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SEDIMENTATION IN PARASITOLOGICAL COPROSCOPY

Maria da Luz Ribeiro MOITINHO (1) & Cláudio Santos FERREIRA (2)

SUMMARY

A sedimentation technique is described, in which a fecal suspension is placed on top of an aqueous sucrose solution of specific gravity 1.015 g/cm^3. Using 100 by 15 mm test tubes, duplicate gravity sedimentation experiments were made using homogenized fecal suspensions (single-columns) and fecal suspensions placed on top of clear columns (double-columns). Egg- and cyst-counts, and turbidity determinations were made in the sediments obtained after definite time intervals. Most **Ascaris lumbricoides, Trichuris trichiura** and Ancylostomidae eggs sedimented within 20 minutes in single — and between 30 and 60 minutes in double-columns. **Giardia duodenalis** cysts required longer periods to sediment in double — than in single-columns; after 180 minutes (the maximum period of observation), double-column sediments produced 60.0% of the counts of single-columns. Double-column sediments were consistently less turbid than single-column ones.

KEY WORDS: Parasitological coproscopy; Sedimentation techniques; LUTZ's technique.

INTRODUCTION

Microscopic parasite objects sediment from diluted aqueous fecal suspensions at higher rates than fine, turbidity-producing particles. Thus, migration through a clear liquid column is expected to separate such particulate materials more efficiently than usual fecal sedimentation procedures.

It was decided to compare gravity sedimentation according to the current single-column method (LUTZ[4], 1919; HOFFMAN et al[3], 1934), with a double-column technique, the fecal suspension as the upper column and a sucrose solution as the lower one. The specific gravity of the latter column should be somewhat higher than that expected for the fecal suspension. As the efficiency of a coproscopic technique depends on the number of parasites recovered and the turbidity of the material being observed, results were evaluated in terms of parasite counts and turbidity determinations made in the sediments.

MATERIAL AND METHODS

Samples of normally passed, formed human feces kept under refrigeration (4°C) and processed within 72 hours after collection were used. The experimental material consisted of: 1) sam-

(1) Departamento de Análises Clínicas da Universidade Estadual de Maringá, Paraná, Brasil.
(2) Departamento de Parasitologia, Universidade de São Paulo, São Paulo, SP, Brasil.
Address for correspondence: Dr. Cláudio Santos Ferreira. Departamento de Parasitologia, Instituto de Ciências Biomédicas da Universidade de São Paulo. Edifício Biomédicas II. Av. Prof. Lineu Prestes, 1374. CEP 05508 São Paulo, SP, Brasil.

ples with cysts of **Giardia duodenalis** and eggs of **Ascaris lumbricoides, Trichuris trichiura** and Ancylostomidae. 2) samples with eggs of **A. lumbricoides, T. trichiura** and Ancylostomidae. 3) samples not necessarily with parasites.

Suspensions in distilled water (2% v/v) were prepared from all samples and strained through a Nylon net (aperture, 0.7 mm). Two sets of 100 by 15 mm test tubes, previously marked at 50 and 80 mm from the bottom, were respectively labeled "single-column" and "double-column". Tubes of the first set were filled up to the 50-mm mark with distilled water and up to the 80-mm mark with fecal suspension. The liquids were mixed by inversion. Tubes of the second set were filled up to the 50-mm mark with a sucrose solution (1.015 g/cm^3 specific gravity). Fecal suspension was carefully poured on top of the sucrose solution, care being taken to avoid mixing the two liquids. A time-count for each individual tube was started. After prescribed time-intervals, the upper 75-mm layer of the contents of each tube (both sets) was removed by suction, a fixed volume of sediment remaining.

Parasite-counts and turbidity determinations were performed in counting-chambers[1] made to contain 48.96 mm^3 (18.00 by 16.00 by 0.17 mm). Turbidimetry readings were made with the aid of a simple microphotometer equipped with a blue filter (peak transmittance at 480 nm)[2]. Distilled water was used as a standard.

Sedimentation experiments, using both single and double columns, included:

Suspensions of 89 samples with cysts of **G. duodenalis** and eggs of **A. lumbricoides, T. trichiura** and Ancylostomidae, sedimented for 60, 120 and 180 minutes. Egg- and cyst-counts were done in samples of homogenized suspensions (zero-time sedimentation) and of sediments corresponding to the three periods of time.

Suspensions of 37 samples with eggs of **A. lumbricoides, T. trichiura** and Ancylostomidae, sedimented for periods of 5, 10, 15, 20, 25 and 30 minutes. Egg-counts were done in samples of homogenized suspensions and of sediments corresponding to the six periods of time.

Suspensions of 8 samples with or without parasites, sedimented for periods of 20, 40, 60, 80, 100, 120, 140, 160 and 180 minutes. Turbidity was evaluated in homogenized samples and in sediments corresponding to each of the nine periods of time. Results were expressed as absorbance values.

The chi-square test (5% level of significance) was used to test the experimental results.

RESULTS AND DISCUSSION

Egg-counts associated with either single- or double-column sedimentation for periods of 60, 120 and 180 minutes (Table 1) did not vary significantly (chi-square: 6.9 for single-columns; 10.4 for double-columns, with 6 df.: $p > 0.05$). The hypothesis was thus accepted that "all" previously suspended **A. lumbricoides, T. trichiura** and Ancylostomidae eggs settled within the first 60-minute interval. Egg-counts in sediment samples collected at five-minute intervals during 30 minutes from each set of tubes provided further information about sedimentation rates of these parasite objects (Table 2). "All" **A. lumbricoides** eggs in single- or double-columns settled within 10 and 15 minutes. Counts of Ancylostomidae and **T. trichiura** eggs in single-column sediments corresponding to intervals from 20 to 30 minutes, did not vary significantly (chi-square: 3.0 with 4 df.: $p > 0.05$). On the other hand, a statistically significant variation was observed during the same interval in double-column sediments (chi-square: 28.1 with 4 df: $p < 0.05$). Thus, a 20-minute period sufficed for sedimentation of helminth eggs in general in 80-mm high single-columns. Double-columns of the same total height required sedimentation periods of at least 30 minutes for the same results.

TABLE 1
Parasite-counts in sediment samples from single- and double-column tubes at 60-minute intervals.

Parasites	Columns	Intervals in minutes Totals			
		0	60	120	180
A. lumbricoides	s	47	1,562	1,335	1,415
	d	0	1,384	1,428	1,329
Ancylostomidae	s	10	282	218	211
	d	0	246	245	192
T. trichiura	s	13	513	453	435
	d	0	532	504	449
G. duodenalis	s	272	1,043	1,812	2,368
	d	0	291	877	1,418

s = Single-columns; d = Double-columns.

TABLE 2
Helminth egg-counts in sediment samples from single- and double-column tubes at 5-minute intervals.

Parasites	Columns	Intervals in minutes Totals						
		0	5	10	15	20	25	30
A. lumbricoides	s	35	1,324	1,421	1,377	1,305	1,372	1,361
	d	0	783	1,191	1,506	1,315	1,237	1,337
Ancylostomidae	s	16	298	430	579	566	589	620
	d	0	57	229	311	449	483	671
T. trichiura	s	11	269	418	471	529	508	511
	d	0	107	260	370	380	454	501

s =Single-columns; d = Double-columns.

As shown in Table 1, counts of **G. duodenalis** cysts increased steadily in both sets of tubes during the entire 180-minute observation period. It was therefore not possible to evaluate the interval of time required to sediment "all" cysts in either set. Within each period of time, cyst-counts were consistently lower in double- than in single-column sediments, but differences were less pronounced in the last period. After 60-minute sedimentation, double-column produced less than 30.0% of the cysts counted in single-column sediments after the same period. A 180-minute sedimentation period produced 59.9% of the cysts counted in single-column 180-minute sediments.

Turbidity levels of well mixed suspensions and of sediments collected at 20-minute intervals, from both single- and double-column tubes, were evaluated as absorbance values. Single-column sediment absorbance values start from that of mixed suspensions and increase steadily with time. As double-column fecal particles migrate through a previously particle-free column, lower sediment turbidity levels are to be expected. This was experimentally confirmed: double-column sediments were (for the same fecal samples) consistently less turbid (i. e. produced lower absorbance readings) than single-column ones (Tables 3 and 4).

TABLE 3
Absorbance values corresponding to eight fecal suspension samples sedimented in single-column tubes, at 20-minute intervals.

Samples	Intervals in minutes Absorbance readings x 100									
	0	20	40	60	80	100	120	140	160	180
1	7.6	12.8	15.2	22.9	17.1	22.2	22.3	27.6	26.1	28.4
2	7.6	13.7	19.4	24.4	21.5	27.6	26.4	32.8	30.5	34.8
3	9.2	19.0	22.3	27.2	27.2	32.4	34.7	29.3	40.6	45.9
4	10.8	20.4	26.0	26.0	26.0	34.7	35.7	32.0	37.2	46.1
5	7.6	16.7	20.1	19.4	19.4	22.2	23.7	25.2	25.2	26.8
6	8.6	15.5	19.4	27.6	31.0	30.1	26.8	26.8	30.1	31.0
7	9.2	14.6	15.5	21.1	23.7	27.0	24.0	26.8	24.8	30.1
8	10.0	11.9	17.3	16.3	24.8	26.0	24.8	26.8	29.2	33.8
Mean	8.8	15.6	19.4	23.1	23.8	27.8	27.3	28.4	30.5	34.6
SD	1.2	3.0	3.6	4.0	4.5	4.5	5.1	2.7	5.7	7.5

TABLE 4

Absorbance values corresponding to eight fecal suspension samples sedimented in double-column tubes, at 20-minute intervals.

Samples	Intervals in minutes Absorbance readings x 100									
	0	20	40	60	80	100	120	140	160	180
1	0.0	7.6	5.6	10.8	9.7	9.7	12.5	19.4	18.7	16.7
2	0.0	3.6	9.2	12.5	14.9	14.9	16.7	14.9	16.7	21.5
3	0.0	6.1	7.6	9.2	10.8	16.7	20.1	27.6	20.1	30.1
4	0.0	4.6	9.7	11.4	14.9	16.7	18.7	22.9	21.5	25.2
5	0.0	6.1	6.1	7.6	8.6	10.8	11.4	9.7	12.5	14.9
6	0.0	7.6	13.1	14.9	12.5	17.4	17.4	17.4	19.4	25.2
7	0.0	5.6	5.6	9.7	9.7	17.4	14.3	14.9	15.5	15.5
8	0.0	7.1	7.6	9.7	14.9	15.5	12.5	14.9	22.2	18.7
Mean	0.0	6.0	8.1	10.7	12.0	14.9	15.5	17.7	18.3	21.0
SD	0.0	1.4	2.6	2.2	2.7	3.0	3.2	5.6	3.3	5.5

In routine laboratory practice, low turbidity levels mean sharp images and less time required for diagnosis. Protozoan cysts, which sediment at lower rates, are not as easily separated from fine particles as helminth eggs. The results of this study indicate that double-column sedimentation is advantageous procedure for helminth eggs concentration.

RESUMO

Sedimentação em coproscopia parasitológica.

Descreve-se uma técnica de sedimentação, na qual a suspensão fecal é colocada no topo de uma solução de sacarose de massa específica igual a 1,015 g/cm^3. Em tubos de ensaio de 100 x 15 mm, experimentos em duplicata de sedimentação por gravidade foram realizados usando-se suspensões fecais homogeneizadas (colunas simples) e suspensões fecais sobrepostas às soluções de sacarose (colunas duplas). Contagens de cistos e de ovos e determinações turbidimétricas foram feitas nos sedimentos obtidos após definidos intervalos de tempo. A maioria dos ovos de **Ascaris lumbricoides, Trichuris trichiura** e Ancylostomidae sedimentaram dentro de 20 minutos em coluna simples e entre 30 e 60 minutos em coluna dupla. Cistos de **Giardia duodenalis** demandaram períodos muito mais longos para sedimentar em coluna dupla do que em coluna simples; após 180 minutos (período máximo de observação), sedimentos de coluna dupla produziram 60,0% da contagem da coluna simples. Sedimentos de coluna dupla mostraram constantemente menor grau de turbidez do que os da coluna simples.

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