# Veterinaria Cuyana

**Vol. 6 diciembre 2011**
**Publicación de la**
**Facultad de Ciencias Veterinarias**
**Universidad Católica de Cuyo (San Luis) Argentina**

**Versión impresa ISSN 1850-0900**
**versión en línea ISSN 1850-356X**
**ISBN 978-950-559-218-0**

**Dirección postal**
Veterinaria Cuyana
Felipe Velázquez 471 (D5702GZI)
San Luis, Argentina



**Evaluadores de trabajos de Veterinaria Cuyana**

La revista Veterinaria Cuyana consulta distintos expertos en las áreas temáticas de cada trabajo. Agradecemos el trabajo desinteresado de los evaluadores.

La revista Veterinaria Cuyana es una publicación anual de la Facultad de Ciencias Veterinarias de la Universidad Católica de Cuyo, Argentina.
Está destinada a la difusión de trabajos científicos, de revisión e información institucional de esta y de otras instituciones.

*The Journal Veterinaria Cuyana is a annual publication of the School of Veterinary Sciences of the Catholic University of Cuyo, San Luis, Argentina.*
*It is destinated to the diffusion of scientific reports in the field of the Veterinary Sciences, generated in this and in other institutions.*

# Índice

# EXPERIMENTAL EVALUATION OF THE BIOTIC POTENTIAL AND OTHER BIOLOGICAL VARIABLES OF *TOXOCARA CATI* (SCHRANK 1788): A PRELIMINARY EXPERIMENT

**Radman NE[1], Gamboa MI[1,2], Risso MA[2,3], Burgos L[1], Archelli SM[1].**

[1]Cátedra de Parasitología Comparada, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. Calle 60 y 118 La Plata (1900),
[2]Comisión de Investigaciones Científicas de la Provincia de Buenos Aires.
[3]Cátedra de Bioestadística,
Carrera de Microbiología Clínica e Industrial,
Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata.

**ABSTRACT***: The zoonotic parasite Toxocara cati (Schrank, 1788) is a nematode for domestic cats that also infects humans, with the soil being the principal common reservoir. T. cati, along with other Toxocara species plus Ascaris suum and Baylisascaris procionis, are causative agents of larva-migrans syndrome. Our aim was to determine experimentally the following biological variables for T. cati: time of egg development, prepatent period, and biotic potential. Two cats were inoculated: one with infective T.-cati eggs (IE), the other with mouse meat after deliberate T.-cati infection (M). Quantitative data were compared by the Bayesian mean-differences test and expressed as mean ± standard error. Complete egg development through the infective stage required 23 days; the prepatent period lasted 23 days in both animals; and the mathematically calculated biotic potentials obtained were lower than the values previously reported. Significant differences were observed in the distribution of the mean of eggs/female/day in favor of the M animal (M = 10,033 ± 1,281, vs. IE = 8,599 ± 937, Z = 3.620; P <0.01). The nematodes parasiting the IE cat showed a much more prolonged oviposition than the M that extended until the day after the administration of an antihelminth drug (piperazine). The M animal had stopped oviposition 15 days before that point. Nevertheless, at final of the experience (67 days postinoculation) the eggs per g for the M cat was higher than for the IE. Future studies will elucidate which mode of infection is more significant epidemiologically. Further investigations on the biology of T. cati are needed to enable an efficient epidemiological control of this zoonotic parasite.* **Key Words**: *Toxocara cati*, paratenic host, biotic potential.

# AVALIAÇÃO EXPERIMENTAL DO POTENCIAL BIÓTICO E OUTRAS VARIABLES BIOLÓGICAS DO TOXOCARA CATI (SCHRANK 1788). UMA EXPERIÊNCIA PRELIMINAR.

RESUMO: El parásito Toxocara cati (Schrank, 1788), é um nematodo freqüentemente achado em felinos domésticos que também pode infectar humanos, sendo o solo o principal reservorio. T.cati, ao igual que outras espécies do género, Ascaris suum, Toxascaris leonina y Baylisascaris procionis, ocasiona a Síndrome de Larva Migrans. O objectivo de este estudo foi determinar experimentalmente as seguintes variables biológicas para T.cati: tempo de desenvolvimento dos ovos, periodo prepatente e potencial biótico. Se inocularon dois felinos: um com ovos larvados de T.cati (IE) e outro com carne de um rato infectado experimentalmente com ovos larvados do nemátodo (M). Os dados cuantitativos comparam-se usando uma prova de diferença de médias Bayesiana e expressaram-se em média ± erro extandar. O desenvolvimento dos ovos atê seu estádio infectante obteve-se aos 23 días. O período prepatente fue de 23 días em ambos animais e os potenciais bióticos obtidos mediante cálculo matemático foram inferiores aos valores informados previamente. Teve diferenças significativas na distribuição de médias da variable Potencial Biótico a favor do animal inoculado com carne de rato infectada. (M=10,033 ± 1,281, vs. IE=8,599 ± 937, Z=3.620; P<0,01). Os nematodos que parasitaron o gato IE mostraran uma oviposición mas prolongada, que estendeu-se até o día posterior a administração da droga antihelmíntica (piperazina). No animal M interrompeu-se a oviposición 15 días antes de que IE. No entanto, ao final da experiência (día 67 post-inoculación), o gato M eliminou uma maior quantidade de ovos por grama de materia fecal que o gato IE. Transladando a natureza, a situação proposta em forma experimental, podería inferirse que os animaies infectados com carne proveniente de hospedadores paraténicos têm maior importância epidemiológica por contaminar más o ambiente. Serían necessários más estudos sobre a biología de Toxocara cati a efeitos de poder realizar um controle epidemiológico eficiente sobre este parásito zoonótico.

## INTRODUCTION

*Toxocara cati* (Schrank, 1788) is the most common nematode enteroparasite of domestic cats worldwide. Studies conducted in certain South American countries reported prevalences ranging from 10 to 65%—*e. g.*, Valdivia (65.1%) and Santiago de Chile (10%;), Chile (Torres *et al.*, 1995 López *et al.*, 2006), Rio de Janeiro (25.2%), Brazil (Labarthe *et al.*, 2004); and Buenos Aires (35.7%), Argentina (Sommerfeld *et al.*, 2006). Certain peculiarities concerning the parasite's biology, such as transplacental and transmammary transmission in *T. canis* (Burke *et al.*, 1985) and transmammary transmission in *T. cati* (Parsons *et al.*, 1987; Coati *et al.*, 2004), hinder the obtainment of parasite-free offspring. Furthermore, the production of resistant eggs and the role of small mammals as transport hosts facilitate parasite transmission (Parsons 1987; Dubinsky *et al.*, 1995). The infection and duration of *T.-cati* larvae in the tissues of paratenic hosts (small mammals and/or birds) have been studied experimentally by different investigators (Prokopik *et al.*, 1982; Akao *et al.*, 2000; Azizi *et al.*, 2007). In natural environments, these reservoir animals serve as significant contributors to the maintenance and distribution of infection in both settled and wild habitats (Dubinsky *et al.*, 1995). Like other ascarids, *T. cati* is a zoonotic pathogen and also is reported to cause the *larva-migrans* syndrome in humans so as to produce various pathologies (Petithory *et al.*, 1994; Taylor, 2001).

The soil behaves as a reservoir for this parasite, maintaining the infective forms for long periods of time. Numerous studies have been conducted on soils worldwide that indicate high levels of contamination under different environmental conditions (Uga *et al.*, 1995; Fonrouge *et al.*, 2000; Radman *et al.*, 2000; Sommerfelt *et al.*, 2006; Córdoba *et al.*, 2002).

Several authors measured the quantity of eggs produced by a female of the different *Toxocara* species. In *T. cati*, Dubey (1967) re-

ported between 19,000 and 24,000 eggs per day, though Magnaval *et al.* (2001) reported some 200,000 eggs per day. These differences in egg production have not been sufficiently studied.

The aim of the present work was to determine the following experimental biological variables of *T. cati*: time of egg development, prepatent period from two different inocula (larvated eggs and tissues of a paratenic host), and biotic potential.

## MATERIALS AND METHODS

### *EXPERIMENTAL ANIMALS*

Three 2-month-old cats from the same litter, whose mother had been deparasited before conception and during pregnancy, were used. Two of the animals were inoculated—one with infected mouse meat (M), the other with infective *T.-cati* eggs (IE)—while the third was left as a control (T). The animals were then kept in separate cages under appropriate maintenance conditions with water and food *ad libitum* for 196 days.

### *INOCULUM*

Eggs of *T. cati* were collected from adult female worms by dissection. The eggs were transferred to 2% (v/v) neutral formalin saline, kept at room temperature, and observed daily until their maturation into infective stages. Once the eggs were larvated, the inoculum was prepared.

Inoculum for cat M: Five *Balb/c* mice were inoculated by means of a gastric tube with 1,000 infective eggs each. After inoculation, one mouse was sacrificed per day to observe the presence of larvae in the mouse tissues. One of the mice, sacrificed 3 days after egg inoculation (which harbored 21 larvae in the lung and in the liver) served as the inoculum for cat M. The larvae were not counted in the murine tissues.

Inoculum for cat IE: A suspension of infective eggs was concentrated to a density of 38 eggs per 100 µl and was kept refrigerated until inoculation. Of the suspension, 200 µl were placed in a gelatin capsule (76 eggs).

Both cats were then inoculated: M spontaneously consumed the inoculum, and IE was given the gelatin capsule orally.

### *FECES*

Feces were collected daily during the following periods:

- Preinoculation: for 60 days before the inoculation. Feces were processed by the flotation technique in Sheather solution (∂, 1,300 gr/ml)
- Postinoculation: for 127 days after the inoculation. Feces were processed by the same technique until eggs of *T. cati* were present. When eggs were found, macroscopic observations were recorded and an analysis performed by the Mac Master technique.
- Posttreatment: for 9 days after treatment, macroscopic and microscopic observations were conducted and the eliminated worms selectively counted for each gender.
- Treatment: Consisted in a single dose of 200 mg/kg piperazine by mouth on day 127 postinoculation.

### *ANALYSIS AND DATA TREATMENT*

The mean of eggs eliminated by female per day (MEPG) was quantified, while the biotic potential (BP)—*i. e.*, the number of eggs produced by a single *T.-cati* female on the day of maximum production)— was also calculated (Chapman, 1928).

The following formula was used to estimate the biotic potential of both cats M and IE: the EPG (day of maximum production) / number of female worms obtained.

Quantitative data were compared by the Bayesian mean-differences test (Bolstad, 2007; Ntzoufras, 2009) and expressed as the mean ± the standard error. When no homogeneity of variances was registered, a Napierian-logarithm transformation was used (Sheskin, 2003). Bayesian inference is exact for any sample size (Kery, 2010).

The Bayesian mean-differences test: To compare mean values for the EPG and the biotic potential, a noninformative so-called *a-priori* distribution (m = 0, standard deviation = 1,000) is needed. First a Bayesian credibility interval based on the parameters of posterior distribution and then a unilateral Z test were constructed. A probability value of $p < 0.05$ was considered significant (Bolstad, 2007; Ntzoufras, 2009).

Animal maintenance and all experimental procedures performed on animals, including the euthanasia of mice, were conducted in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). This research has been approved by the Institution (Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata).

## RESULTS

*T. cati* larvae were observed in all the tissues of the sacrificed mice but were not counted. Although no eggs or adults were recovered at any time from the control mice, 21 larvae of the parasite were found in two of its reference organs (liver and lung).

No eggs of *T. cati* were observed in the feces of the kittens during the preinoculation period. *T. cati* eggs in the feces from both cats M and IE, however, first became detectable at day 23 after inoculation (prepatent period).

*T. cati* eggs in the feces from both cats M and IE were then observed intermittently during the patent period. Cat IE eliminated eggs until day 128 postinoculation (one day after the administration of the antihelminth drug) along with 4 adult *T.cati* females plus 1 adult male. In contrast, cat M stopped eliminating eggs 15 days before drug treatment, but after purgation expelled 3 adult females plus 3 adult males. No eggs or adults were recovered at any time from the control animal. Table 1 lists the EPG counts obtained over the entire period in both experimental cats.

No significant differences were found in the distribution of the mean values for the variable EPG between the two cats: [M (n = 94): 927.1 ± 114.7 *vs.* IE (n 14 = 88): 736.9 ± 77.4; Z = 1.349; P >0.05].

Significant differences were found in the MEPG values between M and IE (M = 10,032.5 ± 1,281.3 *vs.* IE =17 8,598.6 ± 936.9; Z = 3.620; P <0.01), while the BP values for the two cats [M: 52,381 (3,370 EPG x 46.63 g / 3 females) *vs.* IE: 56,906 (3,220 EPG x 70.69 g / 4 females)].

## DISCUSSION

In the present preliminary investigation, egg development at room temperature was completed after 23 days, in agreement with reports by other authors (Parsons, 1987; Gamboa, 2005).

The inoculation fed the IE cat was sparse (76 larvated eggs), while the number of larvae that the M cat consumed was not quantified. That animal, however, was given meat from a mouse that had been previously inoculated with 1,000 larvated eggs and subsequently harbored 21 larvae in the lungs and liver. This observation would imply that the M cat very likely received a heavier inoculum than the IE animal.

The prepatent period was the same in the two animals (day 23 postinoculation). This result does not, however, agree with the observations of Boch *et al.* (1982), who reported a prepatency of 8 weeks.

Although no significant differences were observed in the distribution of the mean EPG value between the M and the IE cats, the former animal exhibited significantly greater MEPG values than the latter. The BP value—as reflected in the maximum egg elimination per female per day—was much higher than the MEPG for both cats and was within the same order of magnitude (*i. e.*, *ca.* 50,000). This figure is intermediate relative to the range reported by Magnaval *et al.* (2001) and Dubey (1967), where the respective values were 200,000 and between 19,000 and 24,000 eggs per day per female.

The IE cat evinced a much more prolonged oviposition than the M animal, since this process extended up to the end of the experiment (*i. e.*, the administration of piperazine), whereas oviposition in the latter ceased 15 days earlier. Nevertheless, at 67 days postinoculation the M cat's EPG was higher than that of the IE. Future studies are needed to elucidate which of the two modes of infection is epidemiologically the more significant.

As indicated in the results, all the feces scrutinized macroscopically throughout the course of the experiment contained only few adult worms. Probably some external condition or influence during the *in-vitro* maintenance of the experimental inoculum was responsible for the low mean biotic potential of the parasites and thus the finding of relatively few such

adult specimens of *T. cati* in our experimental cats. Moreover, some worms could have gotten mascerated within the digestive tract so as to become unrecognizable upon elimination. Furthermore, the administration of a second dose of piperazine might have been useful in collaborating the absence of nematodes in the animals at the conclusion of the experiment.

In this experiment, a comprehensive analysis of the different variables could not be conducted owing to the unknown number of larvae consumed by the M feline. We may nevertheless infer that *T. cati* undergoes numerous larval losses during the completion of its life cycle in the intestine.

In addition the female *T. cati* arising from the meat-fed M cat exhibited a higher biological potential than did those from the IE inoculate given the larvated eggs. Despite the interruption of egg-laying 15 days earlier in the M cat, the females that parasitized that animal eliminated a greater total number of eggs than did those from the IE cat in the end. The interruption of oviposition in the M-cat females could be interpreted as a deterioration of individuals through migration into the tissues of the paratenic host. This would contribute to a reduction of their fertility period, though not to the number of eggs produced.

Extrapolating these experimental findings to the natural environment, we would infer as a conclusion that animals infected with meat from parasitically infected hosts would constitute a more epidemiologically consequential source of environmental contamination.

Further investigations on the biology of *T. cati* are needed involving a greater number of animals, quantitated inocula, and including a second dose of piperazine in order allow the observed results to approximate the corresponding circumstance under natural conditions. In that way appropriate epidemiologic-control measures for this parasite could be implemented since the epidimiology of its infection could be considered zoonotic.

## ACKNOWLEDGEMENTS

This work was supported by the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina and the Universidad Nacional de La Plata. The authors are grateful to Dr. Donakl F. Haggerty, a retired career investigator and native English speaker, for editing the final version of the manuscript.

## REFERENCES

Akao N., Takayanagi T.H., Suzuki R., Tsukidate S. & Fujita K. 2000. Ocular larva migrans caused by *Toxocara cati* in Mongolian Gerbils and a comparison of ophtalmologic findings with those produced by *T. canis*. J. Parasitol. 86:1133-1135.

Azizi S., Oryan A., Sadjjadi S.M. & Zibaei M., 2007. Histopathologic changes and larval recovery of *Toxocara cati* in experimentally infected chickens. Parasitol. Res. 102:47-52.

Boch J. & Supperer R., 1982. Parasitología en Medicina Veterinaria. 1º Ed español. 627 p.

Burke T.M. & Roberson E.L. 1985. Prenatal and lactational transmission of *Toxocara canis* and *Ancylostoma caninum*: Experimental infection of the bitch at midpregnancy and at parturition. Int. J. Parasitol. 15:485-490.

Chapman R.N. 1928. The quantitative analysis of environmental factors. Ecology. 9:111-122.

Coati N., Schnieder T. & Epe C. 2004. Vertical transmission of *Toxocara cati* Schrank 1788 (Anisakidae) in the cat. Parasitol. Res. 92:142-146.

Commission on Life Sciences, 1996. Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, National Research Council, National Academy Press, Washington, D.C. 164 p.

Córdoba A., Ciarmela L., Pezzani B., Gamboa M., De Luca M., Minvielle M. & Basualdo J. 2002. Presencia de parásitos intestinales en paseos públicos urbanos en La Plata, Argentina. Parasitol. Latinoam. 57:25-29.

Dubey J.P. 1967. Egg production of *Toxocara cati*. Vet. Rec. 81:671-672.

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

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. Bol. Chil. Parasitol. 55:83-85.

Gamboa M.I. 2005. Effects of temperature and humidity on the development of eggs of *Toxocara canis* under laboratory conditions. J. Helminthol. 79:327-331.

Kery M. 2010. Introduction to Winbugs for Ecologists: Bayesian Approach to Regression, Anova, Mixed Models and Related Analyses: Elsevier Science & Technology. 1-302.

Labarthe N., Serrao M.L., Ferreira A.M., Almeida N.K. & Guerreo J. 2004. A survey of gastrointestinal helminths in cats of the metropolitan region of Rio de Janeiro, Brazil. Vet. Parasitol. 123:133-139.

Magnaval J.F., Glickman L.T., Dorchies P. & Morassin B. 2001. Highlights of human toxocariasis. Korean. J. Parasitol. 39:1-11.

Ntzoufras I. 2009. Bayesian Modeling Using WinBUGS. John Wiley & Sons, Inc., Hoboken, New Jersey, p.83-124.

Parsons J.C. 1987. Ascarid infections of cats and dogs. Vet. Clin. North. Am. Small. Anim. Pract. 16:1307-1339.

Petithory J.C., Beddok A. & Quedoc M. 1994. Zoonoses dórigine ascaridienne: les syndromes de Larva migrans visceral. Bull. Acad. Natle. Méd. 178:635-647.

Prokopik J. & Figallova V. 1982. Migration of some roundworm species in experimentally infected white mice. Folia Parasitol. (Praha). 29:309-13.

Radman N.E., Archelli S.M., Fonrouge R.D., Guardis M. del V. & Linzitto O.R. 2000. Human Toxocarosis. Its prevalence in the city of La Plata. Mem. Inst. Oswaldo Cruz. 95:281-285.

Sheskin D. 2003. The Handbook of Parametric and Nonparametric Statistical Procedures. Third edition, Chapman & Hall/CRC Press, London, p.396-443.

Sommerfelt I.E., Cardillo N., Lopez C., Ribicich M., Gallo C. & Franco A. 2006. Prevalence of *Toxocara cati* and other parasites in cat´s faeces collected from the open spaces of public institutions: Buenos Aires. Argentina. Vet. Parasitol. 140:296-301.

Taylor M.R. 2001. The epidemiology of ocular toxocariasis. J. Helminthol. 75:109-18.

Torres P., Franjola R., Perez J., Auad S., Hermosilla C., Flores L., Riquelme J, Salazar S, Miranda J.C. & Montefusco A. 1995. Intestinal geohelminthosis in man and domestic animals in the riverside of the Valdivia River Basin, Chile. Bol. Chil. Parasitol. 50:57-66.

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 J. Trop. Med. Publ. Health. 26:730-734.