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Studies on the incidence of *Toxocara* and *Toxascaris* spp. ova in the environment. 1. A comparison of flotation procedures for recovering *Toxocara* spp. ova from soil

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SUMMARY

Seven different flotation fluids were assessed for their efficiency in recovering *Toxocara canis* ova from artificially seeded soil samples. Using the most efficient (a saturated solution of magnesium sulphate plus 5% potassium iodide) 25 g amounts of 234 environmental soil samples were examined for the presence of *Toxocara* spp. and *Toxascaris* ova. Twenty-six samples (11·1%) yielded ova of one or other species. There was no discernible pattern of distribution of positives with relation to the source of the samples. The maximum number of ova recovered in any one sample was 19. All the ova recovered from the environment were considered viable and potentially infective.

INTRODUCTION

Toxocara canis and *Toxascaris leonina* are common intestinal helminth parasites of dogs – in a survey of dogs in the Glasgow area 51% carried at least one of these species (Girdwood *et al.* 1978). The existence of viable *Toxocara* spp. ova in the superficial layers of soil presents a potential public health hazard – it is suggested that the ingestion of such contaminated soil may be important in the aetiology of human visceral larva migrans (Borg & Woodruff, 1973; Woodruff, 1970). In addition, in experimental animal studies infections with *Toxascaris leonina* ova produce a similar visceral larva migrans picture (personal unpublished data). The role of *Toxascaris leonina* in the pathogenesis of human disease has still to be defined. In order to assess the amount of environmental contamination of the soil by *Toxocara* and *Toxascaris* spp. ova a simple, accurate and reproducible egg recovery technique is necessary. The present study was undertaken to determine the relative efficiency of various electrolyte solutions in recovering these ova from soil using a modified flotation technique.

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MATERIALS AND METHODS

Artificially seeded soil samples

Six 1 kg batches of soil were screened for the presence of helminth ova using a standard zinc sulphate flotation technique (Faust *et al.* 1938). Ten 5 g samples from each batch were examined by this technique. Eight hundred grams of soil from a batch which failed to reveal ova on screening was further subjected to heat sterilization at 150 °C for 30 min in a hot air oven to destroy any undetected *Toxocara* and *Toxascaris* ova. This sterilized soil was seeded with viable *Toxocara canis* ova at a concentration of 400 ova per 25 g of soil. The ova were obtained from the uteri of fertile female *Toxocara canis* worms recovered at dog autopsies. The seeded samples were thoroughly mixed before use.

Natural soil samples

A total of 234 samples were collected from public parks including play areas, flowerbeds and verges and private gardens in the Strathclyde Region of Scotland. A trowel was used to remove the top layer of soil to a depth of 3 cm. The soil samples were stored in sealed polythene bags in a dark dry cupboard at room temperature until they were examined.

Flotation fluids

The solutions under test were made up and the specific gravities determined at 25 °C.

The following aqueous solutions were used: 33% zinc sulphate (SG 1·09), saturated zinc sulphate (SG 1·27), 33% magnesium sulphate (SG 1·07), 50% magnesium sulphate (SG 1·14), saturated magnesium sulphate (SG 1·275) and saturated sodium chloride (SG 1·205).

Procedures

Soil samples of 25 g were weighed and passed through a coarse sieve of pore size 4 mm^2 to remove stones and grass. To each sample 25 ml of 0·0025% (v/v) Tween 80 (Gurr) in tap water were added as a wetting agent to facilitate separation of the ova. The sample was homogenized in an M.S.E. homogenizer for 5 min. It was then passed through a second sieve of specification 20 mesh 31 SWG (B.S.U.)* into a Buchner flask by means of a suction pump attached to the flask and the residue was washed with a further 25 ml of Tween 80 in water. The sample was then transferred to a 50 ml round bottomed centrifuge tube and centrifuged at 2000 ***g*** for 10 min. The supernatant was discarded. The precipitate was resuspended in the solution to be tested and was again centrifuged at 2000 ***g*** for 10 min. It had been determined previously that no ova were present in the first (water) supernatant. The flotation solution in the centrifuge tube was 'topped up' to form a positive meniscus and a coverslip was superimposed and left for 5 min. The coverslip was then removed, placed on a 3 × 1 in. microscope slide and examined microscopically for the presence of ova. The residue of each sample was subjected to the same

* Endecotts (Filters) Limited, London, S.W. 19.

concentration procedure on three further occasions in order to assess the usefulness of repeated flotations of individual samples.

RESULTS

The efficiency of the various fluids tested in recovering ova of *Toxocara* and *Toxascaris* spp. using a series of four flotations for each sample is summarized in Table 1.

From this pilot study it was found that saturated magnesium sulphate performed most efficiently – 82·5 % of ova in the test sample being recovered after a series of four flotations. Moreover, 69·75 % of ova were recovered at the first flotation.

By the addition of 5 % potassium iodide (w/v) to saturated magnesium sulphate at 25 °C a solution of specific gravity 1·33 was obtained. The efficiency of this solution in recovering ova was compared with that of saturated magnesium sulphate, using ten less heavily seeded soil samples containing 12 ova per 25 g. (It was felt that such lighter concentrations of ova would more closely reflect the conditions to be found in naturally infected soil samples.) The results are presented in Table 2. It can be seen that the saturated solution of magnesium sulphate plus 5 % potassium iodide consistently recovered more ova than the saturated solution of magnesium sulphate alone. Accordingly, the former solution was used to examine the natural soil samples. Only one flotation per 25 g sample was performed. The individual results obtained on examining the first 100 such samples are presented on Table 3. It can be seen that 11 samples yielded *Toxocara* or *Toxascaris* ova or both and that the maximum number of ova recovered from any one sample was 19.

In all 234 environmental soil samples were examined using the above technique. The condensed findings are recorded in Table 4. Twenty-six (11·1 %) soil samples yielded ova of *Toxocara* and/or *Toxascaris* spp. and again it was noted that the ova were present in fairly small numbers, less than 6 ova per 25 g of soil being found in 20 out of the 26 positive specimens (77 %). All ova recovered from the environmental samples were washed in tap water and retained in the laboratory; they all developed to the L2 larval stage within 3 weeks and all larvae showed active movement within the egg shell. All eggs were therefore regarded as viable and potentially infective.

DISCUSSION

Seven flotation fluids were assessed for their efficiency in recovering *Toxocara* ova from artificially seeded soil samples. From the results obtained it appeared that, using a one-flotation procedure, only 50 % saturated magnesium sulphate, saturated magnesium sulphate and saturated magnesium sulphate plus 5 % potassium iodide were capable of recovering more than 50 % of the ova known to be present in the samples. It was noteworthy that 33 % zinc sulphate, a solution widely used for the recovery of ova and cysts from faecal specimens

Table 1. *Comparison of the efficiency of various flotation fluids in recovering* Toxocara *ova from seeded soil samples*

Flotation fluid	Specific gravity	Number of ova recovered on four serial flotations of a 25 g seeded soil sample (1)	(2)	(3)	(4)	Total no. recovered	Recovery after 4 flotations (%)	Recovery after 1st flotation (%)
33% zinc sulphate	1·09	2	1	4	0	7	1·75	0·5
Saturated zinc sulphate	1·27	66	36	8	0	110	27·5	16·5
33% magnesium sulphate	1·07	61	37	8	1	107	26·75	15·25
50% magnesium sulphate	1·14	220	65	3	0	288	72	55
Saturated magnesium sulphate	1·275	279	31	19	1	330	82·5	69·75
Saturated sodium chloride	1·205	120	64	20	1	205	51·25	30

Table 2. *Comparison of the number of* Toxocara *and* Toxascaris *ova recovered from ten seeded soil samples* (25 *g*) *using saturated* $MgSO_4$ *solution or a saturated* $MgSO_4$ *solution plus* 5% *KI*

Soil sample number	$MgSO_4$ (SG 1·275) Serial flotation number (1)	(2)	(3)	(4)	Soil sample number	$MgSO_4$ + 5% KI (SG 1·33) Serial flotation number (1)	(2)	(3)	(4)
1.	4	2	—	—	6.	6	1	1	—
2.	5	2	1	—	7.	7	2	1	—
3.	4	—	—	—	8.	8	1	—	—
4.	3	2	1	—	9.	6	2	—	—
5.	3	1	2	—	10.	7	2	—	—

Table 3. Toxocara *and* Toxascaris *ova recovered from first* 100 *natural soil samples*

Soil code number	Ova recovered	Soil code number	Ova recovered	Soil code number	Ova recovered	Soil code number	Ova recovered	Soil code number	Ova recovered
1.	2 T1	21.	—	41.	—	61.	—	81.	—
2.	—	22.	—	42.	—	62.	—	82.	—
3.	1 T1	23.	—	43.	—	63.	—	83.	—
4.	—	24.	—	44.	—	64.	—	84.	—
5.	—	25.	—	45.	—	65.	—	85.	—
6.	19 Tc	26.	—	46.	—	66.	—	86.	4 T1
7.	1 Tc	27.	—	47.	—	67.	—	87.	—
8.	1 Tc	28.	—	48.	—	68.	7 Tc, 1 T1	88.	—
9.	1 Tc	29.	—	49.	—	69.	1 Tc	89.	—
10.	—	30.	—	50.	—	70.	—	90.	—
11.	—	31.	—	51.	—	71.	—	91.	—
12.	—	32.	—	52.	—	72.	2 Tc	92.	—
13.	—	33.	—	53.	—	73.	—	93.	—
14.	—	34.	—	54.	—	74.	—	94.	—
15.	17 Tc	35.	—	55.	—	75.	—	95.	—
16.	—	36.	—	56.	—	76.	—	96.	—
17.	—	37.	—	57.	—	77.	—	97.	—
18.	—	38.	—	58.	—	78.	—	98.	—
19.	—	39.	—	59.	—	79.	—	99.	—
20.	—	40.	—	60.	—	80.	—	100.	—

T1 = *Toxascaris leonina* ova. Tc = *Toxocara spp.* ova.

Table 4. *Numbers of ova detected in 234 environmental soil samples (25 g) using $MgSO_4$ + 5% KI*

	Numbers of ova per soil sample (25 g)				Total number of positive samples	Positive (%)
	1–5	6–10	11–15	16–0		
Toxocara	12	2	1	2	26	11·1
Toxascaris	8	0	1	0		

(Spencer & Monroe, 1975) performed extremely poorly. This solution has also been used for processing soil samples (Woodruff & Shah, 1976 and Borg & Woodruff, 1973). It was also noted that the efficiency of the various solutions was not solely dependent on their specific gravities. Other physico-chemical properties of magnesium sulphate solutions must be postulated to explain their greater efficiency in recovering *Toxocara* ova from soil. Using the most efficient of the solutions investigated (saturated $MgSO_4$ + 5% KI) 234 environmental soil samples yielded 26 (11·1%) containing viable *Toxocara* and/or *Toxascaris* ova; the majority of samples (77%) contained less than six ova per sample. It should be noted that the ova of *Toxocara canis* and *Toxocara cati* are practically indistinguishable and therefore the role of the domestic cat in environmental contamination has yet to be resolved. The public health significance of such findings in random environmental soil samples must, at present, remain the subject of debate. Insufficient is known about the ecology of such ova in the soil – the effects of temperature, rainfall, sunlight and time of deposition have still to be elucidated. In addition, the physical limitations of the techniques described in this paper must be appreciated. It is felt that the nature of the soil sample, i.e. amount of herbage, consistency, water and geological composition, must influence the efficiency of the recovery procedures and, more importantly, the public health significance of such findings. Further studies are being undertaken in an attempt to define the importance of such factors.

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