Diagnostic Algorithm for the Diagnosis of Pediatric Parasitic Gastroenteritis

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Background: Current practices for ordering stool studies in patients with abdominal and gastrointestinal symptoms are not standardized. We hypothesized that an algorithm involving first-line use of a *Cryptosporidium*/*Giardia* combination antigen test and stricter use of ova and parasite (O&P) examinations would be clinically and cost effective. **Methods:** In this study, stool O&P test results for pediatric patients in Dallas, Texas, were reviewed. All results obtained between 2009 and 2012 were included. Patient charts were reviewed to determine test results, symptoms, treatment, travel, and past medical history. Using these data, a retrospective modeling study was done to evaluate the utility of a diagnostic algorithm that limits O&P testing to those patients who are immunocompromised or have travelled

outside the United States. **Results:** Over the 3-year period of this study, we found that the prevalence of gastrointestinal parasitic disease in children was 1.9%. Analysis of the diagnostic algorithm for the judicious use of stool O&P showed that as much as 65% of testing may be unnecessary and could be eliminated. **Conclusions:** Our findings show that the prevalence of pediatric gastrointestinal parasitic disease in Texas may be lower than expected. In addition, these data show that a diagnostic algorithm limiting O&P testing may be both clinically and cost effective in low-prevalence settings. However, such an algorithm would miss a significant number of infections due to *Dientamoeba fragilis* and *Blastocystis hominis*. J. Clin. Lab. Anal. 30:155–160, 2016. ©

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Key words: parasites; ova and parasite; *Giardia*

Abbreviations

CDC = Centers for Disease Control
CMC = Children's Medical Center
O&P = ova and parasite
TAT = turnaround time
Zn-PVA = zinc containing polyvinyl alcohol

INTRODUCTION

The current epidemiology of pediatric parasitic disease in the United States has not been well studied. The Centers for Disease Control (CDC) publishes regular surveillance data for parasitic disease and associated outbreaks, but these data do not provide specific information about disease rates in children and focus only on *Cryptosporidium* and *Giardia* (1–3). Other studies have assessed ova and parasitic testing but do not provide pediatric specific

analyses (4,5). A 2006 publication by Klein and colleagues prospectively evaluated patients for diarrheogenic bacteria, and specimen volume permitting, they investigated parasitic etiologies as well (6). While this is a high-quality study, only 656 specimens were evaluated and produced only seven positive results. Given the paucity of data defining disease prevalence in children in the United States, the goal of our study was to define the epidemiology of pediatric parasitic infection at a large children's medical center in North Texas.

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In addition, it has been proposed that microscopic ova and parasite (O&P) examination should be limited to those patients who have either travelled outside the United States or are immunocompromised (7). This proposal is based on data demonstrating that parasitic disease other than *Cryptosporidium* and *Giardia* is rare in the United States. Therefore, it has been proposed that only those patients at high risk for non-*Cryptosporidium*/*Giardia* infection be granted full microscopic ova and parasitic exam. If clinically effective, this proposal would be of great utility because it would limit dependence on stool microscopic exams that are both expensive and labor intensive. As a result, a secondary goal of this study was to evaluate the utility of an algorithm that would limit full O&P testing to those pediatric patients who had either travelled to an endemic area or were immunocompromised.

PATIENTS AND METHODS

In this study, we retrospectively reviewed the data from patients seen at Children's Medical Center (CMC) of Dallas who had at least one microscopic stool O&P exam between 2009 and 2012. CMC consists of two inpatient facilities: a comprehensive 487-bed hospital and a 72-bed satellite hospital located 21 miles away. There are also several outpatient general and specialty clinics, including an international adoption clinic, which performs physical examination (including an O&P and the *Cryptosporidium*/*Giardia* antigen test) on children 1–2 weeks after they arrive in the United States. O&P testing was conducted at a large reference laboratory in the United States. Although the lab's test menu suggests only ordering the test if the patient is immunocompromised or has travel history, CMC does not screen orders or enforce any limitations. Stool specimens are divided and preserved in both 10% formalin and zinc containing polyvinyl alcohol (Zn-PVA) within an

hour of collection before submission to a reference laboratory. Both the formalin and Zn-PVA specimens were concentrated by filtration and centrifugation using a SpinCon concentration system (Meridian Biosciences, Cincinnati, OH). A wet-mount preparation contrasted with iodine was prepared from a drop of the formalin concentrate, and a permanently stained trichrome slide was prepared from the concentrated Zn-PVA concentrate for each individual specimen. Of note, orders for full O&P testing and *Cryptosporidium*/*Giardia* testing are not linked and can be ordered separately.

We reviewed the charts of all patients with positive O&P test results as well as a representative subset of patients with negative results. These negative control patients were evenly distributed (the first two patients per month) over the duration of the study period ($n = 68$). All patients were assessed for the following information: organisms identified by O&P, travel history, immune status, and whether an antigen test for *Giardia* and *Cryptosporidium* was performed, and the results of that test. We also recorded if treatment had been given for patients with organisms seen on O&P.

Over 3 years, 1,963 patients underwent at least one O&P. Patients who had multiple tests within 30 days were only counted once, and the results of the second and subsequent tests were not included in our evaluation unless secondary testing was positive. Prevalence was calculated as the number of positive results divided by the total number of patients ($n = 1,963$). This study was approved by the University of Texas Southwestern Medical Center institutional review board.

RESULTS

Thirty-seven (1.9%) patients were found to have a positive O&P (see Table 1). The following organisms were

TABLE 1. Positive O&P Examinations Seen in Pediatric Patients in North Texas Over 3 Years, 2009–2012^a

Organism	N	Treatment	Travel history	Immunocompromised	Concurrent organisms
<i>Giardia</i>	13 (0.66%)	11	3	0	<i>B. hominis</i> —1 <i>D. fragilis</i> —1
<i>Cryptosporidium</i>	1 (0.05%)	1	0	0	0
<i>B. hominis</i>	14 (0.71%)	6	3	2	<i>E. nana</i> —2 <i>E. nana</i> and <i>E. coli</i> —1 <i>Giardia</i> —1
<i>E. coli</i>	5 (0.26%)	4	3	2	<i>E. nana</i> and <i>B. hominis</i> —1 <i>E. nana</i> , <i>Strongyloides</i> , <i>E. hartmanni</i> —1
<i>E. nana</i>	6 (0.3%)	2	1	2	<i>B. hominis</i> . —2 <i>B. hominis</i> and <i>E. coli</i> —1 <i>E. coli</i> , <i>Strongyloides</i> , <i>E. hartmanni</i> —1
<i>D. fragilis</i>	5 (0.26%)	2	3	0	<i>Giardia</i> —1
<i>Strongyloides</i>	1 (0.05%)	1	1	1	<i>E. coli</i> , <i>E. nana</i> , <i>E. hartmanni</i> —1

^aOnly one positive test from each episode of disease was included (tests of cure were excluded). Exams with two organisms were included in both categories.

Fig. 1. Algorithm for stratifying O&P testing.

seen: *Blastocystis hominis* ($n = 14$ [0.7%]); *Giardia* ($n = 13$ [0.66%]); *Dientamoeba fragilis* ($n = 5$ [0.26%]); *Endolimax nana* ($n = 6$ [0.3%]); *Entamoeba coli* ($n = 5$ [0.26%]); and *Cryptosporidium* ($n = 1$ [0.05%]); *Strongyloides*

($n = 1$ [0.05%]), and *Entamoeba hartmanni* ($n = 1$ [0.05%]). Patients with two or more organisms in one specimen were counted in both categories. Fifteen patients (41%) had a history of travel outside the United States or were immunocompromised, compared to 35% in the negative control group. Of the 37 patients with positive O&P findings, 20 also had a concurrent antigen test performed, of which 8 were positive—7 for *Giardia* and 1 for *Cryptosporidium*. There was 100% correlation between O&P results and *Giardia* antigen testing. Because *Cryptosporidium* is not commonly identified by routine O&P testing, we are unable to assess the correlation between antigen and O&P testing.

The proposed algorithm would allow all patients with suspected parasitic gastrointestinal disease to be screened by *Cryptosporidium*/*Giardia* antigen testing (see Fig. 1). If negative for *Cryptosporidium*/*Giardia*, patients would be screened for travel or immunocompromised status before granting a full O&P. For those who are immunocompetent or have not traveled, the algorithm workup would stop with *Cryptosporidium*/*Giardia* antigen testing.

Based on our data, we evaluated what our patients' results would have been if this algorithm had been in practice over that same 3-year period (see Fig. 2). Of the 37 patients, 14 of them would have had a positive antigen test for *Giardia* ($n = 13$) or *Cryptosporidium* ($n = 1$). The

Fig. 2. Comparison of organism detection in current practice and with the proposed algorithm. *One of these "misses" was a patient with *Giardia* also, so workup would have stopped after a positive antigen test. **Assumes 100% sensitivity for antigen detection *Cryptosporidium*/*Giardia* assay.

TABLE 2. Number of O&P Specimens Collected for Each Positive Patient

No. of positive examinations	Number	Percentage (%)
1/1	28	76
2/2	3	8
3/3	3	8
1/2	2 ^a	5
3/4	1 ^b	3
Total	37	100

^aOne of two had a negative initial result.^bThe fourth specimen submitted was negative.

remaining 23 patients would have been screened based on travel or immunocompromised status, and 11 of these would have been granted a conventional O&P. The results of these would have included the following: *B. hominis* ($n = 6$), *E. coli* ($n = 4$), *E. nana* ($n = 2$), *D. fragilis* ($n = 2$), *E. hartmanni* ($n = 1$), and *Strongyloides* ($n = 1$). The remaining 12 patients would not have been granted a full O&P and therefore these organisms would have been “missed”: *B. hominis* ($n = 8$), *E. coli* ($n = 1$), *E. nana* ($n = 4$), and *D. fragilis* ($n = 3$). This included two patients with *Giardia* and a second organism (one with *B. hominis* and one with *D. fragilis*). In our proposed algorithm, these additional organisms would not have been detected because workup of these patients would have stopped with a positive antigen test for *Giardia*.

It is commonly accepted dogma that three stool specimens are required before intestinal parasitic infection can be ruled out. To assess whether this practice was supported by our data, we surveyed all positive patient episodes. Positive episodes were stratified by number of specimens collected (Table 2). The majority of positive specimens came from patients from whom only a single specimen was submitted ($n = 28$ (76%)). Another six patients (16%) had multiple specimens submitted, of which all were positive. In three patients, multiple specimens were submitted but one of them was negative. In only one of those patients was the first specimen negative. Thus, of the nine patients with parasitic infection, who had multiple specimens collected, only one benefitted from the multiple specimen collection strategy. In other words, the collection of multiple specimens for the diagnosis of intestinal parasitic infection increased the yield by 11.1%. This is consistent with the findings of others, including those which were referenced by the CDC manual *Laboratory Procedures for the Diagnosis of Intestinal Parasites* (4, 8). Despite the fact that the majority of patients included in this study had only a single specimen submitted, we do not believe that this led to a significant underestimate of prevalence. Even if all patients had multiple stool specimens submitted, the expected increase in yield would be approximately 10%. With 37 positive patients, that

would have led to an additional four positive patients that would not significantly change the calculated prevalence.

The proposed algorithm is intended to limit resource utilization and as a result, reduce laboratory costs. As part of this analysis we assessed the impact of the algorithm on laboratory costs. The reference laboratory charge for a full O&P is approximately \$40. The ImmunoCard STAT! *Cryptosporidium*/*Giardia* antigen test (Meridian Bioscience), which is performed in-house, costs \$13. In the past 3 years, 2,176 O&Ps were performed. Because the ordering patterns of physicians in this institution are not generalizable to other settings, we did not include the cost of the antigen test from the pre-algorithm group. Many of these patients did have a *Cryptosporidium*/*Giardia* antigen test ordered and as a result our cost analysis likely underestimates the cost savings that would be realized by implementing this algorithm. Total lab expenditure for full O&P testing over the 3-year study period was \$87,040. Using our algorithm, we would have performed 2,176 antigen tests ($2,176 \times \$13 = \$28,288$). We estimate that 0.7% (14) of these tests would be positive, so 2,162 patients would be screened for travel or immunocompromised history. Using the value from our control group, 35% (756) would be granted an O&P at the cost of \$40 each—for a total cost of \$30,240. Adding this to the antigen test cost, the total over 3 years would be \$58,528, giving a difference in costs of \$28,512 for 3 years, or \$9,504 per year. We did not take into account the labor costs associated with an increase in in-house antigen testing or a decrease in preparing samples for shipment.

The average turnaround time (TAT) was calculated for those tests that generate positive results. This TAT was calculated as the time between specimen collection and reporting of the positive result in the medical record. On average a positive O&P result took 105 h or 4.4 days (data not shown). The average TAT for the *Cryptosporidium*/*Giardia* antigen test (batched once daily 7 days a week) was 20 h (data not shown). The difference between the TAT's reflects what many labs sending out their microscopic O&P testing will experience. These results may not be applicable to those labs performing microscopic O&P's in-house. Although outsourcing microscopic O&P is becoming common, those still performing the test in-house could expect a significant reduction in O&P volume and therefore labor requirements for this time-consuming and labor-intensive test.

DISCUSSION

Overall the prevalence of parasitic pathogens was very low in this study (~1.9%). This is an interesting finding as it conflicts with the higher prevalence rates found in other studies of parasitic disease in the United States (4, 5, 9). However, others have found similar prevalence rates albeit

in a different area of the United States and in a different patient population (6, 10). Another important finding here is the lack of *Entamoeba histolytica/dispar* disease in our patient population. This is very different from the findings of others that found higher prevalence rates of *E. histolytica/dispar* (5). These findings are particularly surprising given that this was a North Texas patient population, where a high percentage of patients are from or have traveled to Mexico and/or are part of immigrant families. It is generally thought that these patients are at higher risk for parasitic infection (11–13). To determine if this phenomenon was seen in adult patients, we conducted a review of stool O&P tests conducted over the same time frame from a neighboring community hospital. In this patient population, 29 positive specimens for *E. histolytica/dispar* were found out of a total of 9,475 tested. This suggests that the prevalence of disease is extremely low in this area (~0.3%) and may explain why no positive results were found in our patient population (data not shown).

The algorithm assessed in this study was designed to limit parasitic testing. The primary goal of such an algorithm would be to reduce overall hospital costs and to implement an efficient diagnostic process. Not surprisingly, our TAT data showed that antigen testing yielded significantly faster results. It is difficult to know how physicians are using the full O&P testing in our institution but it seems likely that ordering patterns are based primarily on habit rather than a specific need. Indeed, a number of survey-based studies have concluded that physician ordering practice for the diagnosis of intestinal parasitic disease is not consistent nor is it necessarily based on a patient's likelihood of having parasitic disease (14–17). A benefit of implementing the proposed algorithm would be to guide physicians to the most appropriate diagnostic process.

An important concern with implementing strict testing criteria is that significant infections might be missed. In the retrospective modeling study, 11 possible infections (eight *B. hominis*, three *D. fragilis*) would have been missed if O&P testing were restricted to travel and immunocompromised patients. *B. hominis* is a controversial pathogen with some literature suggesting it is disease-causing while others disagree (18, 19). Similarly, the pathogenic potential of *D. fragilis* has been questioned but most now feel that it is a definitive pathogen (20–22). Despite their controversial nature, most would agree that in many instances these organisms are capable of causing disease. Therefore, missing their diagnosis may be clinically significant. Of note, all but two of the patients with *D. fragilis* or *B. hominis* who would have been missed by the algorithm were prescribed treatment, suggesting that a physician deemed them to be pathogens. In the final analysis, the proposed algorithm would have missed 57% of *B. homi-*

nis infection and 60% of *D. fragilis* infection making it of limited clinical utility. One might argue that despite these “misses,” the low prevalence of these pathogens in this study may still support the use of such an algorithm. This approach would not be appropriate for institutions with higher prevalence of *D. fragilis* and *B. hominis* disease. To explore this argument further and gain a broad perspective on parasitic epidemiology throughout the United States, we collected data from a large US reference laboratory between November 1, 2009, and December 14, 2012. In total, 167,375 total specimens were tested with either a microscopic O&P exam or an antigen test where appropriate. Of these, 1,633 were positive for *B. hominis*, 1,043 positive for *Giardia* (538 by O&P, 505 by antigen), 146 were positive for *D. fragilis*, 70 were positive for *E. histolytica/dispar* (4 by O&P, 66 by antigen), and 116 positive for *Cryptosporidium* spp. (98 by antigen, 18 by modified acid fast stain). These data show that on a national level *D. fragilis* and *B. hominis* are among the most prevalent parasites identified with microscopic O&P examination. If these organisms are to be considered pathogenic, then restricting O&P testing to those who are immunocompromised or have traveled may miss a significant amount of disease.

The concept of restricting parasitic testing in low-prevalence settings is not unprecedented. Morris et al. evaluated the practice of restricting parasitic testing to one specimen rather than multiple specimens (4). They concluded that such practice in a low-prevalence setting was an effective means for reducing costs without adversely affecting patient care. Branda et al. came to similar conclusions with this approach and speculated that the practice of limiting testing was appropriate up to a prevalence of 20% (5). With only 1.9% of all patients yielding positive results, the prevalence in our patient population was far less than 20%. Further, the prevalence of those clinically relevant organisms that may have been missed by the proposed algorithm was even lower at 0.6%. Thus, it may be that the significant savings in cost and labor outweigh the relatively small number of misdiagnoses that would be incurred by the algorithm.

Our study is limited by a small number of patients with positive tests. Also, in our model projections we assume that the antigen test had 100% sensitivity. Although no test is 100% sensitive, this assumption is reasonable as the full O&P did not detect a single *Cryptosporidium* or *Giardia* not detected by the antigen test. Finally, we excluded patients who only had an antigen test performed. In our study period, there were 42 patients with a positive *Giardia* antigen test and 10 with a positive *Cryptosporidium* antigen test. These numbers were not factored into our prevalence because the goal of this study was to assess the utility of stratifying microscopic O&P testing by travel and/or immunocompromised status.

CONCLUSIONS

We conclude that due to their low positivity rate, O&P examinations could be restricted to those patients with risk factors, including travel or immunocompromised status, and a negative *Cryptosporidium*/*Giardia* antigen test. However, implementing such an algorithm may miss a significant percentage of *B. hominis* and *D. fragilis* infection. Further studies are needed to prospectively assess the utility of this diagnostic algorithm, and the clinical significance, if any, in failing to detect *D. fragilis* and *B. hominis*. Lastly, a higher prevalence of parasitic infections such as *Ascaris lumbricoides*, *Balantidium coli*, *Diphylobothrium latum* as well as others may be observed in other areas of the country. The proposed algorithm would fail to detect these infections and laboratories should make provisions for these exceptional cases.

CONFLICT OF INTEREST

There are no conflicts of interest for any of the authors and no funding source.

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