

BRIEF COMMUNICATION

EFFECT OF "IN VITRO" CULTIVATION TIME ON THE INFECTIVITY OF *Toxocara canis* EGGS

Susana Zevallos LESCANO (1), Maria Cristina NAKHLE (1) & Pedro Paulo CHIEFFI (1)

KEYWORDS: *Toxocara canis*; "in vitro" cultivation; Infectivity; Immune response.

Human Toxocariasis is a serious epidemiological problem in many countries². It is elicited by larvae of nematodes of the genus *Toxocara*; *T. canis* a parasite of canines is the major agent of human toxocariasis, whereas *T. cati*, infecting felines, causes this disease less frequently⁶. An important role in maintaining toxocariasis of carnivores may be played by small mammals-paratenic hosts of *Toxocara* spp. The occurrence of toxocaral antibodies in small mammals strongly suggests environmental contamination with infective eggs of *Toxocara* spp. The antibody level may be an indicator of the number of eggs ingested by the paratenic hosts, as a correlation has been ascertained between the infective dose and the antibody level^{1,8}.

The eggs of *T. canis* are extremely resistant to chemical and climatic agents and it is suspected that they may survive in an appropriate external environment for about 6 years like the related nematode *Ascaris lumbricoides*⁹. Since they are quite sensitive to desiccation and temperatures above 37 °C, we do not know how many eggs passed by dogs with patent infections remain infective in the soil.

The first references about the "in vitro" development of ascarids eggs are from STEWART¹⁴ who obtained the partial segmentation of *Ascaris* eggs submitting them to a saturated atmosphere by adding water to an hermetic system. PITTS¹¹ got the total development of *A. suum* eggs maintained in saline solution at 37°C during four weeks. Since then, different substances were assayed looking for an optimal medium for experimental development of these eggs till the using of formalin solution. GONZALEZ⁷ did not find any significative difference among saline solution, formalin at 1%, Ringer-Lactate solution and Phosphate-buffered saline when used as media to incubate *T. canis* eggs.

Toxocara eggs are unembryonated and not infectious when passed in the feces of dogs and cats into the environment. Within a period of between 3 to 6 weeks to several months, depending on soil type and climatic conditions, such as temperature and humidity, eggs develop to an infectious stage that it is supposed to survive under

optimal circumstances for at least 1 year¹⁰. Several studies from all over the world demonstrated high rates of soil contamination with *Toxocara* eggs in parks, playgrounds, sandpits and other public places with positivity values ranging from 1.3% found in Switzerland¹² up to 87.5% found in Japan¹³. Many factors interfere on longevity of these eggs; it is known that they develop better in clay soil, with poor sun light exposure and the rain intensity is considered important to concentrate them. Here in Brasil CASEIRO³ made a survey on soil contamination with *Toxocara* spp. eggs in public places in Santos, State of São Paulo, between September 1995 and September 1996 and had found higher frequencies of viable eggs between September and November 1995 and between May and June 1996. A possible explanation for this fact would be the occurrence of lower rain index, and the lower temperature when compared with summer months, facilitating eggs survival in soil and justifying the presence of higher number of living eggs in these periods of time. Another explanation would be that proposed by CHIEFFI & MÜLLER⁴ who had found the same kind of annual variation in the soil of public places in Londrina, State of Paraná., ascribing it to the fact of being more frequent the birth of puppies in these periods, as a consequence of normal periodical variations in the occurrence of bitches rut.

The present study deals on the effect of cultivation time on the infectivity of *T. canis* eggs and the level of specific antibodies generated by its inoculation in the murine model. We used 2% formalin to cultivate *T. canis* eggs at 28°C; larval development and viability were confirmed microscopically prior to inoculation. Inbred male BALB/c mice aged 6 weeks were orally infected with three doses of 100 eggs each in a week period. Mice were divided randomly into 3 groups: GI, 12 animals inoculated with 1 month cultivated eggs; GII, 10 animals inoculated with 11 months cultivated eggs, and 6 mice were maintained without infection as control group. The animals were bled from retroorbital plexus on days 23, 38 and 70 post infection. Antibodies were determined in mice sera using an enzyme-linked immunosorbent assay (ELISA) in microtitre plates using excretory-secretory (ES) antigen, obtained by in vitro cultivation of infective *T. canis* larvae⁵.

(1) Instituto de Medicina Tropical de São Paulo, LIM 06, São Paulo, SP, Brasil.

Correspondence to: Susana Zevallos Lescano, Instituto de Medicina Tropical de São Paulo, Lab. Imunopatologia da Esquistossomose, Av. Dr. Enéas C. Aguiar 470, 05403-000 São Paulo, SP, Brasil.

