Parasitology: diagnostic yield of stool examination

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To assess the need for routinely submitting three stool samples per patient for recovery of enteric parasites, we reviewed the records of our parasitology laboratory for 1985–87 to determine the number of parasites that would not have been detected if only one or two samples had been submitted. A total of 16% of all stool samples were positive. For each sample that was positive for a parasite (index sample) a search was done for other stool samples, positive or negative, received from the same patient within 6 days of reception of the index sample. We identified 676 sets of two (276) or three (400) samples of which at least 1 was positive. A total of 93% of the enteric parasites were detected in the first sample in the two-sample sets. Among the three-sample sets 90% of the parasites were detected in the first sample, 8% in the second and 2% in the third. We recommend waiting for the result from the first stool sample rather than routinely submitting three samples for recovery of enteric parasites.

Il est habituel de prélever trois échantillons de selles pour l'analyse parasitologique. Afin de savoir si les trois sont nécessaires, nous déterminons à partir des dossiers de notre laboratoire pour 1985 à 1987 combien de recherches auraient été positives si on n'avait examiné qu'un ou deux échantillons par sujet. De tous les échantillons 16% sont positifs. Pour chacun de ceux-ci on cherche les rapports concernant les autres échantillons provenant du même sujet et parvenus au laboratoire dans les 6 jours de la réception de l'échantillon positif. On trouve ainsi 276 groupes de deux échantillons et 400 de trois dont au moins un est positif. Des diagnostics positifs 93% le sont dès le premier échantillon s'il y en a eu deux, 90% s'il y en a eu trois. Dans ce dernier cas le diagnostic est posé au deuxième échantillon 8% des fois et au troisième 2% des fois. C'est pourquoi nous conseillons de prendre connaissance du résultat d'une première analyse plutôt que d'en prescrire trois d'emblée.

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It is common practice to ask patients suspected of having a parasitic enteric pathogen to submit three stool samples for microscopic examination in the laboratory. This practice was adopted following the work of Sawitz and Faust,[1] who showed that the rate of recovery of *Entamoeba histolytica* was under 50% if only one stool specimen was examined. If six specimens were examined the rate was over 90%. For the recovery of helminths examination of one or two stool samples was usually sufficient.

We performed a study to assess the need for routinely collecting three stool samples for recovery of enteric parasites and to estimate how many and which type of enteric parasites would not have been detected if only one sample had been submitted per patient.

Methods

We reviewed the records for 1985–87 at our laboratory. During the study period 13 478 stool samples were examined, and the positivity rate was 16%. For each sample that was positive for a parasite (index sample) a search was done for other stool samples, positive or negative, received from the same patient within 6 days of reception of the index sample. A "set" was defined as a group of two or three samples of which at least one was

positive for a parasite. The decision to collect one, two or three samples was made by the ordering physician and was not dictated by the results of sample examination. If the first sample in the set was negative the set was called "first negative". If the first two samples were negative the set was called "first two negative". A set was called "first positive" if the first sample was positive, regardless of the results of the other samples.

In our laboratory each stool specimen preserved with sodium acetate–formalin is concentrated by the formalin–ether technique. If requested, or if the patient is young, has travelled outside the country recently or is immunocompromised, a modified sugar flotation–centrifugation screening technique is performed to detect *Cryptosporidium* spp. Any positive result of screening is confirmed by means of a Ziehl–Neelsen stain. Every preserved specimen is examined as described, but in addition an iron–hematoxylin stain is performed.

Table I — Distribution of categories of stool sample sets

Category	No. (and %) of sets: Two samples (n = 276)	Three samples (n = 401)
First negative	18 (6)	30 (8)
First two negative	–	10 (2)
First positive	258 (93)	361 (90)

Table II — Distribution of parasites among the two-sample sets

Parasite	No. (and %) of sets: First sample positive	Second sample only positive	Total
Protozoa			
Entamoeba histolytica	2 (100)	0 (0)	2
Entamoeba spp.	45 (94)	3 (6)	48
Giardia lamblia	53 (88)	7 (12)	60
Dientamoeba fragilis	57 (93)	4 (7)	61
Cryptosporidium	47 (98)	1 (2)	48
Other	2 (50)	2 (50)	4
Helminths	8 (89)	1 (11)	9
Mixed	44 (100)	0 (0)	44
Total	258 (93)	18 (6)	276

Results

The distribution of the various categories of sets is shown in Table I. If each patient who submitted two stool samples had submitted only one sample 6.5% of the enteric parasitic infections would not have been diagnosed. The corresponding rate for the patients who submitted three stool samples was 10%. Among the latter group, submitting two samples would have been sufficient to establish a diagnosis in 97.5% of cases.

Table II shows the distribution of the various parasites among the two-sample sets: 223 sets (81%) contained a protozoan, 9 (3%) a helminth and 44 (16%) more than one parasite per sample (mixed). *Giardia lamblia* was least likely to be detected in the first sample. On the other hand, when a patient had cryptosporidiosis, only rarely (in 2% of cases) was it not diagnosed by examination of the first sample.

The distribution of the various parasites among the three-sample sets is shown in Table III. The proportions of sets containing protozoa and helminths and of mixed sets were similar to those for the two-sample sets (78%, 3% and 19% respectively). Similar trends for *G. lamblia* and for *Cryptosporidium* spp. were found. In addition, *Dientamoeba fragilis* was least likely to be recovered by examination of the first sample.

Discussion

Our results show that in at least 90% of cases examining only one stool sample is sufficient to diagnose an enteric parasitic infection. This finding

Table III — Distribution of parasites among the three-sample sets

Parasite	No. (and %) of sets: First sample positive	First and second samples positive	Third sample only positive	Total
Protozoa				
E. histolytica	4 (100)	0 (0)	0 (0)	4
Entamoeba spp.	78 (89)	7 (8)	3 (3)	88
G. lamblia	69 (87)	8 (10)	2 (2)	79
D. fragilis	68 (83)	9 (11)	5 (6)	82
Cryptosporidium	49 (94)	3 (6)	0 (0)	52
Other	4 (80)	1 (20)	0 (0)	5
Helminths	12 (92)	1 (8)	0 (0)	13
Mixed	77 (99)	1 (1)	0 (0)	78
Total	361 (90)	30 (8)	10 (2)	401

is quite different from the conclusion of Sawitz and Faust,[1] who stated that the yield of examining one stool sample was under 50% for *E. histolytica*. The discrepancy can be at least partially explained by differences in the techniques used in stool preparation and examination. Sawitz and Faust examined only fresh specimens. For each sample they prepared a direct fecal film, unstained and iodine stained, a hematoxylin-stained film and a film prepared after zinc sulfate concentration. Although this concentration technique may be comparable to the formalin–ether technique that we used in our laboratory, examining preserved specimens undoubtedly increases the rate of diagnosis of parasitic infections.

Our results are in accordance with a report by Montessori and Bischoff,[2] who found that 95.6% of enteric parasites were identified in the first specimen and an additional 3.5% and 0.9% in the second and third specimens respectively.

Why was *G. lamblia* least likely to be detected in the first sample? It is well known that in giardiasis intermittent shedding is not uncommon: there can be periods of up to 21 days during which the protozoan cannot be recovered from the stool. In addition, the median prepatency period of giardiasis has been estimated to be 14 days.[3] An investigating physician who strongly suspects giardiasis should order at least two more samples[4] if the first one examined is negative, since in our experience 12% of cases of giardiasis were diagnosed by examination of the second sample in the two-sample sets and 13% by examination of the second or third sample in the three-sample sets.

We therefore recommend waiting for the result from the first sample rather than sending several samples at the same time to the parasitology laboratory. When the first sample examined is negative for enteric parasites, up to two additional samples should be examined to rule out this diagnosis more accurately.

The effect of our recommendations on the volume of stool specimens received can be estimated. Table IV shows that 1020 tests were done unnecessarily in the sets in which at least one sample was positive. This represents 7.6% of the total number of samples examined during the study period. Table V shows that 8721 additional tests would have to be done in the one-sample and two-sample sets in which the first or the first and second samples were negative. Subtracting the 1020 "unnecessary" tests from this figure, we find that our recommendations would generate 7701 additional tests, an overall increase of 57.1%.

Peters and colleagues[5] recently suggested that for cost containment, samples received from a given patient be pooled before processing. Our findings show that such a practice is unnecessary since examining one sample is usually sufficient to diagnose parasitic infection.

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References

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Table V — Number of additional unnecessary tests if three tests were done for patients in the one-sample and two-sample groups

Type of set	No. of tests actually done	No. (and %) of additional unnecessary tests
One-sample	3060	6120
Two-sample	2601	2601
Three-sample	5661	—
Total		8721 (64.7*)

*As in Table IV.

Table IV — Number of necessary and unnecessary repeat tests in the two-sample and three-sample sets in which at least one sample was positive

Type of set	No. of tests	No. of sets in which first sample positive	No. (and %) of repeat tests	
			Necessary	Unnecessary
Two-sample (n = 276)	552	258	18	258
Three-sample (n = 401)	1203	361	40	762
Total			58	1020 (7.6*)

*Proportion of grand total of samples (13 478).