JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1984, p. 1192–1193
0095-1137/84/121192-02$02.00/0

Vol. 20, No. 6

Detection of *Cryptosporidium* Oocysts in Sputum During Screening for Mycobacteria

RICHARD A. MILLER,[1,2]* JUDITH N. WASSERHEIT,[1,2] JEAN KIRIHARA,[2,3] AND MARIE B. COYLE[2,3,4]

Division of Infectious Diseases, Department of Medicine, Pacific Medical Center, Seattle, Washington 98144[1]; Departments of Microbiology[3] and Laboratory Medicine,[4] Harborview Medical Center, Seattle, Washington 98104; and University of Washington, Seattle, Washington 98195[2]

Received 25 June 1984/Accepted 13 August 1984

We report a case in which *Cryptosporidium* oocysts were detected during routine auramine screening of a sputum specimen for acid-fast bacilli. The patient was a 28-year-old male with acquired immune deficiency syndrome and intestinal cryptosporidiosis. Microbiology laboratory workers should be alert to the possibility that these oocysts may be present in sputum specimens from patients with acquired immune deficiency syndrome.

Gastrointestinal infection with protozoa of the genus *Cryptosporidium* has been increasingly recognized as a cause of severe diarrhea in patients with acquired immune deficiency syndrome. Extraintestinal infection has been well documented in animals (4), but is rarely reported in humans (3, 6). We recently identified structures consistent with cryptosporidial oocysts in the sputum of a patient with chronic intestinal cryptosporidiosis.

A 28-year-old male homosexual who was an intravenous drug abuser was diagnosed as having acquired immune deficiency syndrome after experiencing a 3-month history of severe diarrhea, intermittent fever, progressive fatigue, and a 45-lb (ca. 20.4-kg) weight loss. Early during the course of the patient's illness, *Giardia* and *Shigella* spp. were detected in his stools by conventional stain and culture techniques. *Cryptosporidium* spp. were identified in stools a short time later by staining as described below. Quinacrine, metronidazole, and trimethoprim-sulfamethoxazole were administered. At the time of hospitalization, the patient also had a 2-week history of pleuritic chest pain and cough productive of thick, white sputum. He reported no episodes of aspiration of gastric contents, seizure, or loss of consciousness.

The physical examination was remarkable only for the findings of diffuse lymphadenopathy and oropharyngeal lesions consistent with thrush. The leukocyte count was 5,500/mm^3, with a helper-suppressor T cell ratio of 0.19. The chest radiograph was normal, but sinus films revealed bilateral maxillary opacification. An auramine stain of a direct stool smear contained round structures (3 to 5 μm in diameter), which were consistent with *Cryptosporidium* oocysts (5; L. K. Rabe, A. M. Larson, and M. B. Coyle, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C272, p. 282). Identification was confirmed by modified Kinyoun stain (1), which showed oocysts with typical internal morphological features. An auramine stain of the sputum was performed for mycobacteria. No bacilli were found, but numerous round bodies, 3 to 5 μm in diameter, which avidly retained the fluorescent stain, were seen (Fig. 1). A modified Kinyoun-stained slide prepared from the same specimen confirmed both the identification of typical *Cryptosporidium* oocysts and the absence of acid-fast bacilli. Auramine stains of material swabbed from the tonsillar crypts and posterior nasopharynx, as well as a sinus aspirate, were negative. *Mycobacterium avium* complex was subsequently cultured from sputum, blood, and lymph node aspirate samples. Other potentially pathogenic microorganisms identified included *Clostridium difficile* from stool, *Candida albicans* from sputum, group B streptococcus and *Haemophilus influenzae* from the sinus aspirate, and herpes simplex virus from a rectal swab.

Although the severity of the diarrhea fluctuated for the next few months, it never completely ceased, and cryptosporidial oocysts were always present in the stools. There was no diminution of symptoms or oocyst excretion in response to therapy with quinacrine, metronidazole, trimethoprim-sulfamethoxazole, or oral vancomycin. Multiple drug therapy for *M. avium* complex was initiated, but compliance was minimal because of toxic reactions. The patient was readmitted because of recurrent fever and dyspnea 3 months later, at which time Kaposi's sarcoma was diagnosed. Pneumonia involving *Pneumocystis carinii* and *Candida albicans* was diagnosed by open lung biopsy. No *Cryptosporidium* oocysts were seen in the biopsy specimen, although they were still present in respiratory secretions. Despite aggressive therapy, the patient died from progressive respiratory failure. The pathological examination showed *P. carinii* pneumonia with intense inflammatory pneumonitis. There was also diffuse Kaposi's sarcoma throughout the gastrointestinal tract. *Cryptosporidium* spp. were identified in the bowel lumen, but there was no evidence of tissue invasion.

The size, morphology, and staining characteristics of the round bodies identified on the auramine and modified Kinyoun stains of sputum samples were consistent with cryptosporidial oocysts (1, 5; Rabe et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984). Yeast cells do not retain auramine (Rabe et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984), and we have not encountered false-positive results due to morphologically similar auramine-positive objects previously in sputum. The negative results on the auramine stains of material from the nasopharynx and sinuses indicated that the sputum specimen had probably not been contaminated by *Cryptosporidium* organisms colonizing the oropharynx. The pathogenic potential of *Cryptosporidium* oocysts in human lungs is unknown, but in a similar case, metaplastic changes without inflammation were noted in a bronchoscopy speci-

* Corresponding author.

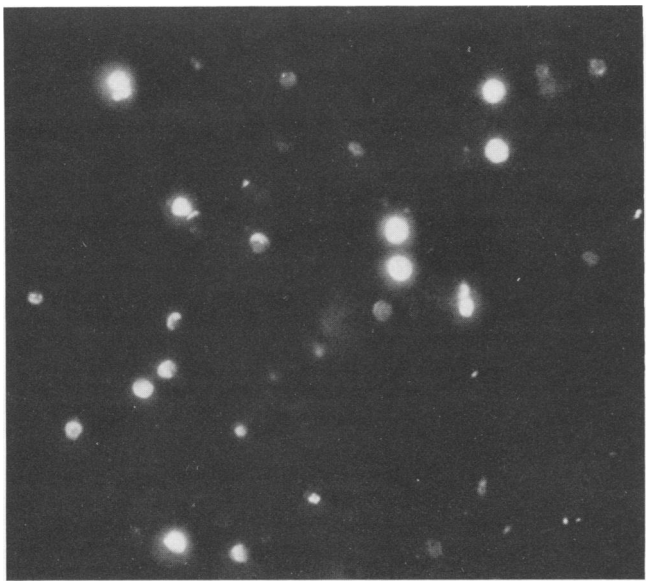

FIG. 1. Auramine-stained smear of sputum. Numerous *Cryptosporidium* oocysts and oocyst fragments can be seen (×400).

men (3). The route by which the infection spread to the respiratory tract, whether via aspiration of infected gastric contents or via the blood, is also unknown. Therapy with conventional antibiotics and antiprotozoal agents was ineffective against the cryptosporidiosis in our patient. Spiramycin has since emerged as a possibly effective agent for the treatment of cryptosporidiosis in immunocompromised hosts (2, 2a).

Given their high incidence of pulmonary and disseminated mycobacterial infections, it is likely that most acquired immune deficiency syndrome patients will have auramine or conventional acid-fast stains performed on respiratory-tract specimens. Microbiologists, as well as clinicians caring for these patients, should be aware of the potential for the presence of cryptosporidial oocysts in these specimens.

LITERATURE CITED

1. **Bronsdon, M. A.** 1984. Rapid dimethyl sulfoxide-modified acid-fast stain of *Cryptosporidium* oocysts in stool specimens. J. Clin. Microbiol. **19**:952–953.
2. **Centers for Disease Control.** 1984. Update: treatment of cryptosporidiosis in patients with acquired immunodeficiency syndrome (AIDS). Morbid. Mortal. Weekly. Rep. **33**:117–119.

2a. **Collier, A. C., R. A. Miller, and J. D. Meyers.** 1984. Cryptosporidiosis after marrow transplantation: person-to-person transmission and treatment with spiramycin. Ann. Intern. Med. **101**:205–206.

3. **Forgacs, P., A. Tarshis, P. Ma, M. Federman, L. Mele, M. L. Silverman, and J. A. Shea.** 1983. Intestinal and bronchial cryptosporidiosis in an immunodeficient homosexual male. Ann. Intern. Med. **99**:793–794.
4. **Hoerr, F. J., F. M. Ranck, and T. F. Hastings.** 1978. Respiratory cryptosporidiosis in turkeys. J. Am. Vet. Med. Assoc. **173**:1591–1593.
5. **Payne, P., L. A. Lancaster, M. Heinzman, and J. A. McCutchan.** 1983. Identification of cryptosporidium in patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. **309**:613–614.
6. **Pitlik, S. D., V. Fainstein, A. Rions, L. Guarda, P. W. A. Mansell, and E. M. Hersh.** 1983. Cryptosporidial cholecystitis. N. Engl. J. Med. **308**:967.