

Recommended method for recovery of *Toxocara* and other geohelminth eggs from soil

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ABSTRACT. The flotation method elaborated for recovery of *Toxocara* and other geohelminth eggs from soil is described. Soil samples of about 500 ml volume are picked from 3-cm superficial layer of the ground. In the laboratory, 40 g of dry and sifted material is analysed according to following procedure: 1 h standing, 20 minutes shaking and 3 minutes centrifugation (1500 rpm) in 5% sodium hydroxide (NaOH), then centrifugation, like above, with H₂O for washing the sample and next with the saturated sodium nitrate (NaNO₃) for flotation the eggs. Specimen is prepared by placing a cover slip on the positive meniscus of the flotation liquid.

Key words: eggs, flotation method, geohelminths, soil examination, *Toxocara*.

There are many methods for recovery of geohelminth eggs from soil but none of them is perfect. The literature shows that the examinations are performed with different techniques and so, the results are difficult to compare.

For several years we have established the level of soil contamination with geohelminth eggs in different regions of Poland, in an urban and rural areas. We have examined almost 4000 soil samples altogether. Our experience and techniques described earlier [1, 2, 3, 4] led us to elaborate the procedure for isolation of *Toxocara* and other geohelminth eggs from soil which we advise. The method has many advantages: makes possible to isolate different geohelminth eggs from soil, allows to discriminate between dead and living eggs and observe the development of the embryos. Moreover the slides are clear, suitable for microphotography and easily stored. The efficiency of the method for recovery of *Toxocara* spp. eggs ranges from 36.6 to 56.0%.

Lately, the interest in biological soil contamination is growing up. Having been requested about the details of the method used for recovery of *Toxocara* eggs I find it worthwhile to publish the description of the whole technique. The use of the procedure by other researches will make it possible to compare the results.

Sampling

At the beginning a draft plan of the site examined should be made (this facilitates the interpretation of results). Site selection should follow a specific

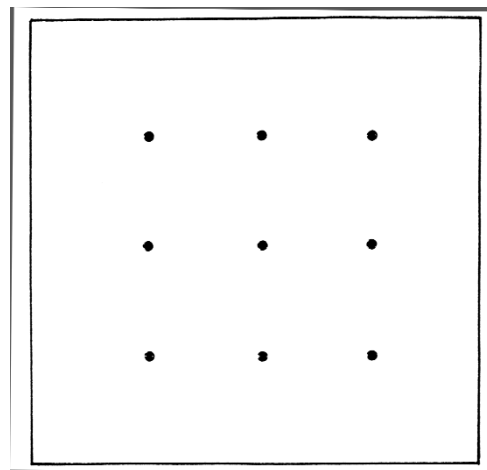


Fig. 1. Pattern of sampling

schedule (Fig. 1). Samples of about 500 ml volume are picked from the superficial layer of the ground of about 3 cm depth. This is because geohelminth eggs do not penetrate the soil profile easily and stay for a long time near the surface [5]. It is most com-

fortable to put soil samples into plastic bags with a number and description.

Laboratory examination

Soil samples should be examined in the laboratory directly after being collected. Material is spilt out on the tray and dried in the room temperature for 1 to 2 days (depending on soil humidity). Once dried the soil sample is mixed and sifted to remove solid objects. Then, a 40 g portion is weighed and put into a 250 ml Erlenmeyer's flask with a broad, smooth opening. To separate the eggs from the particles of soil 60, ml of 5% sodium hydroxide (NaOH) is poured into the sample and left for 1 h (0.05% Tween 80 is less effective than 5% NaOH). After this time the sample is shaken for 20 minutes on a swinging shaker (100 swings per minute). The whole content of the flask is energetic poured into a

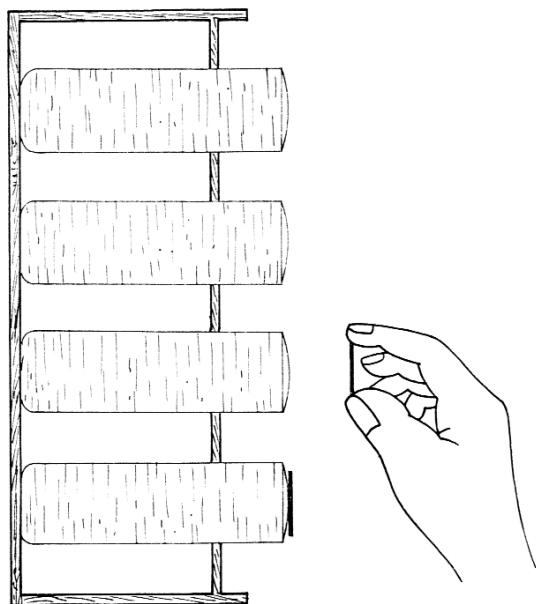


Fig. 2. Putting the cover slip on the flotation fluid with positive meniscus in the centrifuge tube

100 ml thick-walled centrifuge tube. To settle the eggs on the bottom the sample is centrifuged for 3 minutes with 1500 rotation per minute (rpm). The supernatant is discarded and centrifugation is repeated with 60 ml of water (1500 rpm, 3 minutes). After washing the material, the sediment is suspended in 60 ml of flotation fluid – saturated sodium nitrate (NaNO_3) with specific gravity 1.30 and centrifuged again (1500 rpm, 3 minutes). The eggs should set off on the surface. The tube is transferred into the stand and the flotation fluid is added with a pipette to form a positive meniscus (Fig. 2). Then on the surface of the fluid, a 24 x 24 mm cover slip is placed and left for 10 minutes. During this time geohelminth eggs stick to the glass. Then the cover slip with the hanging drop on the underside is placed on the slide. The specimen thus prepared is ready for microscopic observation. The specimens should be kept in the boxes with 100% relative humidity.

The laboratory cycle described above should not be interrupted as this might influence the result.

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