Isolation of helminth eggs from composted sludge

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From each sample of composted sludge, 10 subsamples of 10 grams each were examined as described below. Weighed samples were transferred to the container of a blender, and a few drops of detergent and 300-400 ml of tap water were added. The subsample was homogenised by running the blender for at least 15 seconds. The resulting mixture was poured through three metal test sieves (diameter 200 mm; height 50 mm) stacked in a sink. The sieves at the top, in the middle and at the bottom of the stack had mesh sizes of 70 mesh (aperture 212 µm), 140 mesh (aperture 106 µm), and 400 mesh (aperture 38 µm), respectively. The container of the blender was rinsed with 200-300 ml of tap water, and the resulting washing fluid was also poured through the sieves.

The material that was retained in the bottom sieve was collected in one corner of the sieve by gentle flushing with water from a tubing connected to a tap. The material collected was poured and flushed into a 100 ml centrifuge tube. The container of the blender and the sieves were then thoroughly flushed before processing the next subsample.

After centrifugation at 2000 rpm for 10 minutes, the supernatant was siphoned off, leaving about 5 mm of fluid above the sediment. Twenty millilitres of 10% formalin were added to the tube, and thoroughly mixed with the sediment and remaining supernatant with a glass rod. Five millilitres of ether were then added to the tube, forming a discrete layer on top of the suspension. The centrifuge tube was capped and vigorously agitated for about 30 seconds. The tube was then left in a rack for at least 1 h to allow sedimentation and separation of fat from the suspension. After sedimentation, three layers had formed. The top layer consisted mainly of fat, and formed a plug in the tube, the middle layer contained a mixture of water and formalin, and the bottom layer was the sediment. The fat-rich plug was separated from the tube wall by means of a needle and poured out and collected together with the middle layer.

One or two drops of detergent were added to the centrifuge tube along with approximately 70 ml of saturated sucrose solution (1400 grams sucrose pr. 1000 ml of water). The sediment was mixed with the sucrose solution and additional sucrose solution was added to 2-3 cm below the top of the tube. Then 20 ml of water was gently placed on top of the sucrose layer by means of a 20 ml syringe.

After centrifugation at 2000 rpm for 10 minutes, the interface between the sucrose layer and the water layer was gently siphoned off by means of a 20 ml syringe (without a needle). A total of 14-15 ml of fluid was withdrawn from the interface by moving the tip of the syringe in circles across the whole area. The syringe was connected to a membrane filter holder containing a nitrocellulose filter (diameter 25 mm, pore size 8 µm), and the contents of the syringe were gently pressed through the filter.

The syringe was detached, 10 ml of water was drawn into it from a beaker and pressed through the filter after the syringe had been reconnected to the filter holder. The syringe was detached again, and filled with air, which was pressed through the filter after reconnection of the syringe to expel water.
The filter was transferred to a microscope slide using forceps with flat ends and left to air dry at room temperature. Four to five drops of immersion oil were added to the filter and a cover slip placed upon it. The filter was then systematically examined for parasite eggs under a

microscope at 100× magnification.
Eggs were identified to genus, family or other grouping, depending on type. The eggs were classified as dead (clearly damaged), potentially alive (unembryonated, with no visible defects), or alive (moving larvae inside). Eggs of different types and condition were enumerated. After examination of all 10 subsamples in the manner described, the average number of eggs of different types per 10 gram of composted sludge was calculated.
Unembryonated nematode eggs, usually ascarid eggs, found in fresh samples of composted sludge, were sometimes difficult or impossible to classify with regard to viability. In these instances, another portion of the original sample was incubated at 27°C for 4 weeks. After incubation, another series of 10 subsamples of 10 grams each were examined as described above. If unembryonated live eggs had been present in the fresh sample, live eggs containing larvae should now be found in the incubated sample.
This method can also be used for isolation of helminth eggs from samples of raw sludge (using subsamples of 25 grams), dewatered sludge, and soil.

Acknowledgement
This method is mainly based on a method developed by cand.med.vet. Karl Bergstrøm during his research work at the Norwegian School of Veterinary Science from 1977 to 1979 on the occurrence of parasite eggs in sewage sludge. Bergstøm, for his part, modified a method published in 1977 by RJ Jørgensen as a stencil entitled 'Isolation of nematode eggs in soil and sediments. Principles and exercises' at the Royal Veterinary and Agricultural University, Copenhagen, Denmark.