A standardized method for detecting parasite eggs and oocysts in soils

L. ZENNER*, J.M. GOUNEL and C.M. CHAUVE

SUMMARY

A modification of the flotation technique for detecting and estimating egg or oocyst load of 4 different parasites from soil (Toxocara canis, Trichuris ovis, Heterakis gallinarum and Eimeria mulardi) was developed in our laboratory. We compared the egg recovery rate with two different flotation fluids (mercuric iodide and magnesium sulphate), various successive centrifugations, and pollution level of the sample. The level of recovery depends both on the parasite species and the flotation media. Recovery rates increased with the number of successive centrifugations. However, 45.2 to 92.2 % of eggs were recovered yet after the first centrifugation. The influence of mineral and organic matter on the recovery rate were 17.6 % for T. canis, 4.7 % for H. gallinarum, 7.6 % for T. ovis and 49.3 % for E. mulardi. Finally, the lower limit of our technique corresponded to infestation rates of less than 50 T. canis, T. ovis and H. gallinarum eggs, and 100 E. mulardi oocysts in 50 g of soil.

KEY-WORDS : soil - eggs - oocystes - detection - parasites.

RÉSUMÉ

Standardisation d’une méthode de détection d’œufs et d’oocystes de parasites sur les sols. Par L. ZENNER, J.M. GOUNEL et C.M. CHAUVE.

Nous avons développé au laboratoire une technique de détection d’œufs et d’oocystes de parasites sur le sol pour en estimer la charge parasitaire, en adaptant la technique de flottation. Pour cette mise au point, nous avons utilisé des œufs de Toxocara canis, Trichuris ovis et Heterakis gallinarum, ainsi que des oocystes d’Eimeria mulardi. Nous avons comparé les résultats obtenus avec 2 liquides de flottaison : l’iodomercurate de potassium et le sulfate de magnésium, puis ceux obtenus en augmentant le nombre de centrifugations. La quantité d’œufs dénombrés est fonction de l’espèce parasitaire et du liquide de flottation. Elle augmente avec le nombre de centrifugations successives. Ainsi 45.2 à 92.2 % des œufs sont retrouvés dès la première centrifugation. L’influence de la matière minérale et organique dans le prélèvement est de 17,6 % pour T. canis, 4,7 % pour H. gallinarum, 7,6 % pour T. ovis et 49,3 % pour E. mulardi. Enfin l’estimation de la limite inférieure de sensibilité pour 50 g de terre est de 50 œufs pour T. canis, T. ovis et H. gallinarum, et de 100 oocystes pour E. mulardi.

red the recovery rates of ova alone or in a soil mix, utilizing two different flotation media. We then tested the sensitivity of this technique at different ova concentrations in samples soiled with dirt.

**Materials and methods**

**PREPARATION OF PARASITE OVA**

Toxocara canis eggs were obtained from the feces of dogs brought for consultation and monitored in our laboratory. Trichuris ovis ova were provided by the «Laboratoire Vétérinaire Départemental de l’Ain» (Bourg en Bresse, France). Heterakis gallinarum ova were isolated from droppings collected on a Heterakis - contaminated poultry farm. Eimeria mulardi oocysts were produced in our laboratory [3]. For each parasite, the fecal suspension was filtered through successive sieves of progressively smaller pore diameter depending of the ova collected. Ova were removed from the retaining sieve with tap water. The suspension was collected and centrifuged at 950 g for 5 min. The pellet was then suspended in magnesium sulphate (d = 1.28) and centrifuged at 950 g for 5 min ; the supernatant liquid containing the ova was filtered. Finally ova were rinsed from the sieve with tap water and left to sediment for 2 hours. The final pellet was re-suspended in 50 ml of a 1 % formalin solution then stored at + 4°C. Ova content of an aliquot was estimated prior to experimentation with a Malassez’s count chamber. Ova counts were repeated five times.

**COMPARISON OF TWO FLOTATION FLUIDS**

Egg recovery rates (Total Recovery : TR) in two different flotation solutions, magnesium sulphate (d = 1.28) and mercuric iodide (d = 1.44), were compared by adding 300-600 eggs to 60 ml of flotation fluid. The mixture was then divided into four 15 ml centrifuge tubes, which were each covered with an 18x18 mm coverslip. The tubes were centrifuged at 150 G for 5 minutes. The coverslips were then removed, placed on a slide, and examined microscopically, and the number of eggs adhering to each coverslip was recorded. The inside of each tube was scraped with a wire in order to re-suspend any eggs which had adhered to its walls. Tubes were topped up with the same flotation media, covered, centrifuged, and the coverslips examined as before. All together, the coverslip recovery and count process was repeated five times. The protocol was conducted five times with each flotation solution.

**SOIL SAMPLES**

Batches of soil were screened for the presence of parasite ova using the methods described above. Egg-free soils (theses soils were previously screened for the absence of parasite eggs or oocysts) were then artificially seeded with parasite eggs or oocysts and used to test the recovery method described below.

**STANDARDIZED RECOVERY TECHNIQUE**

The technique is illustrated in figure 1. A sample of 50 g of egg free soil was placed in a 250 ml glass beaker, toped with water to a final volume of 200 ml and artificially seeded with known quantities of helmint eggs or coccidia oocysts. The mixture was thoroughly homogenized with a glass stick and left to decant for 20 seconds before being filtered through a coarse sieve ( pore size 0.1 mm) to remove large size debris. The mixture was homogenized again and transferred into two 100 ml centrifuge tubes which were centrifuged at 150 G for 5 minutes. One of the tubes was randomly selected; the supernatant liquid was discarded while the precipitate was suspended again in 60 ml of flotation fluid. This suspension was divided into four 15 ml tubes which were each filled to the brim. The mouth of the tubes was covered with a 18x18 mm coverslip in contact with the fluid meniscus. The 4 tubes were centrifuged at 150 G for 5 minutes. The four coverslips were then removed, placed on a slide, and examined microscopically. In the case of low eggs density, the presence of soil on the coverslip rendered a complete egg count impossible. Thus, a coverslip was considered positive if at least one egg was detected. Recovery rates (TR) were controlled by reproducing the exact technique without the adjunction of soil. Here, the number of eggs on each coverslip could be counted. The process was repeated five times for each of three egg loads (50, 100 and 200).

The sensitivity of the technique was defined as the smallest soil parasite load that could be detected in a sample. The probability $P$ of all four coverslips being negative is equal to 4 times the probability that an individual coverslip is negative.

**STATISTICAL ANALYSIS**

Statistical analysis were performed using Student’s $t$-test for means comparison. Probability calculations are explained above.

**Figure 1. — Flow diagram showing the method for eggs recovery from soil.**
Results

COMPARISON OF THE EFFICIENCY OF TWO FLOTATION FLUIDS AND OF CENTRIFUGATION NUMBERS

The efficiency of mercuric iodide and magnesium sulphate as a flotation medium for the recovery of different parasite ova after 5 successive flotations is summarized in Figure 2A. The total recovery (TR) level depends both on the parasite species (and therefore the characteristics of its eggs), and also on the flotation medium employed. With the exception of *E. mulardi* in magnesium sulphate, recovery rates greater than 60% were obtained irrespective of fluids and parasite species after the first centrifugation (Figure 2B). Higher *T. canis* and *T. ovis* recovery rates were obtained with magnesium sulphate (p < 0.02 and p < 0.01 respectively). On the other hand, *E. mulardi* recovery rates were significantly better with mercuric iodide (p < 0.03) while, recovery rates were similar for *H. gallinarum*. In view of our results, a single centrifugation in magnesium sulphate was used in the subsequent experiments.

RECOVERY OF PARASITE OVA IN SOILS

The influence of mineral and organic matter on the recovery rate (TR) is summarized in Figure 3. Obviously, recovery rates were lower in the soil samples versus the water samples. This difference was significant (p < 0.02) for *T. canis, T. ovis* and *E. mulardi* but not for *H. gallinarum*. Egg losses ranged from 4.7% to 49.3% (17.6 ± 0.1 for *T. canis*, 4.7 ± 4.0 for *H. gallinarum*, 7.6 ± 3.7 for *T. ovis* and 49.3 ± 3.5 for *E. mulardi)*.

Recovery rates in water (Table I) were good, even at the rate of 50 eggs per 200 ml water. For these concentrations of eggs, recovery rates (TR) were not assessed for soils; we determined the dilution level at which at least one coverslip was negative, that is, no eggs were detected. This level occurred with 50 eggs / 50 g soil for *T. canis, H. gallinarum* and *T. ovis*. Moreover, at this dilution the probability that the 4 coverslips resulting from one 100 ml tube are all negative, are respectively 0.01%, 9.15% and 2.56% (Table II). With *E. mulardi*, negative coverslips occurred at 100 eggs / 50 g soil sample with the probability of all negative of 1.50%.

Discussion

Few studies describe techniques for assessing environmental contamination by parasite eggs. When techniques are recorded, as is the case with *Toxocara* species, recovery rates vary greatly among authors [1, 4, 6, 9, 12, 13, 17, 20]. A standardized technique, which can be applied regardless of the parasite species, is essential for evaluating parasite loads and the resulting health hazards to humans and animals. We therefore developed and tested a centrifugal flotation technique for quantifying soil contamination by parasite eggs.

We tested this technique on four morphologically differing egg types: *T. canis* (pitted, sub-globular to oval, 85x75 µm) [7], *T. ovis* (oval, 70-80 µm, thick wall with bi-polar plugs) [18]; *H. gallinarum* (ellipsoidal, thick shelled, 63 to 71 µm by 38 to 48 µm) [18] and *E. mulardi* (ovoid, 21x17 µm) [3].

Centrifugal flotation is a qualitative diagnostic tool which is commonly utilized by medical parasitologists. Here, we sought to quantify recovery rates. Some authors observed

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<th>% recovery at the following eggs or oocysts concentration</th>
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<td><strong>T. canis</strong></td>
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<td><strong>H. gallinarum</strong></td>
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<td><strong>T. ovis</strong></td>
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<td><strong>E. mulardi</strong></td>
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Table I. — Percentage of recovery (TR) of eggs or oocysts at different initial concentrations (means ± standard deviation).

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<th>number of blank coverslip at the following eggs or oocysts concentration in soil samples</th>
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Table II. — Number of blank coverslips at different egg or oocysts concentration in soil samples. n represent the number of blank coverslip / total number of read coverslip. P represent the probability that the 4 coverslips resulting from one ml tube are all negative.
FIGURE 2. — A. Percentage of recovery of eggs or oocysts after each centrifugation with mercuric iodide or magnesium sulphate. B. Comparison of the total recovery (TR) and the first centrifugation recovery with mercuric iodide or magnesium sulphate.
that the recovery of eggs by centrifugal flotation is affected by a number of parameters, including soil texture, sample size, degree of soil contamination, pretreatment, flotation solution and flotation time [14]. We first aimed at determining the best flotation solution and protocol. Authors have recommended several flotation media including saturated solution of magnesium sulphate [16], mercuric iodide [4, 5], and sodium dichromate [4]. We compared recovery rates with both magnesium sulphate and mercuric iodide, and found that recovery rates depended on the egg species. Although magnesium sulphate was not definitively superior to the other solution, we chose it as flotation medium for the standardized recovery technique. Nevertheless, if field researchs would focus on coccidia oocysts in soils, it would be interesting from our study to choose mercuric iodide.

Repeated centrifugations are often utilized in egg flotation recovery protocols [4, 5, 16]. We also counted the total number of eggs that were recovered after five successive centrifugations. Understandably, recovery rates increased with the number of centrifugations. However, for the sake of simplicity, one centrifugation has been retained in our recovery technique. We determined the limits of this technique by varying the degree of soil contamination and identifying the threshold at which no eggs were present on all 4 coverslips.

Soil texture is the only parameter which cannot be predetermined. NUNES et al. [14] studied the influence of soil texture on the recovery rates of T. canis eggs, and determined that the best recovery rates were obtained from sandy soils. Soil particules interfere with the recovery of eggs. During preliminary trials, we noticed that the highest egg loss occurred during sieving, when soil aggregates trapped parasite ova.

In simplifying the technique, we randomly eliminated one of the initial two 100 ml tubes. However, we previously ensured that egg distribution was similar in both tubes by comparing recovery rates between them at 5 % error rate. There was no significant difference between the two tubes : we could, indeed simplify the recovery protocol in this manner. Finally, the lower limit of our technique is less than 50 T. canis, T. ovis and H. gallinarum eggs, and 100 E. mulardi eggs in 50 g of soil.

In conclusion, this technique appears to be fully compatible with field studies. However, we have already successfully applied it to determine parasitic eggs load in free-range animal production.

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References


