BRIEF COMMUNICATION

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

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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 lumbricoides9. Since they are quite sensitive to dessiccation 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 anual 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 ocurrence 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.

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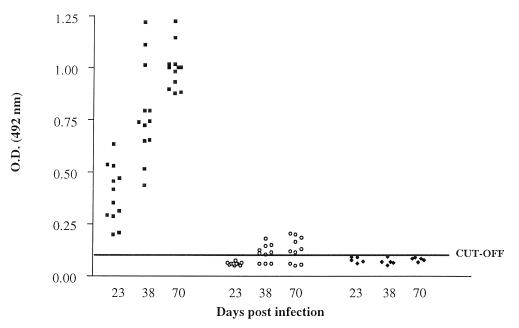


Fig. 1 - The dynamics of ELISA estimated circulating *T. canis* antibodies in BALB/c mice infected with 1 (**n**) and 11 (**o**) months eggs cultures. The represents the control group in differents days post infection.

Figure 1 shows the Optical Density (OD) values from sera at a 1:160 dilution in different days post infection. It can be seen that in mice infected with 1 month cultivated eggs the level of specific antibodies increases continuosly and all the sera were positive with the maximum response on day 70 post infection. GII mice exhibited a weaker overall immune response and it can be seen that at day 23 p.i. all the sera were still negative. On day 38p.i. 70% of sera showed positive results but the OD values were lower than that observed in GI; the same happened with the OD values on day 70 p.i.. Even though the eggs of the two groups harboured infective larvae and the viability was confirmed microscopically the infective degree observed was quite different.

Analyzing our results we can suppose that the degree of infectivity of *T. canis* eggs maintained in optimal "in vitro" conditions may be variable, decreasing sharply after, at least, 11 months of laboratory maintainance. On the other hand, we can presume that in field conditions, usually more agressive than those found in our experiment, the survival time of *T. canis* eggs could be shorter. New studies should be done to confirm this hypothesis.

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