–734.

Uga, S., Minami, T. & Nagata, K. (1996) Defecation habits of cats and dogs and contamination by *Toxocara* eggs in public park sandpits. *American Journal of Tropical Medicine* **54**, 122–126.

Uga, S., Nagnaen, W. & Chongsuvivatwong, V. (1997) Contamination of soil with parasite eggs and oocysts in southern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **28**, 14–17.

Wharton, D. (1980) Nematode egg-shells. *Parasitology* **81**, 447–463.

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