Application of Rejection Criteria for Stool Ovum and Parasite Examinations

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We retrospectively determined the yield of 2,015 stool ovum and parasite (O&P) examinations performed over an 11-month period. Two aspects were evaluated: the yield of positive results from multiple specimens per patient versus the result of a single examination, and the yield of positive results from stools submitted after 3 days of hospitalization. There were 131 (6.7%) positive O&P examinations from 75 patients: for 35 patients the single examination was positive; for 18, two of two examinations were positive; and for 15, three of three examinations were positive. The remaining seven patients had at least one negative examination in a series, but pathogenic intestinal parasites were detected in only three of these patients. Seventeen patients with positive O&P examinations were inpatients: seven of nine patients examined for O&P within 3 days of admission had stool specimens that contained recognized pathogens, in contrast to only two of eight patients examined after 3 days of hospitalization. After reviewing the data and informing hospital clinics, wards, and physicians, we instituted the following policy for screening stool specimens submitted for O&P examination. Only one O&P examination was performed for each outpatient visit and for inpatients hospitalized for 3 days or less, and examinations were not performed on stools submitted after 3 days of hospitalization unless the clinician arranged for the examination on the basis of clinical need. Over the 6-month follow-up period, 29.9% of O&P requests were rejected, 22% for patients in hospital for longer than 3 days and 7.9% for multiple O&P requests. Of 265 initially rejected specimens, 22 (8.3%) were examined after the referring physician contacted the laboratory. None of these specimens was positive. We conclude that eliminating O&P examinations of patients hospitalized for more than 3 days and initially performing only one examination per patient significantly reduces the number of examinations performed and reduces laboratory charges without adversely affecting patient care.

Clinical laboratories commonly receive two or three stool specimens per patient for ovum and parasite (O&P) examination in search of parasitic causes of diarrhea. Several studies are quoted as evidence that multiple specimens are required to achieve adequate sensitivity (2, 3, 7, 13, 16, 19). Most of these studies, however, are epidemiological investigations aimed at detecting asymptomatic parasitic infection, particularly with *Entamoeba histolytica* (2, 3, 7, 13, 19). In addition, some investigations did not use a stool concentration method (3, 7). It is possible, therefore, that the multiple-examination concept is epidemiologically flawed when applied to the task of identifying parasitic causes of diarrhea in a tertiary-care institution.

Siegel et al. recently published data on inappropriate testing for diarrheal diseases in patients hospitalized for more than 3 days (15). None of their 90 positive O&P examinations over a 3-year period were performed with specimens from patients who had been hospitalized for more than 3 days. Because we were aware that many of our O&P examinations were being performed with specimens from patients who had been in hospital for more than 3 days, we conducted a retrospective review of an 11-month period to determine the proportion of such O&P examinations that revealed parasites. We also reviewed all positive O&P examinations to determine whether submitting multiple stool specimens increased the diagnostic yield over that of a single O&P examination.

MATERIALS AND METHODS

Patient population. Duke University Medical Center is a 1,125-bed tertiary-care hospital that in fiscal year 1990 to 1991 admitted 308,549 inpatients and had 507,913 outpatient visits.

Specimen processing. All stools were received in the Para-Pak Stool System (Meridian Diagnostics, Cincinnati, Ohio) containing stool in 10% formalin in one container and in polyvinyl alcohol (PVA) fixative in the other. Formalin-preserved stools were concentrated by the formalin-ethyl acetate method with the Fecal Concentrator kit (Remel, Lenexa, Kans.). PVA-preserved stool was used to make a permanent trichrome-stained smear (MicroScan, Mahwah, N.J.).

Data analysis. The O&P bench daily logbook for the period from January through November 1990 was inspected for the following: total number of specimens received, number of specimens received per patient, day of hospitalization on which each specimen was collected, number of specimens processed, number of positive examinations, and identity of the parasites that were present. Stool specimens submitted within 7 days from the same patient were considered a series from that patient; stool specimens submitted more than 7 days apart were recorded as if they were from different patients. Examinations for *Enterobius vermicularis* by the adhesive-tape method were not included, nor were examinations for *Cryptosporidium* spp. The results of two previous surveys (March 1984 through May 1985 and December 1979 through December 1981) were also inspected.

Rejection criteria. Once the results were analyzed, a circular detailing rejection criteria for O&P examinations

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TABLE 1. Number of stool specimens received for O&P examinations per patient

No. of specimens/ patient	No. of patients	No. (%) of patients with positive examination	Total no. (%) of specimens
1	579	35 (6.7)	579 (26)
2	281	23 (8.2)	562 (25)
3	223	17 (7.6)	669 (30)
4	44	0	176 (8)
5	13	0	65 (3)
6	23	0	138 (6)
7	4	0	28
10	1	0	10
11	1	0	11
Total	1,169	75	2,238 (100)

was sent to all wards, clinics, and medical practitioners in the medical center. The circular stated that (i) the previous recommendation for performing three O&P examinations per patient was based on old epidemiological studies involving asymptomatic infections and therefore was not applicable to most patients with symptomatic parasitic infections; (ii) beginning 1 October 1991, only one O&P examination would be performed per outpatient visit or for inpatients hospitalized for 3 days or less; (iii) if the first specimen was negative and *Giardia lamblia* was thought clinically likely, a second specimen could be submitted no earlier than 5 days later; (iv) stool specimens submitted for O&P examination from patients who had been in hospital for longer than 3 days would not be processed; (v) if clinical reasons existed for a stool O&P examination outside these criteria, the clinician should contact the on-call Medical Microbiology Fellow or Clinical Pathology Resident.

Once the rejection criteria were in place, a daily record was kept of the number of specimens rejected and the reason for rejection. The number of specimens processed after a physician call to the Medical Microbiology Fellow was also recorded.

RESULTS

Over the past decade there has been a 310% increase in the number of O&P examinations performed at Duke University Medical Center, from 57 a month in 1981 to 129 a month in 1984–1985 to 181 a month in 1990. Table 1 shows how many specimens were received from each patient during January through November 1990. Two or more examinations were requested for 50% of patients. Of the 75 patients found to have enteric parasites, 68 had no negative examinations: for 35, their single examination was positive; for 18, two of two examinations were positive; and for 15, all three examinations were positive. Therefore, all 68 of 75 (90.7%) patients would have had their enteric parasitic infection detected on the basis of a single O&P examination. Of the seven patients who had at least one negative examination, three had one of two examinations positive for *B. hominis*, three had one of two examinations positive for *Dientamoeba fragilis*, *Entamoeba hartmanni*, and *G. lamblia*, respectively, and one had two of three examinations positive for *G. lamblia* (all three stool samples from this patient were submitted on the same day). Therefore, only 7 of 75 (9.3%) patients required more than one O&P examination to detect their enteric parasitic infection, and none required more than two examinations.

TABLE 2. Identity and frequency of parasites found in 131 O&P examinations from 75 patients

Parasite	No. (%) found
<i>Blastocystis hominis</i>	62 (39)
<i>Giardia lamblia</i>	52 (33)
<i>Endolimax nana</i>	17 (11)
<i>Entamoeba coli</i>	8 (5)
<i>Entamoeba hartmanni</i>	6 (4)
<i>Dientamoeba fragilis</i>	6 (4)
<i>Strongyloides stercoralis</i>	3
<i>Chilomastix mesnili</i>	2
<i>Entamoeba</i> sp.	1
<i>Entamoeba histolytica</i>	1
Total	158 (100)

Table 2 lists the 158 parasites seen in the 131 positive O&P examinations from the 75 patients. Fourteen patients had mixed infections, nine with two organisms and five with three organisms. Table 3 details the number of positive O&P examinations for each parasite.

Table 4 lists the parasites found in the 17 inpatients with positive examinations. Accepted pathogens were detected in two of these patients after 3 days of hospitalization. One patient, a 27-year-old man with AIDS, was admitted with dehydration, renal insufficiency, metabolic acidosis, anemia, and cytomegalovirus retinitis. Eight months earlier, as an outpatient, he had had all three O&P examinations positive for *G. lamblia*, *B. hominis*, and *D. fragilis* and had received 750 mg of metronidazole twice a day for 10 days. During the present admission, stool examinations for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Clostridium difficile* toxin, fecal leukocytes, and *Cryptosporidium* spp. were negative. *Mycobacterium avium* complex was recovered from his stool. During this hospitalization only one stool specimen was submitted for O&P examination, and *E. histolytica* was present. His diarrhea improved, and he was discharged without specific therapy for the *E. histolytica*. A few days after discharge he presented to the Emergency Room with fever, cough, and continuing diarrhea. Three O&P examinations were negative. It is thought that the *E. histolytica* was not responsible for his diarrhea but rather that asymptomatic carriage was unmasked by it.

The second patient, a 54-year-old man with nodular diffuse

TABLE 3. Number of O&P examinations positive for each parasite recovered

Parasite	No. of parasites present	No. of positive examinations					
		1/1	2/2	3/3	1/2	1/3	2/3
<i>Blastocystis hominis</i>	62	11	8	10	5		
<i>Giardia lamblia</i>	52	14	10	5	1		1
<i>Endolimax nana</i>	17	4	2	2	2	1	
<i>Entamoeba coli</i>	8	3	1	1			
<i>Entamoeba hartmanni</i>	6	2		1	1		
<i>Dientamoeba fragilis</i>	6	2		1	1		
<i>Strongyloides stercoralis</i>	3	1	1				
<i>Chilomastix mesnili</i>	2		1				
<i>Entamoeba</i> sp.	1					1	
<i>Entamoeba histolytica</i>	1	1					
Total	158	38	23	20	11	1	1

TABLE 4. Positive O&P examinations on 17 inpatients

Day(s) of hospitalization	Parasite detected
1.....	<i>Giardia lamblia</i>
1.....	<i>Giardia lamblia</i>
2.....	<i>Giardia lamblia</i>
2.....	<i>Giardia lamblia</i>
2.....	<i>Dientamoeba fragilis</i>
2.....	<i>Blastocystis hominis</i>
3.....	<i>Entamoeba nana</i>
3.....	<i>Giardia lamblia</i> , <i>Entamoeba nana</i>
3, 5.....	<i>Giardia lamblia</i>
5.....	<i>Blastocystis hominis</i> , <i>Endolimax nana</i>
6.....	<i>Entamoeba histolytica</i> ^a
6, 9.....	<i>Blastocystis hominis</i>
7.....	<i>Entamoeba hartmanni</i>
8, 14.....	<i>Strongyloides stercoralis</i> ^a
13.....	<i>Blastocystis hominis</i>
14, 17.....	<i>Blastocystis hominis</i>
14.....	<i>Blastocystis hominis</i>

^a Only two positive examinations after 3 days in hospital were with accepted pathogens. See the text for case histories.

large-cell lymphoma, was admitted for chemotherapy. His admission leukocyte count was 11.8×10^9 with a 55% eosinophilia. Chemotherapy was begun on day 2, and the patient developed fever on day 3 and then diarrhea on day 4. Blood cultures taken on day 8 grew *Clostridium tertium* and *Enterobacter cloacae*. A specimen sent for O&P examination on day 8 was positive for *Strongyloides stercoralis*, and the patient was given thiabendazole for 3 days. An O&P examination on the day therapy stopped showed that his stools still contained *S. stercoralis*, and he was re-treated until his leukocyte count became 1.0×10^9 . Follow-up stool O&P examinations on days 19 and 32 were negative.

During the first 6 months following implementation of the rejection criteria, a total of 811 stool specimens were submitted for O&P examination. The number of submitted specimens decreased from 184 in the first month to 120 by the third month, after which the number remained approximately stable. If the number continues to remain at or about 120 per month, this would represent a 34.4% decrease in the number of specimens received for O&P examination. Of the specimens submitted, 265 (32.6%) were rejected; 22 specimens were initially rejected but were examined after the ordering physician contacted the Medical Microbiology Fellow or Clinical Pathology Resident. Therefore, the overall decrease in O&P examinations was from approximately 180 a month to 100 a month, a 44.4% decrease.

Most of the rejected specimens (179 of 243 [74%]) were rejected because the patient developed diarrhea after day 3 of hospitalization. The other 64 specimens (26%) were rejected because multiple specimens were submitted. Only 22 of 265 specimens (8.3%) initially rejected were subsequently processed per the ordering physician's request. None of these contained parasites.

DISCUSSION

The Centers for Disease Control manual *Laboratory Procedures for the Diagnosis of Intestinal Parasites* (8) cites five papers supporting the statement that the possibility of finding intestinal parasites is increased by examining multiple specimens (2, 3, 13, 16, 19). One of these references is a review and does not contain original data (16). In another, examination of the first stool specimen detected 89% of *E.*

histolytica and 95% of *G. lamblia* infections (2). Although examination of three stool specimens increased the number of parasites detected, the increase in yield was modest (5 to 17%) (2). The four papers with original data are reports of investigations of asymptomatic populations (2, 3, 13, 19). For investigators involved in similar epidemiological studies of asymptomatic persons in endemic situations, the collection of multiple stools per patient may be appropriate. However, slavish adherence to this approach when investigating cases of acute diarrhea in a tertiary-care hospital seems to us to be epidemiologically flawed. Indeed, contemporary data show that modern methods detect more than 90% of parasites in the first examination (9, 14). Moreover, others have reported that one examination can be sufficient when screening some asymptomatic populations (5).

In this study, most patients who had enteric parasites required only a single O&P examination to detect the infection, and all infections were detected by two O&P examinations. In addition, only three hospitalized patients had pathogenic parasites detected in stool specimens submitted after the patient had been hospitalized for more than 3 days. One isolate, *G. lamblia*, had been previously detected in a stool specimen submitted on hospital day 3 (Table 4). A second isolate, *E. histolytica*, appears to have been carried asymptotically by the patient and was not responsible for his diarrhea. The third parasite, *S. stercoralis*, was therefore the only accepted pathogen that would have been missed had we rejected all stool specimens submitted from patients hospitalized for more than 3 days. Clearly, development of diarrhea in an immunocompromised patient, especially if it is associated with bacteremia, pulmonary infiltrates, or eosinophilia, is an indication to search for *S. stercoralis*.

It is of interest that only 22 of 265 specimens (8.3%) initially rejected for either criterion were subsequently processed because the ordering physician contacted the Medical Microbiology Fellow. An initial concern among our technical and administrative staff was that ordering physicians would "override" the system so often as to undermine the effectiveness of the policy. Clearly, this did not happen. Moreover, such interactions enhance the consultative role of the Medical Microbiology Fellow and Clinical Pathology Resident and augment that aspect of their training. The same role is also the responsibility of the professional clinical microbiologist.

The most clinically important parasite likely to be missed by the application of our policy is *G. lamblia*, because of sampling either in the prepatent period or when cyst or trophozoite numbers are small (4, 6, 11). For this reason we suggest that if the first O&P examination is negative and giardiasis is still considered likely, a second examination be performed several days later. An alternative approach would be to pool multiple specimens (10, 18) or to use an enzyme immunoassay that allows detection of *G. lamblia* antigen even when the O&P examination is negative (1, 12).

We do not agree with the conclusion of Thomson et al. that multiple stool specimens are necessary to detect parasites with sufficient sensitivity (17). In their study, only patients who had at least one positive result among two or three examinations were included; patients with a single positive result were excluded (17). Our data (Table 1) show that most patients with a positive examination had only one specimen examined.

Within 3 months of implementation, application of the rejection criteria resulted in a sustained 34.4% decrease in the number of stool specimens submitted for O&P examination. With the 29.9% rejection rate, this gives an overall

workload reduction of 44.4%. The significant decrease in workload in the parasitology section enabled us to reallocate personnel to meet increasing workloads in other areas of the laboratory. Moreover, on the basis of workload data of 184 specimens per month for the previous 11 months, such a sustained reduction would eliminate more than \$64,000 per year for charges billed to patients and third-party carriers. Given the low diagnostic yield from the specimens that were rejected, this seems a prudent and warranted savings to the health care system.

REFERENCES

1. Addiss, D. G., H. M. Mathews, J. M. Stewart, S. P. Wahlquist, R. M. Williams, R. J. Finton, H. C. Spencer, and D. D. Juranek. 1991. Evaluation of a commercially available enzyme-linked immunosorbent assay for *Giardia lamblia* antigen in stool. *J. Clin. Microbiol.* **29**:1137-1142.
2. Alicna, A. D., and E. J. Fadell. 1959. Advantage of purgation in recovery of intestinal parasites or their eggs. *Am. J. Clin. Pathol.* **31**:139-142.
3. Andrews, J. 1934. The diagnosis of intestinal protozoa from purged and normally-passed stools. *J. Parasitol.* **20**:253-254.
4. Danciger, M., and M. Lopez. 1975. Numbers of *Giardia* in the feces of infected children. *Am. J. Trop. Med. Hyg.* **24**:237-242.
5. Gyorkos, T., J. McLean, and C. Lor. 1989. Absence of significant differences in intestinal prevalence estimates after examination of either one or two stool specimens. *Am. J. Epidemiol.* **130**:976-980.
6. Jokipii, A., and L. Jokipii. 1977. Prepatency of giardiasis. *Lancet* **i**:1095-1097.
7. Marsden, A. T. H. 1946. The detection of *Entamoeba histolytica* in the feces by microscopic examination. *Med. J. Aust.* **1**:915-916.
8. Melvin, D. M., and M. M. Brooke. 1982. Laboratory procedures for the diagnosis of intestinal parasites, 3rd ed. U.S. Department of Health, Education, and Welfare publication no. (CDC) 82-8282. Centers for Disease Control, Atlanta.
9. Montesson, G., and L. Bischoff. 1987. Searching for parasites in stool: once is usually enough. *Can. Med. Assoc. J.* **137**:702.
10. Peters, C. S., L. Hernandez, N. Sheffield, A. L. Chiffom-Swiatlo, and F. E. Kocka. 1988. Cost containment of formalin-preserved stool specimens for ova and parasites from outpatients. *J. Clin. Microbiol.* **26**:1584-1585.
11. Rendtorff, R. C. 1954. The experimental transmission of human intestinal protozoan parasites: *Giardia lamblia* cysts given in capsules. *Am. J. Hyg.* **59**:209-220.
12. Rosoff, J. D., C. A. Sanders, S. S. Sonnad, P. R. De Lay, W. K. Hadley, F. F. Vincenzi, D. M. Yajko, and P. D. O'Hanley. 1989. Stool diagnosis of giardiasis using a commercially available enzyme immunoassay to detect *Giardia*-specific antigen 65 (GSA 65). *J. Clin. Microbiol.* **27**:1997-2002.
13. Sawitz, W. G., and E. C. Faust. 1942. The probability of detecting intestinal protozoa by successive stool examinations. *Am. J. Trop. Med.* **22**:131-136.
14. Senay, H., and D. MacPherson. 1989. Parasitology: diagnostic yield of stool examination. *Can. Med. Assoc. J.* **140**:1329-1331.
15. Siegel, D. L., P. M. Edelstein, and I. Nachamkin. 1990. Inappropriate testing for diarrheal diseases in the hospital. *JAMA* **263**:979-982.
16. Swartzelder, C. 1952. Laboratory diagnosis of amebiasis. *Am. J. Clin. Pathol.* **22**:379-395.
17. Thomson, R., R. Haas, and J. Thompson. 1984. Intestinal parasites: the necessity of examining multiple stool specimens. *Mayo Clin. Proc.* **59**:641-642.
18. Wahlquist, S. P., R. M. Williams, H. Bishop, D. G. Addiss, J. M. Stewart, R. J. Fenton, D. D. Juranek, and J. J. Sullivan. 1991. Use of pooled formalin-preserved fecal specimens to detect *Giardia lamblia*. *J. Clin. Microbiol.* **29**:1725-1726.
19. Wykoff, D. E., and L. S. Ritchie. 1962. Efficiency of the formalin-ether concentration technic. *J. Parasitol.* **38**(Suppl.): 15-16.