Comparative evaluation of flotation techniques for the detection of soil borne parasites

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Abstract Efficacy of the three conventional flotation techniques in recovering the parasitic forms from soil samples was compared after screening both naturally contaminated and experimentally seeded soil samples. Out of 200 soil samples screened from different locations of Rayalaseema region of Andhra Pradesh, parasitic stages were isolated in 62 (31.0 %), 32 (16.0 %) and 16 (8.0 %) samples by O’Lorcain (J Helminthol, 68:237–241, 1994), Kazakos (Am J Vet Res 44:896–900, 1983) and Santarém et al. (Rev Inst Med trop Sao Paulo 51(3):163–167, 2009) methods, respectively. One hundred and forty-four soil samples were seeded with two different concentrations (100/200) of the eggs of *Toxocara* spp., *Ascaris* spp. and *Ancylostoma* spp. and oocysts of *Eimeria* spp. and were processed by these techniques. Irrespective of parasitic species or concentration, the mean recovery rate of parasitic stages obtained was above 50 ± 4.32 , above 33.67 ± 5.084 and below 22.33 ± 2.37 percent by O’Lorcain (1994), Kazakos (1983) and Santarém et al. (2009) methods, respectively. O’Lorcain (1994) method was found better than the other methods for the isolation of soil borne parasitic stages but none of these methods were found to be cent per cent sensitive.

Keywords Efficacy · Flotation techniques · Recovery · Eggs–oocysts · Soil

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Introduction

Depending on prevailing environmental conditions, developmental stages of sapro-zoonotic parasites may remain viable in soil for several months to years until ingestion occurs (Bowman 1999). The presence of parasitic forms in the soil is potential source of infection to humans and animals (Brooker et al. 2006). Isolation of parasitic stages from soil is a cumbersome process as compared to the fecal and water samples because of strong electrostatic forces acting between parasite eggs and sand particles (Hawksworth et al. 2007). Thus prevalence and epidemiological analysis of soil borne parasitic diseases mainly rely on flotation techniques. Over the past few decades several flotation techniques have been used to recover the Ascarid eggs from the soil samples (Dada 1979; Quinn et al. 1980; Kazakos 1983; Dunsmore et al. 1984; O’Lorcain 1994; Loh and Israf 1998 and Santarém et al. 2008). However, most of these techniques focus mainly on the prevalence of *Toxocara* spp. Comparative performance of these techniques for detection of ova of parasites has rarely been evaluated except by Oge and Oge (2000). In the present study, the efficacy of the three techniques standardized by Kazakos (1983), O’Lorcain (1994) and Santarém et al. (2009) in recovering the parasitic forms from soil samples was determined by processing both naturally contaminated and artificially seeded soil samples.

Materials and methods

Naturally contaminated soil samples

A total of 200 soil samples were collected from parks, playgrounds, veterinary dispensaries, gardens and animal

residing areas in Rayalaseema region of Andhra Pradesh, India. Each sample was processed by the three techniques viz. Kazakos (1983), O’Lorcain (1994) and Santarém et al. (2009). About 200 g of soil from a depth 3–5 cm was collected with shovel and sealed in polythene covers.

Kazakos (1983) method

In this method a saturated solution of NaNO_3 was used as flotation medium. About 20 g of thoroughly mixed soil aliquot was taken into a 50 ml conical centrifuge tube and 30 ml of 0.5 percent Tween40 solution was added. The entire mixture was shaken thoroughly for 2 min and sieved through a nylon sieve of 1 mm^2 pore size. The filtrate was later centrifuged at 1500 rpm ($327 \times g$) for 3 min and the supernatant was discarded. Later the sediment was washed twice with distilled water. After centrifugation the supernatant was discarded and the sediment was suspended in 30 ml of saturated NaNO_3 solution (sp.gr-1.35) and centrifuged for 3 min at 1500 rpm ($327 \times g$).

O’Lorcain (1994) method

An aliquot of 20 g of soil sample was sieved through a mesh of 4 mm^2 pore size to remove coarse particles. Sieved soil sample was transferred into 50 ml conical centrifuge tube and 25–30 ml of one percent Tween80 solution was added to it. Then it was vortexed at high speed for 2 min. After homogenization, the sample was passed through a second 1 mm^2 nylon sieve into Butchner funnel by means of suction pump attached to a flask. The filtrate obtained after sieving was rinsed out of the flask into a 50 ml centrifuge tube and was subjected to centrifugation at 1500 rpm ($327 \times g$) for 5 min. The supernatant was discarded and the sediment washed twice with distilled water and centrifuged as before. The soil sample left at the bottom was resuspended in the saturated NaNO_3 solution (sp.gr-1.35) and subjected for centrifugation at 4000 rpm ($2325 \times g$) for 15 min.

Santarém et al. (2009) method

An aliquot containing 20 g soil was thoroughly mixed and 30 ml of double distilled water was added to the sample and washed twice. Before second washing, samples were allowed to settle down for an hour and then vortexed for one min and centrifuged at 2500 rpm ($908 \times g$) for 8 min. The supernatant was discarded and the sediment was resuspended in distilled water followed by homogenization and centrifugation as described above. After discarding

supernatant, 30 ml of saturated ZnSO_4 solution with sp.gr-1.2 was added and the sediment was resuspended, homogenized and centrifuged at 2500 rpm ($908 \times g$) for 8 min.

Experimentally seeded soil samples

About 1.5 kg of soil sample collected from a depth of 2–3 cm from the ground surface from animal free areas was subjected to heat sterilization at 150°C for 30 min in a hot air oven to destroy the contaminating eggs/oocysts if present and divided into 144 samples of 10 g each. The samples were further subdivided into four groups as A, B, C and D each containing 36 samples. Group ‘A’ soil samples were intended to be seeded with eggs of *Toxocara* spp. and the remaining groups B, C and D were seeded with eggs of *Ascaris* spp., eggs of *Ancylostoma* spp. and oocysts of *Eimeria* spp., respectively. The 36 samples belonging to each group were further divided into two batches (batch I—100 eggs/oocysts and batch II—200 eggs/oocysts per 10 g concentration) of each containing 18 samples. Six trails were conducted under each technique. Soil samples experimentally seeded with the eggs of *Toxocara* spp., *Ascaris* spp. and *Ancylostoma* spp. and oocysts of *Eimeria* spp. at two different concentrations were processed by Kazakos (1983), O’Lorcain (1994) and Santarém et al. (2009) methods and the percent recovery of eggs was calculated by

$$\begin{aligned} \% \text{ Recovery} \\ &= (\text{No. of ova or oocysts recovered} / \\ &\quad \text{No. of ova or oocysts seeded}) \times 100. \end{aligned}$$

Eggs of *Toxocara* spp. were obtained from the uteri of fertile female worms recovered during autopsies. Eggs of *Ascaris* spp. and *Ancylostoma* spp. were obtained from the preserved samples of Veterinary Parasitology Laboratory. Oocysts of *Eimeria* spp. were collected from the intestinal scrapings of sheep brought for postmortem. These eggs and oocysts were separately suspended in 10 ml of 10 % formalin in four individual test tubes and thoroughly mixed by vortexing. The concentration of the stock solution was

Table 1 Recovery percent of three techniques from naturally contaminated soils

Technique	Number of soil samples screened	Number found positive	Recovery percent
Kazakos (1983)	200	32	16
O’Lorcain (1994)	200	62	31
Santarém et al. (2009)	200	16	8

Table 2 Percent recovery of eggs or oocysts from experimentally seeded soil samples by three methods

Parasite species	Parasite load (No. of eggs/oocysts)	Mean recovery percent of eggs/oocysts under six trails by		
		Santarém et al. (2009) Mean $\pm$ SE	O'Lorcain (1994) Mean $\pm$ SE	Kazakos (1983) Mean $\pm$ SE
<i>Toxocara</i> spp.	200	7.42 $\pm$ 1.15	74.7 $\pm$ 2.47	54 $\pm$ 3.78
	100	7.67 $\pm$ 1.56	71 $\pm$ 6.68	46.83 $\pm$ 6.19
<i>Ascaris</i> spp.	200	5.42 $\pm$ 0.76	79.58 $\pm$ 3.25	53.83 $\pm$ 4.25
	100	9.5 $\pm$ 2.74	66.5 $\pm$ 5.27	47 $\pm$ 5.73
<i>Ancylostoma</i> spp.	200	9.67 $\pm$ 0.95	73.33 $\pm$ 3.20	37.33 $\pm$ 2.58
	100	9 $\pm$ 2.082	50.0 $\pm$ 4.32 ^a	39.33 $\pm$ 4.62 ^a
<i>Eimeria</i> spp.	200	22.33 $\pm$ 2.37	82.42 $\pm$ 4.98 ^a	73.42 $\pm$ 5.64 ^a
	100	17.17 $\pm$ 3.79 ^a	53.67 $\pm$ 6.69	33.67 $\pm$ 5.084 ^a

The values bearing same alphabetical superscripts within the row did not differ significantly

estimated prior to seeding by counting (10 times) number per 60 μ l of aliquot.

Analysis of variance was performed to compare the recovery rates of three techniques. When the values were statistically significant ($P < 0.05$) by ANOVA, differences were subsequently analyzed by using the Tukey–Kramer test.

Results and discussion

The recovery rates by three different flotation techniques for the recovery of different developmental stages of different parasites in naturally contaminated soil samples were summarized in Table 1. Out of 200 soil samples screened from different locations of Rayalaseema region of Andhra Pradesh, parasitic stages were isolated in 62 (31.0 %), 32 (16.0 %) and 16 (8.0 %) samples O'Lorcain (1994); Kazakos (1983) and Santarém et al. (2009) methods, respectively. Mean recovery percent $\pm$ SE of the three techniques in recovering four parasitic species at two different concentrations in experimentally seeded soils was summarized in Table 2. Among these techniques, O'Lorcain (1994) method was found more efficient in recovering parasite stages irrespective of parasite species and load. The values were found statistically highly significant at $P \leq 0.0001$. It clearly shows that recovery percent was lower for the four parasite species with Santarém et al. (2009) method as compared to the other two techniques. This difference was significant at $P < 0.01$ at both the concentrations for *Toxocara* spp., *Ascaris* spp. and *Ancylostoma* spp. Mean recovery percent of *Eimeria* spp. at 100 oocyst concentration by Santarém et al. (2009) and Kazakos (1983) method were not significant (17.17 $\pm$ 3.79 and 33.67 $\pm$ 5.084). Likewise the recovery percents were not significant between O'Lorcain (1994) and Kazakos (1983) for *Ancylostoma* spp. at 100 egg concentration and *Eimeria* spp. at 200 oocysts concentration.

A comparison of three existing flotation techniques by naturally contaminated soils revealed that higher positive

samples were recorded by O'Lorcain method followed by Kazakos and Santarem methods. The findings are in accordance with Grover et al. (2000) who isolated more number of *Toxocara* spp. eggs from naturally contaminated soils using O'Lorcain (1994) (6.66 %) method than Garcia (1993) method. In experimentally seeded soils, least recovery was recorded by the technique proposed by Santarém et al. (2009), with mean recovery per cent 22.33 $\pm$ 2.37 %. The lower efficacy rate of this method might be due to parasitic forms being struck in between the coarse sand particles as sieving was not done at any step and secondly, usage of water instead of detergent solution as washing agents. These reasons are in accordance with the observations made by Smith (2005); Hawksworth et al. (2007) opined that electrostatic forces acting between parasitic egg wall and soil particles leads to masking of eggs by soil particles and usage of detergent solution breaks the electrostatic forces to release the ova. Kazakos (1983) centrifugal flotation technique adopted in the current study recorded a moderate recovery of eggs from the seeded soils. Our findings on the efficacy of O'Lorcain (1994) methods in recovery of parasitic forms from soil samples are strongly in agreement with the findings of D'Souza et al. (2002); Anand et al. (2004); Azian et al. (2008) and Das et al. (2009) who adopted this method because of its high efficacy in isolating parasitic stages from environmental samples.

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