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Dark-Field Microscopy of Human Feces for Presumptive Diagnosis of *Campylobacter fetus* subsp. *jejuni* Enteritis

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To determine the value of direct dark-field microscopy for diagnosing enteritis due to *Campylobacter fetus* subsp. *jejuni*, we examined 1,377 human fecal specimens for bacteria with typical *Campylobacter* darting motility, leukocytes, and erythrocytes. Eighty-four specimens (6.1%) grew *C. fetus* subsp. *jejuni*. Of the 48 specimens showing *Campylobacter* motility, 30 (62%) grew *C. fetus* subsp. *jejuni*. The sensitivity, specificity, and predictive value of observing *Campylobacter* motility were 36%, 99%, and 62%, respectively. The predictive value of detecting *Campylobacter* motility was improved if the specimens were diarrheal (23 of 31, 74%), leukocytes were present (25 of 33, 76%), erythrocytes were present (22 of 27, 81%), or if all of the above findings were present (18 of 20, 90%). The sensitivity of detecting *Campylobacter* darting motility was highest if specimens were examined within 2 h of arrival in the laboratory (15 of 30, 50%) as opposed to after 2 h (15 of 53, 28%; $P < 0.01$). Prompt dark-field microscopic examination of diarrheal stool specimens is valuable for the presumptive diagnosis of *Campylobacter* enteritis.

Direct bright-field microscopy of stool specimens to detect leukocytes was recommended in the evaluation of patients with possible bacterial enteritis as early as 1893 by Robert Koch (12). Fecal leukocytes are found most consistently in *Shigella* enteritis, but they are also found in *Salmonella* and *Campylobacter* enteritis, ulcerative colitis, and other intestinal diseases (3, 9, 14). The presence of fecal leukocytes is a nonspecific finding and of little help in guiding therapy.

In contrast, in areas where cholera is common, direct dark-field microscopy accurately predicts the presence of *Vibrio cholerae* in stool specimens, as a result of its characteristic darting motility (1). *C. fetus* subsp. *jejuni*, a common enteric pathogen in the United States, has motility similar to that of *V. cholerae*. Several authors have noted bacteria with this characteristic darting motility in stool specimens of patients with *Campylobacter* enteritis, but the diagnostic value of this finding has not been evaluated prospectively (3, 11). The purpose of this study was to determine the value of direct dark-field examination for predicting the growth of *C. fetus* subsp. *jejuni* from clinical stool specimens.

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MATERIALS AND METHODS

During a 14-month period all stool specimens submitted for routine culture to the Clinical Microbiology Laboratories of the University of Colorado, Denver General, and Denver Children's Hospitals were studied. The consistency of the stool specimen was recorded as formed, soft, loose, or watery. A portion of the specimen was mixed with a drop of tryptic soy broth on a glass slide, overlaid with a cover slip, and examined by dark-field microscopy at 100× and 430× magnification for the presence of organisms with typical *Campylobacter* motility (CM), leukocytes, and erythrocytes. At least 10 low-power fields were examined. Cells and bacteria with CM were quantitated as none, 1+ (<10 per low-power field), 2+ (10 to 25 per low-power field), or 3+ (>25 per low-power field). Typical CM was characterized by rapid darting motility similar to that seen with *Pseudomonas aeruginosa* or by corkscrew motion. If immediate examination was not possible, the specimen was refrigerated and then warmed to room temperature prior to examination.

Stool specimens were cultured non-quantitatively with the use of Hektoen and MacConkey agars, selenite broth, and selective *Campylobacter* blood agar plates consisting of brucella agar base, 5% sheep blood, and the following concentrations of antimicrobials per liter: vancomycin, 10 mg; polymyxin B, 2,500 U; trimethoprim, 5 mg; amphotericin B, 2 mg; and cephalothin, 15 mg (2).

Campylobacter agar plates were incubated at 42°C in a candle jar and examined daily for 3 days. Colonies were presumptively identified as *C. fetus* subsp. *jejuni* if they consisted of gram-negative curved rods with darting motility and demonstrated positive reactions for oxidase and catalase. Final identification was based on previously published criteria (10). The amount of growth of *C. fetus* subsp. *jejuni* was recorded as less than 10, 10 to 100, or greater than 100 colonies per plate.

To determine whether a combination of gross and microscopic findings could improve the predictive value of detecting CM, the following characteristics were analyzed alone and in various combinations: stool consistency, presence of leukocytes or erythrocytes in any quantity, and examination for CM within 2 h. A diarrheal stool was defined as a watery or loose specimen.

Statistical analysis was done by use of the chi-square test and previously published definitions of sensitivity, specificity, and predictive value (7).

RESULTS

The results of dark-field examinations for CM and cultures are shown in Table 1. Of 1,377 stool specimens examined, 84 (6.1%) grew *C. fetus* subsp. *jejuni*. The presence of CM was 36% sensitive and 99% specific, based on positive cultures.

Eighty-two percent of the specimens that grew *Campylobacter* were diarrheal. Although CM was detected in stools of every consistency, it was associated most often with positive cultures when stools were diarrheal (23 of 31, 74%) rather than soft or formed (6 of 15, 40%; $P < 0.05$).

CM was positive more often when specimens were examined within 2 h of receipt in the laboratory (15 of 30, 50%) as opposed to after 2 h (15 of 53, 28%; $P < 0.05$). However, 3 of 9 culture-positive specimens examined 24 to 76 h after receipt still exhibited CM. The time of the examination was not recorded for one specimen.

The microscopic detection of CM correlated with the amount of growth on the *Campylobacter* selective medium. Only 4 of 27 (15%) specimens with fewer than 100 campylobacter colonies demonstrated CM compared with 25 of 54 (46%) specimens with more than 100 colonies ($P < 0.01$).

The sensitivities and predictive values of combinations of gross and microscopic stool findings are shown in Table 2. The predictive values were not substantially improved by prompt examination. Quantitation of CM, leukocytes, or erythrocytes as opposed to their mere presence did not improve their predictive value for growth of *C. fetus* subsp. *jejuni*.

TABLE 1. Results of dark-field microscopy and stool culture for *Campylobacter* in 1,377 clinical specimens

Stool culture for *C. fetus* subsp. *jejuni*	No. of specimens with CM	
	Present	Absent
Positive (n = 84)	30 (36%)	54 (64%)
Negative (n = 1,293)	18 (1%)	1,275 (99%)

TABLE 2. Sensitivity and predictive value of dark-field microscopic and gross stool characteristics for detecting *Campylobacter fetus* subsp. *jejuni*

Specimen characteristic(s)[a]	Culture Result		Sensitivity[b] (%)	Predictive value[c] of positive (%)
	Positive	Negative		
CM	30	18	36	62
CM + diarrheal (D)	23	8	27	74
CM + WBC	25	8	30	76
CM + WBC + D	20	4	24	83
CM + RBC	22	5	26	81
CM + RBC + D	19	2	23	90
CM + WBC + RBC	21	4	25	84
CM + WBC + RBC + D	18	2	22	90

[a] WBC, leukocytes; RBC, erythrocytes; diarrheal, loose or watery.

[b] Based on a total of 84 culture-positive specimens.

[c] Predictive value = no. of specimens demonstrating CM that grew *Campylobacter*/total no. of specimens demonstrating CM.

DISCUSSION

Rapid diagnosis of *Campylobacter* enteritis by direct microscopic examination of feces would be helpful in the management of patients with diarrhea if the test was simple, sensitive, and specific. The use of dark-field microscopy for accurately predicting infection with *V. cholerae*, an enteric pathogen with similar motility, was reported by Benenson et al. (1). In their study, the presence of organisms with typical darting motility in feces correctly predicted a positive culture in 80% of 107 cases of cholera. Karmali and Fleming diagnosed *Campylobacter* infection correctly by phase-contrast microscopy in 24 of 37 (65%) cases of *Campylobacter* enteritis, apparently without false-positive results (11). The relation of phase-contrast results to the time of examination, the presence of leukocytes or erythrocytes, and the stool consistency was not defined. Butzler and Skirrow have also stated that *Campylobacter* enteritis may be diagnosed by dark-field or phase-contrast microscopy (3). Davies and Penfold noted organisms with CM in a urine specimen that subsequently grew *C. fetus* subsp. *jejuni* (5).

In our study 62% of specimens with CM yielded *C. fetus* subsp. *jejuni*, but only 36% of culture-positive specimens demonstrated CM. The predictive value of CM rose from 62% to 90% if the stool had other characteristics typical of bacterial enteritis, e.g., diarrheal consistency,

leukocytes, and erythrocytes. Although earlier studies emphasized the value of detecting fecal leukocytes for predicting bacterial enteritis (6, 9, 13, 14), we found that fecal erythrocytes were as predictive as fecal leukocytes of growth of *Campylobacter* when CM was present.

False-negative CM was related both to the time of examination and to the amount of growth of *C. fetus* subsp. *jejuni*. Although delayed microscopic examination lessened the likelihood of detecting CM in culture-positive specimens, CM was detected in some specimens even after 3 days of refrigeration. Not unexpectedly, stools yielding small numbers of *C. fetus* subsp. *jejuni* were less likely to demonstrate CM compared with stools yielding heavy growth of *Campylobacter*.

False-positive CM may be due to the presence in stool of other bacteria with similar motility. One patient whose stool showed organisms with typical CM, not included in this study, grew *C. fetus* subsp. *intestinalis*. *Vibrio* and *Pseudomonas* species also have darting motility similar to that of *C. fetus* subsp. *jejuni*. We do not know how many of the specimens showing false-positive CM grew either of these other organisms.

In some cases the presence of CM may be more sensitive than culture for detecting *C. fetus* subsp. *jejuni*, as a result of overgrowth by other organisms, inability of certain strains to grow on the selective *Campylobacter* agar, or unusually strict atmospheric requirements (8). One of the specimens in our study that was culture negative but demonstrated CM probably had *Campylobacter* in the specimen since two subsequent stool specimens from the same patient grew *C. fetus* subsp. *jejuni*. Benenson et al. had similar experiences with specimens from two patients that subsequently grew *V. cholerae* (1).

The specificity of the dark-field examination of stool for *V. cholerae* can be increased by demonstration that motile organisms are immobilized with specific antisera. In Benenson's study this technique differentiated noncholera vibrios from the Ogawa and Inaba strains of *V. cholerae* (1). Specific *C. fetus* subsp. *jejuni* antisera potentially could be used in a *Campylobacter* immobilization test. Using a simpler method, Chester and Poulos reported that immobilization by distilled water of pure cultures of *Vibrio* species and *C. fetus* subsp. *jejuni* correctly distinguished these organisms from other motile gram-negative bacteria (4). They postulated that the osmolarity of the diluent mediated this effect. This test also could possibly be adapted to examination of clinical stool specimens.

The low refractility of campylobacters precludes reliable detection of CM in fecal specimens unless a phase-contrast or dark-field technique is used. As suggested by the water-immobilization phenomenon (4), the diluent may also be important. Although tryptic soy broth was used by Benenson et al. and in our studies, the optimal diluent has not been determined (1).

On the basis of this study and the reports of others (3, 11), we conclude that prompt dark-field microscopic examination of diarrheal stools is a valuable procedure that should be performed by clinical laboratories. The presence of CM, leukocytes, and erythrocytes is easily determined and the technique is simple, rapid, and inexpensive. Specimens with positive CM and leukocytes or erythrocytes have a high likelihood of growing *C. fetus* subsp. *jejuni*.

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