

How to efficiently detect DNA of *Toxocara canis* and *T. cati* eggs in sand and soil samples

Wojciech Jarosz[1]*, Jean-François Durant[2], Leonid M. Irenge[2, 3], Renata Fogt-Wyrwas[1], Hanna Mizgajska-Wiktor[1], Jean-Luc Gala[2]

1 Department of Biology and Environmental Protection, Poznan University of Physical Education, Królowej Jadwigi 27/39, 61-871 Poznań, Poland; 2 Center for Applied Molecular Technologies, Institute of Clinical and Experimental Research, Université Catholique de Louvain, Tour Claude Bernard, Avenue Hippocrate 54-55 1st floor, B1.54.01, 1200 Brussels, Belgium; 3 Defense Laboratories Department, ACOS Ops&Trg, Belgian Armed Forces, Martelarenstraat, 181, 1800 Peutie, Belgium

*Corresponding author, e-mail: jarosz@awf.poznan.pl

Toxocarosis is recognized as an important and neglected disease. It is still unclear which of the species, *Toxocara canis* – parasite of canids or *T. cati* – parasite of felids, is more important in transmission of the zoonosis to human. To design the most effective preventive measures in toxocarosis it is important to recognize the source of infection i.e. to determine which of the hosts – dogs or cats spread *Toxocara* eggs with faeces in the given area. Standard, light microcopy observations are not sufficient to distinguish *T. canis* and *T. cati* eggs in soil samples, so there was a need to develop more effective and reliable technique. For the purpose we optimized DNA-based method for detection of geohelminth eggs in large volume samples of soil and sand.

The first step was to determine the most effective lysis of *Toxocara* spp. eggs. We tested: enzymatic, thermal and mechanical lysis and its combinations (6 different protocols were applied). Obtained from egg suspensions DNA was purified using the silica-based NucliSENS® miniMag® kit. Afterwards the specific quantitative real-time polymerase chain reaction (qPCR) targeting T. canis was carried out according to the procedure previously described by Durant *et al.* (2012). After determining that the mechanical lysis of eggs is the most effective for DNA extraction we used it for detecting Toxocara eggs in soil/sand samples. Three techniques were tested on samples spiked with T. canis eggs: i) DNeasy® PowerMax® Soil Kit; ii) direct extraction of DNA from samples with FastDNA® Spin Kit for Soil; iii) flotation-centrifugation method for concentration of eggs followed by DNA extraction with FastDNA® Spin Kit for Feces. The samples of DNA were purified by column and/or beads method and then qPCR amplification was performed. For soil and sand samples DNeasy® PowerMax® Soil Kit offered higher sensitivity than FastDNA® Spin Kit for Soil when the combination of column and beads purifications methods and DNA dilution were used (nearly all qPCR inhibition was eliminated). For soil samples flotation-centrifugation method subsequent DNA extraction and beads purification allowed to achieve the limit of detection of 1 egg / g of soil.

We showed that for DNA extraction the most effective lysis method of *Toxocara* eggs is mechanical disruption of its shells. For recovery of *T. canis* and *T. cati* eggs in sand samples we recommend using of direct DNA extraction with DNeasy® PowerMax® Soil Kit followed with

qPCR and for soil samples examination the following procedures should be consecutively applied: flotation-centrifugation method, DNA extraction with FastDNA® Spin Kit for Feces and beads purification. The methods can be adapted for detecting the eggs of other soil transmitted helminths in soil and sand samples.