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Dientamoeba fragilis Detection Methods and Prevalence: A Survey of State Public Health Laboratories

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Synopsis

Dientamoeba fragilis *is a pathogenic protozoan parasite that has no cyst stage. Because of the lack of a cyst stage, the laboratory detection of* D. fragilis *in stool specimens is dependent on the stool processing and examination methods employed. Failure to use recommended stool fixation and permanent staining techniques almost precludes identification of* D. fragilis, *which is associated with gastrointestinal illness in humans.*

In this survey, questionnaires were mailed to all State and territorial public health laboratories requesting information on the number of ova and parasite examinations, methods of processing and examining stools, and the number of D. fragilis *positive stools for 1985. Forty-three of 54 (80 percent) laboratories responded. Results showed that those laboratories which reported* D. fragilis *detection examined more stools using recommended stool fixation methods and were more likely to stain permanently all stools examined. Permanent staining of all stools, as compared to loose and watery stools only, resulted in a fivefold greater detection of* D. fragilis.

More State and territorial public health laboratories reported finding D. fragilis *infections in 1985 than in a 1978 survey performed by the Centers for Disease Control. However, in 1985 only six laboratories reported 82 percent of all* D. fragilis *detections. To increase the probability of detecting* D. fragilis *in stool specimens, the findings suggest that all stools should be submitted fixed in polyvinyl alcohol fixative, sodium acetate-acetic acid-formalin fixative, or Schaudinn's fixative. Further, all specimens, regardless of consistency, should be permanently stained prior to microscopic examination.*

THE DIAGNOSIS of most intestinal protozoan infections by stool examination requires the detection and identification of cysts or trophozoites. Cysts may survive days to weeks outside of the host, whereas trophozoites degenerate rapidly preventing accurate identification (*1-3*). Certain stool fixation methods, when combined with permanent staining, greatly enhance protozoan detection, especially trophozoites (*3-8*).

Dientamoeba fragilis, a flagellate protozoan with no cyst stage, exists only as trophozoites (*9,10*). Optimal conditions for *D. fragilis* identification require permanently stained preparations of fixed or freshly passed unpreserved stool specimens. Stool fixatives differ in their ability to maintain *D. fragilis* morphology prior to permanent staining and microscopic examination. Stools fixed in polyvinyl alcohol (PVA) fixative, sodium acetate-acetic acid-formalin (SAF) fixative, or Schaudinn's fixative, and fresh stools can be permanently stained, while formalin-fixed stools cannot (*11*). Therefore, PVA, SAF, and Schaudinn's are the preferred fixatives when combined with permanent staining for detection of *D. fragilis*.

While the prevalence of *D. fragilis* in the general population is unknown, its pathogenicity is well supported in the literature. Most common gastrointestinal symptoms, primarily abdominal pain and diarrhea, have been reported in persons infected with *D. fragilis* (*12–19*). Prevalence estimates from selected populations range from 1.4 percent to 53 percent (*12,14,15,20–23*). *D. fragilis* was reported in 0.6 percent of stools examined by State public health laboratories in 1978 (*24*). A variety of stool fixation and examination methods were reported in these earlier surveys. The intent of this study of State and territorial public health laboratories was to determine and compare the stool fixation and examination methods employed by two groups of laboratories, those which did and did not report detecting *D. fragilis* in 1985. In addition, an estimate of the prevalence of *D. fragilis* reported by the responding laboratories was determined.

Materials and Methods

Questionnaires were mailed to 54 State and territorial laboratories in the United States. Information requested for the calendar year 1985 included (*a*) total number of ova and parasite examinations; (*b*) proportions of stools fixed in PVA, SAF, Schaudinn's fixative, in 5 percent or 10 percent formalin, in merthiolate-iodine-formaldehyde (MIF), or no fixative; (*c*) use of permanent stain (all stools, semiformed or liquid stools only, or no stools stained); and (*d*) stools with *D. fragilis* detected. For analysis, the stool fixation and examination methods were grouped in two categories: (a) recommended methods for *D. fragilis* detection (PVA, SAF, Schaudinn's fixatives, or fresh stools with permanent staining) and (*b*) any fixation methods (formalin, MIF, fresh stools without permanent staining, and recommended methods). Continuous variables were analyzed by Student's *t* test, and dichotomous variables were analyzed by chi-square. Probability values (*P*) less than 0.05 were considered significant.

Results

Forty-three of 54 (80 percent) laboratories responded to the survey questionnaire. All regions of the United States and its territories were represented. One laboratory that did not use any stool fixatives was excluded from the analysis. Twenty-three (55 percent) of the responding laboratories reported finding at least one stool with *D. fragilis*. When all methods of stool fixation and examination were considered, there was no significant ($P=0.59$) difference in the number of stools examined by laboratories that did or did not detect *D. fragilis* infection (table 1). However, when laboratories were compared by their use of recommended methods for *D. fragilis* detection (*3–7*), laboratories detecting *D. fragilis* examined a significantly ($P<0.001$) greater number of stools. Furthermore, significantly ($P=0.02$) more laboratories that reported detecting *D. fragilis* routinely employed permanent staining of all stools (table 2).

Analysis of the practice of permanent staining of

Table 1. Number of stools examined by laboratories using various fixation and examination methods for *Dientamoeba fragilis* detection

Stool processing methods	D. fragilis detection	Number of labs	Number of stools: Mean	Median	Range
Any [1]	Yes	23	3,404	2,500	150– 9,226
	No	19	4,400	3,600	144–15,545
Recommended [2]	Yes	23	3,069	2,400	135– 9,133
	No	[3] 14	258	85	0– 2,100

[1] Any includes stools fixed in polyvinyl alcohol (PVA), sodium acetate-acetic acid-formalin (SAF), Schaudinn's fixative, merthiolate-iodine-formaldehyde (MIF), formalin, or submitted fresh unfixed.
[2] Recommended includes PVA, SAF, or Schaudinn's fixative, and permanent staining (references *3–7*).
[3] 5 laboratories used nonrecommended methods, only, to examine stools.

Table 2. Laboratories detecting *Dientamoeba fragilis* by use of permanent staining of stools

Stools permanently stained	D. fragilis detection: Yes	No	Total
All	19	8	27
Only liquid, semi-formed, or no stools	[1] 4	[2] 11	15
Total	23	19	42

[1] All laboratories that detected *D. fragilis* stained all stools or only liquid or semi-formed stools. No laboratory that detected *D. fragilis* failed to permanently stain stools.
[2] 6 laboratories that did not detect *D. fragilis* stained liquid or semi-formed stools, and 5 permanently stained no stools.
NOTE: Chi-square = 5.8, $P=0.02$, $df=1$.

Table 3. Prevalence of *Dientamoeba fragilis* detection by permanent stain use in laboratories

Number of laboratories	Permanent stain usage	Mean number stools examined	Mean prevalence (percent) of D. fragilis detection
19	All stools	3,295	1.6
4	Semi-formed or liquid stools only	2,817	0.3

Table 4. Comparison of two surveys of State and territorial public health departments for *Dientamoeba fragilis* detection

Item	1978		1985	
	Number	Percent	Number	Percent
Labs responding	53 of 55	96	43 of 54	80
Mean number of stools examined by labs	6,270	...	3,839	...
Percent of total stools with *D. fragilis*	...	0.6	...	0.6
States reporting *D. fragilis*	18	34	[1] 23	55
Distribution of *D. fragilis* isolations	[2] 2 States accounted for 87 percent of detections		[3] 6 States accounted for 82 percent of detections	

[1] AZ, CA, CO, CT, GA, ID, IL, IN, IA, KS, ME, MA, MI, MO, NV, NM, OK, OR, PA, PR, TX, WA, WV.

[2] CA, NY.

[3] KS, MO, NM, PR, TX, WA.

stools revealed that the mean number of stools examined was not significantly ($P=0.78$) different; however, the mean prevalence of *D. fragilis* detection was five times greater in laboratories that permanently stained all stools compared with those that permanently stained only loose and watery stools, although this finding was not significant ($P=0.14$, table 3). Comparison of this survey with an earlier survey (*24*), in which 10 more laboratories responded, revealed similar detection rates for *D. fragilis* infection (table 4). However, more States reported detection of *D. fragilis* in this survey of 1985 findings.

Discussion

Several factors may prevent the detection of *D. fragilis* and other protozoans in stool specimens. Prominent among these are the methods used for stool fixation and examination and the training of laboratory personnel (*24–30*). In this study, 23 of 42 (55 percent) responding laboratories reported detection of *D. fragilis* in stools in 1985. Because *D. fragilis* has only a trophozoite stage, the likelihood of detection is improved by using appropriate fixatives in conjunction with permanent staining. While the role of other factors affecting *D. fragilis* detection was not assessed, the reported stool fixation and examination methods most likely explain the failure of some laboratories to detect *D. fragilis*. Laboratories routinely employing proven methods of *D. fragilis* detection were more likely to report its occurrence in stools. PVA, SAF, or Schaudinn's fixatives combined with permanent staining provides greater recovery rates of protozoan trophozoites than other fixatives and techniques (formalin, MIF, zinc sulfate flotation, direct wet mounts) (*3–8*). Several studies have shown the exclusive detection of *D. fragilis* in portions of stool fixed in PVA, SAF, or Schaudinn's fixatives and permanently stained; detection was not reported in the corresponding formalin-fixed or unpreserved, unstained stool portion (*3-7*).

When all methods of stool fixation and examination were considered, a similar number of stools were examined by the laboratories that detected *D. fragilis* and those that did not detect the protozoan. However, when the analysis was limited to stools fixed in PVA, SAF, Schaudinn's, or were unpreserved combined with permanent staining, laboratories that reported finding *D. fragilis* examined more stools than laboratories that did not find *D. fragilis*. Evaluation of the use of permanent stain by the two groups of laboratories revealed that significantly more laboratories detecting *D. fragilis* routinely stained permanently before microscopic examination. Furthermore, permanent staining of all stools detected more *D. fragilis* infections than staining only loose and watery stools.

More State public health laboratories are finding *D. fragilis* in stool specimens. The 1985 survey revealed that 23 of 42 (55 percent) laboratories reported finding *D. fragilis* compared with 18 of 53 (34 percent) in a 1978 survey conducted by the Centers for Disease Control (*24*). While only two laboratories accounted for 87 percent of all *D. fragilis* reported in 1978, six laboratories reported 82 percent of the detections in 1985. The population served by the laboratory, the expertise of the laboratory personnel, and stool processing and examination methods used can all directly affect the reported prevalence. The routine use of certain stool fixation methods and permanent staining was most likely responsible for *D. fragilis* detection and accounted for the differences in *D. fragilis* prevalence reported by this group of laboratories. To increase the probability of detecting *D. fragilis* and to provide a more accurate estimate of its prevalence, laboratories should require that all stools be fixed in PVA, SAF, or Schaudinn's fixative, and regardless of consistency, be permanently stained before microscopic examination.

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