JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1996, p. 2292–2293 Vol. 34, No. 9
0095-1137/96/$04.00+0

Factors Influencing *Cryptosporidium* Testing in Connecticut

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Received 12 September 1995/Returned for modification 17 January 1996/Accepted 28 May 1996

To describe patterns of testing for *Cryptosporidium* oocysts in stool samples, Connecticut laboratories were surveyed. Different detection methods were used. Most laboratories examined stools specifically for *Cryptosporidium* only on physician request. The rate of positive tests varied widely (0 to 28%). Higher rates of positivity were associated with the use of monoclonal antibody methods, the use of two or more staining procedures, and testing of stool specimens in addition to those requested by physicians.

Infection with *Cryptosporidium parvum* can cause a prolonged diarrheal illness which can be life-threatening to persons who have human immunodeficiency virus infection (3). The organism, a small (i.e. 4- to 6-μm) coccidian parasite, can easily be overlooked or confused with yeasts on routine examination of stool specimens for ova and parasites (O&P) (7). Consequently, specific diagnostic procedures have been developed to identify *Cryptosporidium* oocysts (3). In January 1994, Connecticut began public health surveillance (8) for cryptosporidiosis by requiring that all laboratories report *Cryptosporidium* infection to the Connecticut Department of Public Health. However, interpretation of the data collected was difficult because the extent to which *C. parvum* isolates were being tested for or reported was unknown.

In October 1994 we surveyed all Connecticut laboratories licensed to test for fecal parasites. Each laboratory director was mailed a questionnaire to determine (i) the frequency of testing for O&P and for *C. parvum* isolates between 1 January 1994 and 30 September 1994, (ii) barriers to adding *Cryptosporidium* testing to all stool examinations for O&P, (iii) what specimens were selected for *Cryptosporidium* testing (i.e., selection criteria), (iv) laboratory methods used to test for *C. parvum* isolates, and (v) the cost to the patient of adding *Cryptosporidium* testing to all O&P examinations.

Each laboratory's positivity rate for *Cryptosporidium* testing was calculated by dividing the number of tests with positive results by the number of *Cryptosporidium* tests that the laboratory reported performing. Summary positivity rates by laboratory characteristics of interest were also calculated by summing the number of tests with positive results by all laboratories within a given category and dividing by the sum of all the *Cryptosporidium* tests that these laboratories performed.

All 35 laboratories that were licensed to test for fecal parasites and that did so on-site participated in the study. Of these, 30 (86%) were hospital laboratories and 5 were non-hospital laboratories. Two of the 35 laboratories did not test for *C. parvum* during the study period and were not included in positivity rate calculations.

During the 9-month study period the 35 laboratories tested 32,408 stool specimens for O&P (median, 642 per laboratory; range, 93 to 8,779 per laboratory). Thirty-three laboratories tested 1,714 (5.3%) of these specimens for *C. parvum* (median, 18 specimens; range, 0 to 270 specimens); 83 (4.8%) specimens were positive for *C. parvum* (median, 1 specimen; range 0 to 12 specimens) in 21 laboratories. During this period, only 10 *Cryptosporidium*-infected patients were reported to the Connecticut Department of Public Health by seven of these laboratories.

The greatest perceived barriers to adding *Cryptosporidium* testing to all O&P testing were technologists' time (reported by 83% of laboratories), concern about low test positivity rates (77%), the cost of test materials (63%), and a lack of equipment (26%). One laboratory also reported a lack of physician awareness of *Cryptosporidium* testing as a barrier. All but four laboratories reported at least one barrier to additional testing.

All 35 laboratories reported testing for *C. parvum* when it was specifically requested by a physician. Additional criteria requiring testing for *C. parvum* included specimens with structures suspicious of *Cryptosporidium* oocysts on routine O&P testing (22 [63%] laboratories), specimens from persons known to be human immunodeficiency virus positive (6 [17%] laboratories), stool specimens from hospitalized patients (2 [6%] laboratories), watery stool specimens (2 [6%] laboratories), and specimens for which *Giardia* examination was requested (1 [3%] laboratory).

A variety of laboratory methods were used to identify *C. parvum* isolates (Table 1). Kinyoun-modified acid-fast stain was the stain most frequently reported to be used. Eleven (31%) laboratories routinely used more than one staining procedure. Formalin-ethyl acetate concentration with an acid-fast stain was the most common method of identification ($n = 15$).

The positivity rate, by laboratory, ranged from 0 to 28% (median, 3.3%). Twelve (34%) laboratories reported a positivity rate of 0; these laboratories performed 169 (9.9%) of all *Cryptosporidium* tests performed during the study period.

The *Cryptosporidium* positivity rate was 2.8% (12 of 429 tests) for laboratories that tested specimens only if requested by a physician, 5.8% (38 of 654 tests) for laboratories that also tested specimens if O&P testing indicated structures suspicious of *Cryptosporidium* oocysts, and 5.2% (33 of 361 tests) for laboratories that used other, additional test selection criteria. The positivity rate for hospital laboratories in towns with AIDS incidences of <10/100,000 population was 2.4% (9 of 370 tests) and was 5.2% (57 of 1,093 tests) for hospital laboratories in towns with AIDS incidences of ≥10/100,000 population (2).

Positivity rates by laboratory methods are provided in Table 2. Among the laboratories that concentrated the specimens

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TABLE 1. Methods routinely used to identify *C. parvum*

Method	No. (%) of laboratories
Concentration technique	
Used	30 (86)
Not used	5 (14)
Staining procedure	
Acid-fast staining methods[a]	34 (97)
Monoclonal FA	10 (29)
Auramine-rhodamine methods[b]	3 (9)
ELISA[c]	1 (3)

[a] Includes Kinyoun acid-fast, other modified acid-fast, and acid-fast (not modified) staining procedures.
[b] Includes auramine and auramine-rhodamine staining procedures.
[c] ELISA, enzyme-linked immunosorbent assay.

before staining, the positivity rate was higher when a monoclonal fluorescent-antibody (FA) technique was used. The positivity rate for *Cryptosporidium* spp. was also higher when two or more staining procedures were routinely used (positivity rates of 4.0% for one stain and 6.8% for two or more stains). Positivity rates remained higher, after stratifying by AIDS incidence, for hospital laboratories that used multiple selection criteria or two or more staining procedures (Table 3).

The median charge to patients for O&P testing was similar for both hospital and non-hospital laboratories, approximately $50.00. However, when adding *Cryptosporidium* testing to an O&P examination, non-hospital laboratories charged considerably more (median, $50; range, $0 to $60) than hospital laboratories (median, $29; range, $0 to $116).

No laboratories in Connecticut routinely test for *C. parvum*, and only 5% of stool specimens submitted for O&P testing are tested for this organism. Thus, public health surveillance based on current laboratory testing and reporting practices is unlikely to detect outbreaks expeditiously (5) or to allow for an assessment of the magnitude of cryptosporidiosis in Connecticut.

Laboratories reported a wide range of positivity rates (0 to 28%). About one-third of the laboratories had positivity rates of zero, consistent with the concern that laboratories expressed about low positivity rates. The range of positivity rates may have been caused by regional differences in the incidence of cryptosporidiosis within the state or by variations in the sensitivity of testing.

Laboratories that tested stool specimens in addition to those requested by a physician had higher positivity rates. This suggests that using additional criteria to select specimens for *Cryptosporidium* testing is beneficial and that physicians might not be selecting the highest-risk patients for testing. Laboratories that use a monoclonal FA technique also tended to have higher positivity rates, a finding supported by research indicating the greater sensitivities of immunofluorescent-antibody methods (3, 4, 6). Although the routine use of two or more staining procedures increased the positivity rates, it was also associated with higher additional charges for *Cryptosporidium* testing.

TABLE 2. Positivity rates for methods used to identify *C. parvum*

Cryptosporidium testing techniques routinely used[a] (no. of laboratories)	Positivity rate (%)	No. of specimens tested
Acid fast[b] ($n = 15$)	2.7	900
Monoclonal FA ($n = 3$)	6.8	234
Acid fast[b] and monoclonal FA ($n = 7$)	7.6	449
Acid fast[b] and auramine[c] ($n = 2$)	3.4	19
Acid fast[b] and ELISA[d] ($n = 1$)	2.0	50

[a] The routine tests are concentration with staining by the indicated technique.
[b] Includes Kinyoun acid-fast, other modified acid-fast, and acid-fast (not modified) staining procedures.
[c] Includes auramine and auramine-rhodamine staining procedures.
[d] ELISA, enzyme-linked immunosorbent assay.

TABLE 3. Positivity rate by testing factors and AIDS incidence for hospital laboratories[a]

Cryptosporidium testing factors (no. of laboratories)	Positivity rate (%) in locales with the indicated AIDS incidence	
	<10/100,000 population	≥10/100,000 population
Selection criteria		
Physician request only ($n = 9$)	0.0	3.1
MD request and other criteria[b] ($n = 20$)	2.6	6.3
No. of staining procedures		
One ($n = 20$)	2.2	4.0
Two or more ($n = 9$)	8.3	7.1
Total	2.4	5.2

[a] Only hospital laboratories were included in the AIDS incidence (number of new cases reported per year) assessment (2) because hospitals are likely to serve the community in which they are located, whereas clinical laboratories may not.
[b] See text for other selection criteria.

No "gold standard" for testing stool specimens for *C. parvum* has been established, so the absolute sensitivity of currently used methods has not been assessed (9). In addition, the test results reported here were not independently confirmed, nor were data collected on each laboratory's performance in proficiency testing or on the centrifugation times of the laboratories that concentrated specimens.

Cryptosporidiosis has gained increasing attention since infection in humans was first identified in 1976 (1). Despite public health concern, tests for new or emerging pathogens such as *C. parvum* are often not incorporated into existing laboratory testing procedures. Widespread *Cryptosporidium* testing would involve considerable cost, and it would be premature to recommend that all specimens submitted for O&P testing be routinely tested for this organism. Further work is needed to identify testing and selection strategies that would enable laboratories to identify stool specimens most likely to test positive for *C. parvum.*

We gratefully acknowledge the laboratories that participated in the study.

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