A quick and cost-effective method for diagnosing disseminated histoplasmosis in children

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Abstract

The examination of fecal mucus for detecting yeast cells of Histoplasma capsulatum has proved to be a useful tool for diagnosing disseminated histoplasmosis in paediatric patients in a study of 13 cases carried out in Ecuador.

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Histoplasmosis is a fungal infection caused by the dimorphic fungus Histoplasma capsulatum. When the fungus grows saprophytically, it develops mycelium with 2 types of conidia: macro and micro. The infection is acquired by inhalation of microconidia. The parasitic form is characterized by the production of small yeasts 2 to 4 μm in diameter. Most of the cases are subclinical and benign, but some patients, mainly immunocompromised children, may have an acute rapidly fatal course with diffuse reticuloendothelial involvement and systemic infection (Wheat and Kauffman, 2003). Macrophages full of yeasts infiltrate the bone marrow, spleen, liver, and lungs. These patients are critically ill, presenting with prolonged fever, weight loss, nausea, vomiting, abdominal pain and bloody and mucous diarrhea, diffuse lymphadenopathy, hepatomegaly, and splenomegaly. Death is due to a variety of causes, including disseminated intravascular coagulation, gastrointestinal hemorrhages, respiratory insufficiency, and bacterial sepsis. Several techniques are used to diagnose histoplasmosis, including antigen detection, serologic tests, direct microscopy examination, and cultures (Guimaraes et al., 2004; Wheat, 2003; Wheat and Kauffman, 2003). The diagnostic procedures are well described, but recognized deficiencies in these techniques justify ongoing research (Wheat, 2003). For instance, antibodies appear late and serologic tests are less sensitive in patients with disseminated disease because of their underlying immunocompromised state (Wheat, 2003). Cross-reactions with other fungal infections also occur (Kahi et al., 2005). Although cultures provide the strongest evidence for infection and are positive in about 85% of the cases with disseminated histoplasmosis, multiple specimens must be cultured for an accurate diagnosis (Kahi et al., 2005). In addition, H. capsulatum colonies are slow to grow and require up to 6 weeks to develop. For all these shortcomings, the results are most sensitive when a battery of tests is used (Wheat, 2003). Staining clinical specimens with calcofluor white is a time- and cost-saving diagnostic method, but it requires a fluorescent microscope (Hughes et al., 2004).

In a retrospective study (1980–1994) of the cases of disseminated infantile histoplasmosis, diagnosed in the Instituto Nacional de Higiene y Medicina Tropical “Leopoldo Izquieta Pérez” (INHMT-LIP) in Guayaquil, Ecuador, a total of 20 cases were recorded. These cases were diagnosed mainly from bone marrow aspirates, liver biopsy, occasionally from rectum biopsy, bronchial aspirates, ascitic fluid, and blood cultures. All the patients also presented with bloody fecal mucus, even when this clinical manifestation was not the cause of admittance. Surprisingly, when samples of fecal mucus of some of these patients were microscopically examined, numerous yeast cells, compatible with H. capsulatum, were observed. Therefore, taking into account that these yeast cells can be detected easily, quickly, cheaply, and nonaggressively, we performed
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Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)/sex</th>
<th>Geographic origin (city, province)</th>
<th>Clinical symptoms</th>
<th>Evolution (month)</th>
<th>Histoplasma detection</th>
<th>Primary sample</th>
<th>DEFM</th>
<th>BC</th>
<th>DID test</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3/M</td>
<td>Quevedo, Los Rios</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea, pancytopenia</td>
<td>3</td>
<td>Bone marrow +</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7/M</td>
<td>Milagro, Guayas</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea</td>
<td>2</td>
<td>Blood +</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3/F</td>
<td>Quevedo, Los Rios</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea, ascites, jaundice, moderate malnutrition</td>
<td>1</td>
<td>Bone marrow +</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1/F</td>
<td>Buena Fe, Los Rios</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea</td>
<td>2</td>
<td>Liver biopsy +</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2/M</td>
<td>Rio Chico, Manabi</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea, melena, jaundice, moderate malnutrition</td>
<td>2</td>
<td>Bone marrow +</td>
<td>+</td>
<td>+</td>
<td>–</td>
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</tr>
<tr>
<td>6</td>
<td>3/F</td>
<td>Quevedo, Los Rios</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea, ascites, jaundice, edema</td>
<td>3</td>
<td>Bone marrow +</td>
<td>+</td>
<td>+</td>
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<td>2</td>
<td>Liver biopsy +</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
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<td>Guayaquil, Guayas</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea</td>
<td>4</td>
<td>Fecal mucus +</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>9</td>
<td>6 (months)/F</td>
<td>Vinces, Los Rios</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea</td>
<td>1</td>
<td>Fecal mucus +</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
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<td>Quevedo, Los Rios</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea, petechiae, moderate malnutrition</td>
<td>1</td>
<td>Fecal mucus +</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>11</td>
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<td>Quevedo, Los Rios</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea</td>
<td>5</td>
<td>Fecal mucus +</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>Quevedo, Los Rios</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea, abdominal distension, edema, moderate malnutrition</td>
<td>1</td>
<td>Fecal mucus +</td>
<td>+</td>
<td>+</td>
<td>–</td>
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</tr>
<tr>
<td>13</td>
<td>3/F</td>
<td>Babahoyo, Los Rios</td>
<td>Fever, hepatomegaly, splenomegaly, bloody and mucous diarrhea, moderate malnutrition</td>
<td>3</td>
<td>Fecal mucus +</td>
<td>+</td>
<td>+</td>
<td>–</td>
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</table>

DEFM = direct examination of fecal mucus; BC = blood culture.

a prospective study to evaluate the diagnostic value of this procedure.

In the period 1995 to 2004 in the Mycology Laboratory of the INHMT-LIP, a total of 13 children, from different regions of Ecuador, diagnosed with disseminated histoplasmosis were studied (Table 1). From each patient, we took serial samples of fecal mucus for culture and direct examination, and samples of blood for blood culture and double immunodiffusion (DID) test studies.

Samples of mucus were collected from the feces placed in sterile plastic containers and taken to the laboratory as soon as possible. A total of 5 samples were taken per patient. We placed a drop of each sample in the center of each of three 1 × 3-in. glass slides and spread the material with the tip of the pipette. Smears were air dried, fixed by heat, and stained with periodic acid-Schiff and Wright stains. Then they were examined with the ×100 objective to detect intra- and extracellular cells compatible with H. capsulatum. The samples were placed in sterile tubes and diluted with 75% sterile saline. Sulbactam (100 mg) and ampicillin (100 mg) were added to the tubes. After 1 h at 37 °C, samples were centrifuged for 10 min at 2000 × g. The supernatant was discarded, and the pellet was used to inoculate the culture medium. The media used were Sabouraud dextrose agar (SDA) (Difco Laboratories, Detroit, MI) and SDA with chloramphenicol and cycloheximide. Cultures were incubated at 28 °C.

One blood sample per patient was investigated. A total of 0.5 mL of blood without anticoagulant was inoculated directly onto the surface of the 2 media indicated above and incubated at 28 °C. Six tubes (three for each medium) were inoculated with each blood sample. H. capsulatum was identified by a macroscopic study of the colonies and a microscopic examination of the fungal structures mounted on lactophenol-cotton blue (De Hoog et al., 2000; Larone, 2002). DID tests were performed using the Fungal Immunodiffusion Test System (IMMY Immuno-Mycologics, Norman, OK).
The age range of the patients was between 6 months and 7 years, with a mean of 3.1 years; 7 patients were female and 5 male. The most common clinical symptoms were fever, hepatosplenomegaly, and bloody and mucous diarrhea, which were present in all the cases; the less frequent symptoms were jaundice, edema, poor nourishment, and ascites. Of particular note was the presence of greenish mucus and blood in stools, characteristic of enterocolitis, which is frequent in infantile disseminated histoplasmosis (Kahi et al., 2005). *Histoplasma* was present in all the fecal mucous samples of the 13 patients (100%). The stains used revealed the presence of numerous 3- to 4-µm globose or ovoid yeastlike cells, surrounded by a slight halo compatible with *H. capsulatum*, which are free but also inside macrophages and polymorphonuclear cells (Fig. 1). Cultures from the fecal mucus were positive in at least 1 of the 5 samples from each of the 13 patients (100%). Blood culture was positive in all 13 (100%) cases. DID assay was positive only in 1 (7.6%) of the cases. All of these data are summarized in Table 1.

Although this was not the aim of the study, direct microscopic examination of peripheral blood smears stained with Wright-Giemsa was performed in 5 of the 13 patients diagnosed with histoplasmosis, and only 3 of them (60%) were positive for yeast cells of *Histoplasma*. Numerous stool samples from patients with enterocolitis without histoplasmosis were investigated over these years, showing an absence of yeast cells compatible with *H. capsulatum* in all the cases.

Disseminated histoplasmosis in young children is a very severe disease, which is sometimes difficult to detect. Because it is not always diagnosed quickly, its proper treatment is sometimes delayed (Adderson, 2004). The infection is most common in very young poorly nourished children with immature immunologic systems (Ramón-Garcia et al., 1993).

*H. capsulatum* spreads throughout the body, and it can be isolated by invasive procedures such as liver biopsy and bone marrow aspirate (Wheat, 2003). However, in patients with diarrhea and enterocolitis, which are common clinical manifestations of disseminated infantile histoplasmosis, the yeast forms of this fungus can be detected with a simple histologic staining of the fecal mucus. In laboratories less familiar with the staining characteristics of fungal pathogens, these yeast forms can be confused with other yeasts (Wheat and Kauffman, 2003), especially with *Candida glabrata*, which has a similar size to that of *H. capsulatum* (Hughes et al., 2004). However, the isolation of the latter in the blood culture, which in our study was 100%, is a method of confirmation.

In all our cases, systemic histoplasmosis correlated with the presence of yeast cells in fecal mucus. This was confirmed by the culture results. However, the culture results were obtained in 25 days, whereas the direct staining results took only 24 h. Serologic testing was not helpful probably because of the degree of immunosuppression of the patients, their poor ability to develop antibodies, and the acuteness of the illness (Wheat, 2003).

In conclusion, the examination of fecal mucus can be a useful addition to existing methods for diagnosing disseminated histoplasmosis in children. Although morphology alone does not fully identify *H. capsulatum* yeast cells, in childhood cases, their visualization in fecal matter constitutes the 1st clue to the diagnosis of histoplasmosis. One of the greatest advantages of this procedure is that it can be used even in situations in which laboratory facilities are relatively limited. Further studies with more patients are needed to confirm these interesting preliminary results.
References