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Utility of Multiple-Stool-Specimen Ova and Parasite Examinations in a High-Prevalence Setting

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A retrospective analysis of the results of 2,704 ova and parasite (O & P) examinations performed on stool specimens collected from 1,374 patients between October 1996 and September 1997 was performed to evaluate the utility of performing O & P examinations on multiple, independently collected stool specimens in a high-prevalence setting. A total of 995 specimens (36.8%) examined during the study contained parasites; 546 (20.2%) contained pathogenic organisms. The positivity rate (54.5%) for the patients from whom three specimens were examined was significantly higher than for the patients from whom either two specimens (33.3%) or a single specimen (19.8%) was submitted for examination. For the group of patients from whom at least 3 specimens were submitted for O & P examination, 373 independent opportunities for diagnosing infection with intestinal parasites could be analyzed. The first stool specimen collected proved to be adequate in only 75.9% (283 of 373) of evaluated cases; however, examination of two specimens increased the sensitivity of O & P detection to 92% (343 of 373). The third specimen collected provided additional information on only 30 of 373 occasions (8%). These data indicate that in populations with a high prevalence of intestinal parasitic infections, two independently collected stool specimens should be subjected to O & P examination to ensure adequate diagnostic sensitivity.

Most parasitology textbooks and laboratory manuals recommend the examination of at least three independently collected stool specimens to maximize the sensitivity of detection of intestinal ova and parasites (O & P) (1, 2, 5). Such recommendations are based largely on older studies that showed increased rates of detection of organisms, in particular, *Entamoeba histolytica*/*dispar*, when multiple specimens were examined (4, 8).

The current demand for clinical laboratories to improve cost-effectiveness has led a number of investigators to reexamine whether performing multiple O & P examinations is necessary (6, 7, 9, 10). Morris et al. (6) showed that, in a low-prevalence setting (6.7% prevalence rate), there was little benefit in collecting more than a single stool specimen for O & P examination; diagnosis of 91% of intestinal parasitic infections was accomplished by examining the first specimen submitted. Similar results were obtained in a multicenter College of American Pathologists-sponsored Q-Probe investigation (10). The positivity rate for O & P examinations in the Q-Probe study was only 2.7% (detection of nonpathogenic organisms was not included in the analysis), and over 90% of all organisms detected were present in the first specimen examined. In an investigation conducted with a population with a moderate prevalence of parasitic infections, Senay and MacPherson (9) also showed that, for a group of patients from whom at least three stool specimens were available for O & P examination, the diagnostic sensitivity of the first sample was 90%.

Clearly, these data support the use of restrictive policies with regard to stool O & P examinations in institutions serving populations with a low to moderate (≤15%) prevalence of enteric parasitic infections. None of these data, however, were collected in settings in which parasitic infection rates are high and in which any diminution in the sensitivity of O & P detection is likely to have the greatest clinical and epidemiological impact. Furthermore, in studies by both Morris et al. (6) and Senay and MacPherson (9), the distribution of pathogenic parasitic species was heavily skewed toward *Giardia lamblia*; only five infections with *E. histolytica*/*dispar* were reported in both studies combined. It is conceivable, therefore, that in populations in which a more diverse spectrum of parasites is encountered, the results of these studies may not be valid. In addition, these studies contain data collected primarily from symptomatic individuals deemed to be at risk for parasitic infections, and the results may not be applicable to laboratories involved in screening programs designed to detect parasitic infections in incoming refugees, the majority of whom are asymptomatic. Only a single, small study has been published on the utility of performing more than one O & P examination in the setting of screening of asymptomatic individuals (3). The results of the study suggested that there was little benefit to analyzing more than one stool specimen, but the authors indicated that larger-scale studies were necessary before any general recommendations could be made.

A considerable proportion of the stool specimens submitted to the microbiology laboratory at Hennepin County Medical Center (HCMC) for O & P examination is obtained from refugees arriving in Minneapolis from Southeast Asia, East Africa, and republics of the former Soviet Union. Given the conclusions of the previously mentioned studies, we decided to examine whether routinely performing O & P examinations on multiple specimens is necessary even in the setting of screening of our high-prevalence refugee population. Here we report the results of a retrospective analysis of stool O & P examinations carried out in the HCMC microbiology laboratory between October 1996 and September 1997.

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TABLE 1. Numbers of stool specimens received for O & P examination per patient and frequency of positive results

No. of specimens received per patient	No. of patients	No. (%) of patients with at least one positive specimen	Total no. (%) of specimens received
1	651	129 (19.8)	651 (24.1)
2	159	53 (33.3)	318 (11.8)
3	539	294 (54.5)	1,617 (59.8)
4	12	3 (25.0)	48 (1.8)
5	8	3 (37.5)	40 (1.5)
6	5	4 (80.0)	30 (1.0)
Total	1,374	486 (35.4)	2,704 (100)

MATERIALS AND METHODS

Study institution and patient population. HCMC is a 450-bed acute- and tertiary-care teaching facility located in Minneapolis, Minn.; it averages 20,000 patient admissions and 400,000 outpatient visits annually. Included in the patient population at HCMC are approximately 1,000 to 2,000 primary refugees who enter Hennepin County on an annual basis. In 1996, 29.1% of arriving refugees were from Southeast Asia (Laos and Vietnam), 28.8% were from republics of the former Soviet Union, 24.6% were from Somalia, 9.3% were from Bosnia, and 8.2% were from other countries.

O & P examinations. All specimens sent for O & P examinations were received in the ParaPak ULTRA Stool System (Meridian Diagnostics, Cincinnati, Ohio), consisting of stool preserved in 10% formalin in one vial and stool fixed in polyvinyl alcohol in the other. Formalin-preserved material was concentrated prior to examination by use of a formalin-ethyl acetate sedimentation technique as recommended by the manufacturer of the specimen collection kit. Permanently stained preparations of stool specimens were made with material preserved in polyvinyl alcohol by use of Wheatley's (11) modified trichrome stain (Meridian Diagnostics).

Data collection and analysis. The results of all routine stool O & P examinations performed between October 1996 and September 1997 were reviewed. Most stool specimens submitted to our laboratory are obtained from outpatients who have been provided with a set (usually three) of collection receptacles at a clinic visit, with instructions to collect separate specimens over a period of several days. The results of such collections constitute a single clinical evaluation of the patient for enteric parasite infection; therefore, specimens documented as being collected on separate days but received in the laboratory on the same day were considered a single set during data analysis. Specimens arriving in the laboratory as a set without documentation that each specimen was collected on a different data were combined prior to O & P examination.

Since many patients were infected with multiple organisms, the following criteria were applied in an attempt to differentiate potentially significant and insignificant diagnostic events in such individuals. (i) The detection of each different pathogenic species within either a single specimen or a set of specimens was considered to be a diagnostically significant event. (ii) For patients infected solely with nonpathogenic organisms, only the first report of the detection of a parasite or parasites in a set of specimens was deemed significant. (iii) For patients infected with both pathogenic and nonpathogenic organisms, the finding of one or more nonpathogenic species was considered significant only if it occurred in a specimen in which no pathogenic species were detected and if that specimen was collected prior to any specimens for which the identification of one or more pathogenic species was reported.

RESULTS

A review of laboratory records indicated that 2,704 O & P examinations were performed on specimens collected from 1,374 patients between October 1996 and September 1997. Of these specimens, 995 (36.8%) collected from 487 patients (35.4%) contained at least one enteric parasite, and 546 (20.2%) collected from 277 patients (20.2%) contained at least one pathogenic organism. The number of specimens received on a per-patient basis is shown in Table 1. A single specimen was obtained from 651 patients (47.3%), and parasites were detected in 129 of these individuals (19.8%). More than one specimen was collected from 723 patients (52.7%), and O & P examinations were positive in 357 of these individuals (49.4%); this positivity rate was significantly higher (P, <0.001; chi-square test of significance) than that for individuals from whom only a single specimen was collected.

The identities and frequencies of detection of individual species of parasites are shown in Table 2. A total of 12 pathogenic and 6 nonpathogenic species were identified during the study period. Of the 277 patients whose stool specimens contained pathogenic organisms, 217 (78.3%) were infected with one pathogen, 48 (17.3%) were infected with two pathogens, and 12 (4.3%) had three pathogenic organisms identified by O & P examinations. A total of 386 patients had positive O & P examinations for nonpathogens; of these, 214 (55.4%) were infected with more than one nonpathogenic parasite. The mean number of nonpathogenic parasites found per infected patient was 1.73, and the mean number of pathogenic parasites identified in infected individuals was 1.26.

Three or more independently collected stool specimens were obtained from 564 patients, 304 of whom (53.9%) were infected with enteric parasites (Table 1). Parasites were observed in three or more specimens from 200 (65.8%) of these infected individuals, 56 patients (18.4%) had two positive O & P examinations, and in 48 patients (15.8%) only a single specimen contained enteric parasites. Although our current recommendation for the collection of multiple stool specimens from patients being screened for enteric parasites is that they be collected on consecutive days, the time span over which three specimens were collected during the study varied from 3 to 27 days. The median collection time was 4 days and did not differ between populations in which 0, 1, 2, or 3 specimens were positive for parasites (data not shown).

A total of 237 pathogenic organisms were detected in spec-

TABLE 2. Frequency of detection of individual parasite species

Pathogenic species	No. (%) of specimens positive	Nonpathogenic species	No. (%) of specimens positive
Giardia lamblia	264 (40.2)	*Blastocystis hominis*	494 (38.7)
Trichuris trichiura	134 (20.4)	*Endolimax nana*	336 (26.4)
Entamoeba histolytica/dispar	63 (9.6)	*Entamoeba coli*	229 (18.0)
Dientamoeba fragilis	60 (9.3)	*Entamoeba hartmanni*	149 (11.7)
Hymenolepis nana	35 (5.3)	*Iodamoeba butschlii*	53 (4.1)
Ascaris lumbricoides	28 (4.3)	*Chilomastix mesnili*	14 (1.1)
Strongyloides stercoralis	27 (4.1)		
Hookworm spp.	23 (3.5)		
Taenia spp.	12 (1.8)		
Other[a]	10 (1.5)		
Total	656 (100)	Total	1,275 (100)

[a] *Schistosoma mansoni* ($n = 6$), *Chlonorchis sinensis* ($n = 2$), *Diphylobothrium latum* ($n = 1$), and *Enterobius vermicularis* ($n = 1$).

TABLE 3. Frequency of detection of pathogenic parasites in the subset of patients from whom at least three specimens were collected for evaluation

Organism	No. (%) of positive patients			
	Overall	With the following no. of positive specimens:		
		Three or more	Two	One
Giardia lamblia	91 (38.4)	50 (45.9)	20 (30.8)	21 (33.3)
Trichuris trichiura	54 (22.8)	23 (21.1)	15 (23.1)	16 (25.4)
Dientamoeba fragilis	27 (11.4)	9 (8.3)	7 (10.8)	11 (17.5)
Entamoeba histolytica/dispar	21 (8.9)	11 (10.1)	6 (9.2)	4 (6.3)
Hymenolepis nana	12 (5.1)	7 (6.4)	2 (3.1)	3 (4.8)
Ascaris lumbricoides	9 (3.8)	4 (3.7)	5 (7.7)	0 (0)
Hookworm spp.	7 (3.0)	2 (1.8)	2 (3.1)	3 (4.8)
Strongyloides stercoralis	7 (3.0)	0 (0)	5 (7.7)	2 (3.2)
Taenia spp.	3 (1.2)	1 (0.9)	1 (1.5)	1 (1.6)
Schistosoma mansoni	3 (1.2)	1 (0.9)	1 (1.5)	1 (1.6)
Other[a]	3 (1.2)	1 (0.9)	1 (1.5)	1 (1.6)
Total	237 (100)	109 (100)	65 (100)	63 (100)

[a] *Diphylobothrium latum* ($n = 1$), *Chlonorchis sinensis* ($n = 1$), and *Enterobius vermicularis* ($n = 1$).

imens from patients from whom at least three specimens were collected. In 109 (46.0%) of these instances, the pathogen was detected in at least three specimens; on 65 occasions (27.4%), the organism was present in two specimens; and a single specimen contained the parasite 63 times (26.6%). A breakdown of the frequency with which individual pathogenic organisms were detected in this subset of patients and the frequency with which each organism was identified in one, two, or at least three stools in each set of specimens is shown in Table 3. Analysis of these data with the chi-square test of significance revealed no significant difference (at the 95% confidence level) between the frequency of detection in only one specimen in a set of three and the frequency of detection in all three specimens for any individual pathogen.

With the criteria described in Materials and Methods, a total of 373 independent, potentially significant diagnostic events could be evaluated for the subpopulation of patients from whom at least three specimens were obtained for O & P examinations. Had only the first specimen collected been examined, 75.9% (283 of 373) of these diagnoses would have been made successfully. Examination of the first two specimens collected would have increased the diagnostic yield to 92% (343 of 373), and the remaining 8% of these diagnoses (30 of 373) would have required the collection of a third specimen. Essentially identical values were obtained when only the detection of pathogenic organisms was examined, with 74.3% (176 of 237) and 92.8% (220 of 237) of diagnoses being accomplished with the first specimen or with the first and second specimens received, respectively.

DISCUSSION

For the population served by our institution, examination of two independently collected stool specimens was necessary to achieve an acceptable level of sensitivity in diagnosing infection with enteric parasites. The frequency of parasite detection was significantly higher in patients from whom more than one stool specimen was submitted for examination than in individuals from whom only a single specimen was received (49.4 versus 19.8%; P, <0.001). Since the number of specimens submitted is presumably a surrogate marker for clinical suspicion of parasitic infection, this result may in part reflect the fact that the high-prevalence subpopulations (largely immigrants and refugees) seen at our institution are the most likely to have multiple stool specimens submitted to the laboratory. More compelling evidence of the value of examining two specimens is provided, therefore, by analysis of data from patients from whom at least three specimens were collected for O & P examinations. In this group of patients, diagnosis of infection with pathogenic parasites would have been achieved with a sensitivity of only 75% had the first specimen collected been the sole sample submitted to the laboratory. This finding contrasts somewhat with those of several previously published investigations (6, 9, 10), conducted predominantly with lower-prevalence, largely symptomatic populations for which examination of only one specimen resulted in the detection of >90% of all parasites.

Our findings do not, however, support the historical recommendation that the examination of at least three stool specimens is necessary to evaluate a patient for infection with enteric parasites (1, 2, 5). Using a liberal interpretation of what constitutes a diagnostically significant event, the third specimen examined contributed to the overall diagnosis on only 30 of 373 occasions (8%). When all the results of the study are considered, only 18 of 667 pathogenic parasites identified (2.7%) were detected in the third specimen collected. In addition, in only 5 of 546 individuals (1.2%) from whom three specimens were obtained was the presence of pathogenic parasites demonstrated first in the third specimen examined. Interestingly, the frequencies with which individual species of pathogenic organisms were detected were similar irrespective of the total number of specimens reported as positive (Table 3). Certain organisms that previous studies have suggested may be detected with less than optimal sensitivity if a limited number of specimens are examined, namely, *G. lamblia*, *E. histolytica/dispar*, and *Dientamoeba fragilis* (1, 2, 6, 9), were as likely to be found in all specimens submitted as they were in only one or two members of a set of specimens.

In conclusion, the results of this study clearly demonstrate that O & P examination of more than one stool specimen has diagnostic utility in the high-prevalence population served by HCMC. Since previous studies have shown high diagnostic yields for lower-prevalence populations with only a single specimen, our findings illustrate the impact that patient demographics can have on the appropriateness of use of cost-saving algorithms. Such algorithms should be evaluated in situ prior to implementation and modified appropriately if the results do

not support the intended use. Finally, and perhaps most importantly, for a population with a prevalence of enteric parasite infections exceeding 35%, there was no evidence to justify routine O & P examinations of more than two stool specimens. Given the results of this and other studies, it is clear that continued publication in textbooks and laboratory manuals of the recommendation to collect three or more stool specimens for O & P examinations is not warranted. Furthermore, perpetuation of this practice constitutes a cost-inefficient and imprudent use of scarce laboratory resources.

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