Clinical microbiological case: a 35-year-old HIV-positive man with intermittent fever and chronic diarrhea

F.-C. Bange[1], S. Ruttkowski[1], M. Kist[4], S. Bereswill[4], D. Meyer[2], R. E. Schmidt[2], H. Tillmann[3], I. Schedel[3], M. P. Manns[3] and F. Gunzer[1]

[1]Department of Medical Microbiology, [2]Department of Clinical Immunology, [3]Department of Gastroenterology and Hepatology, Medical School of Hannover, Germany and [4]Department of Medical Microbiology and Hygiene, University of Freiburg, Germany

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CASE STUDY

A 35-year-old HIV-positive man was admitted to our hospital with intermittent fever for several months (Figure 1) and chronic diarrhea. He was diagnosed HIV-positive 4 years earlier and had subsequently developed several AIDS-defining diseases such as *Pneumocystis carinii* pneumonia and CMV retinitis. Three months before admission the patient began complaining of watery diarrhea and recurrent fever. His stool showed no blood or mucus. He lost 17 kg of his body weight. On admission, his temperature was 39.3 °C, pulse was 104/min, and blood pressure was 120/80. The patient was cachectic and appeared chronically ill. Further physical examination was unremarkable. Abnormal laboratory results included a CRP of 31 mg/L, haemoglobin of 9.7 g/dL, serum iron of 5 μmol/L and Ferritin of 323 μg/L. The CD4 lymphocyte count was 13/μL, HIV viral load was 30 000 copies/mL plasma. Endoscopic examination of the gastrointestinal tract revealed minimal gastric inflammation and a normal ileum and colon. Blood cultures were negative for pathogens as were stool cultures. The patient was discharged after 9 days. Two months later, re-admission was necessarry because of persistent diarrhea and severe wasting. Again, diagnostic tests were normal. However, three blood cultures taken on the third hospital day became positive in the automated BacT/Alert-System (Organon Technika, Turnout, Belgium) after incubation for 72 h.

Gram staining of positive blood cultures showed no organisms. Subcultures on blood agar and chocolate agar incubated at 37 °C under aerobic and anaerobic conditions remained sterile after 7 days. Staining for acid-fast bacteria was negative. At this point, we discussed the possibility of a false positive blood culture, the frequency of which varies between 1 and 5% in automated blood culture systems. However, a positive signal was obtained after subculturing an aliquot of the positive blood culture in a fresh blood culture bottle. Finally, an acridine orange stain was performed, which showed spiral rods in all positive blood culture bottles (Figure 2). Acridine orange is a fluorochrome that intercalates into nucleic acid, is rapid and sensitive, and is clearly superior to a Gram stain in detecting small Gram-negative rods [1]. We repeated routine subcultures on blood agar under aerobic and anaerobic conditions but this time incubation under micro-aerophilic condition was included. Under the latter condition, all plates were covered with a translucent film of bacteria within 3 days. Single colonies were not discernible. Oxidase reaction and catalase reaction were positive, whereas urease reaction was negative.

SOLUTION

In principle, *Helicobacter/Campylobacter*, *Borrelia*, *Leptospires* and *Treponema* all appear as helical rods on smears stained with acridine orange. In practice, given the patient's history and clinical presentation, *Helicobacter/Campylobacter* was the most likely diagnosis. Differentiation of urease-negative *Helicobacter* by culturing and biochemical analysis is laborious and is typically done in a reference laboratory. Thus we chose a molecular approach for further identification using DNA primers specific for the genus *Helicobacter*, targeting the 16 S rRNA gene sequence and a standard protocol for amplification [2]. The primer pair amplifies a 292-bp product. Genomic DNA of *Escherichia coli, Campylobacter coli* and *C. jejuni* were used as templates to test for specificity of the primers (Figure 3: lanes 1–3). A 292-bp product was amplified only with *H. pylori* DNA or DNA of the patient's isolate (Figure 3: lanes 4, 6), sequencing of which revealed > 98% homology with *H. cinaedi*. Identification was later confirmed by conventional biochemical methods in a reference laboratory.

In the meantime, the patient had become critically ill. After identification of *H. cinaedi* the patient was put immediately on ciprofloxacin. Within 72 h, his temperature dropped below 37 °C (Figure 1). He was discharged 3 weeks later after his diarrhea had stopped and he had gained weight. He remained well when seen as an out-patient 2 months later.

Corresponding author and reprint requests: F.-C. Bange, Institute of Medical Microbiology, Medical School of Hannover, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany
Tel: + 49 511 532 4352

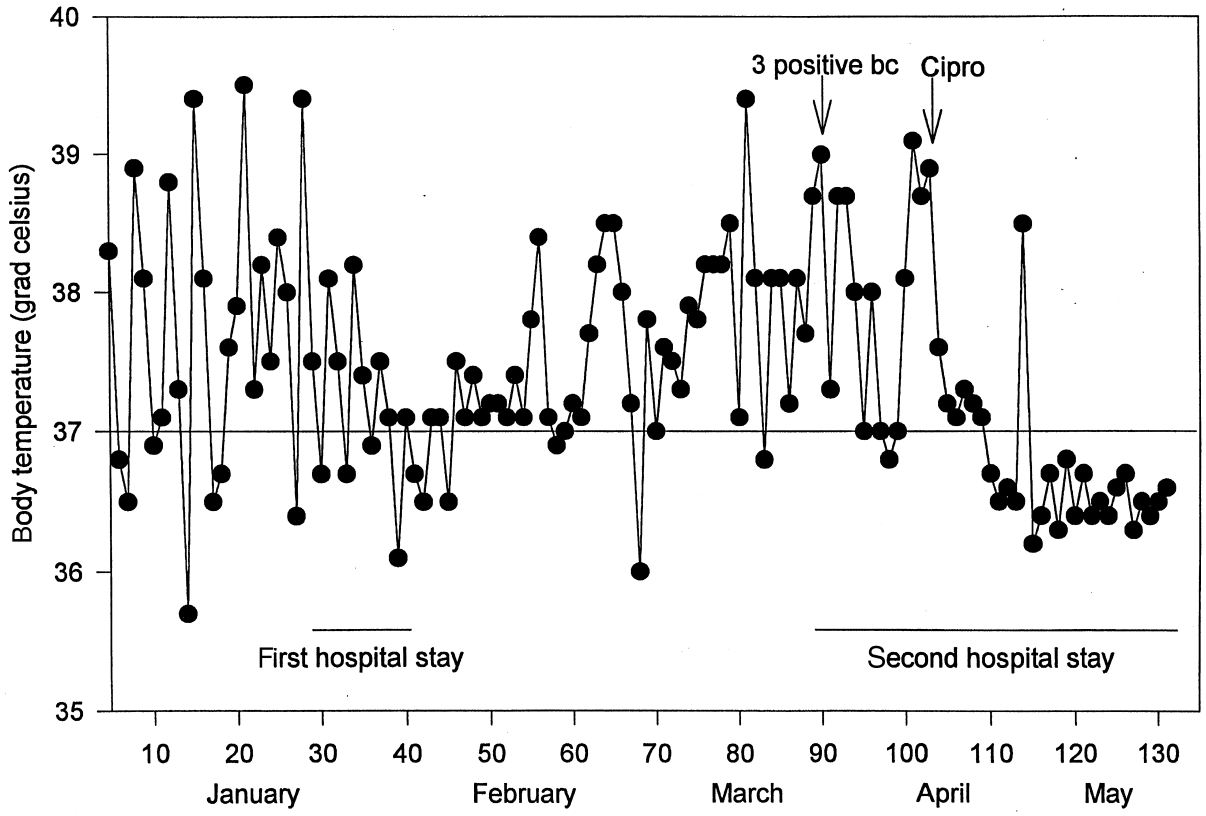

Figure 1 Course of fever between January and May. Time period of the first and second hospital stay is indicated, as is the time point when the three blood cultures became positive (3 positive bc), as well as the start of treatment with ciprofloxacin (cipro) after diagnosis of the causitive pathogen.

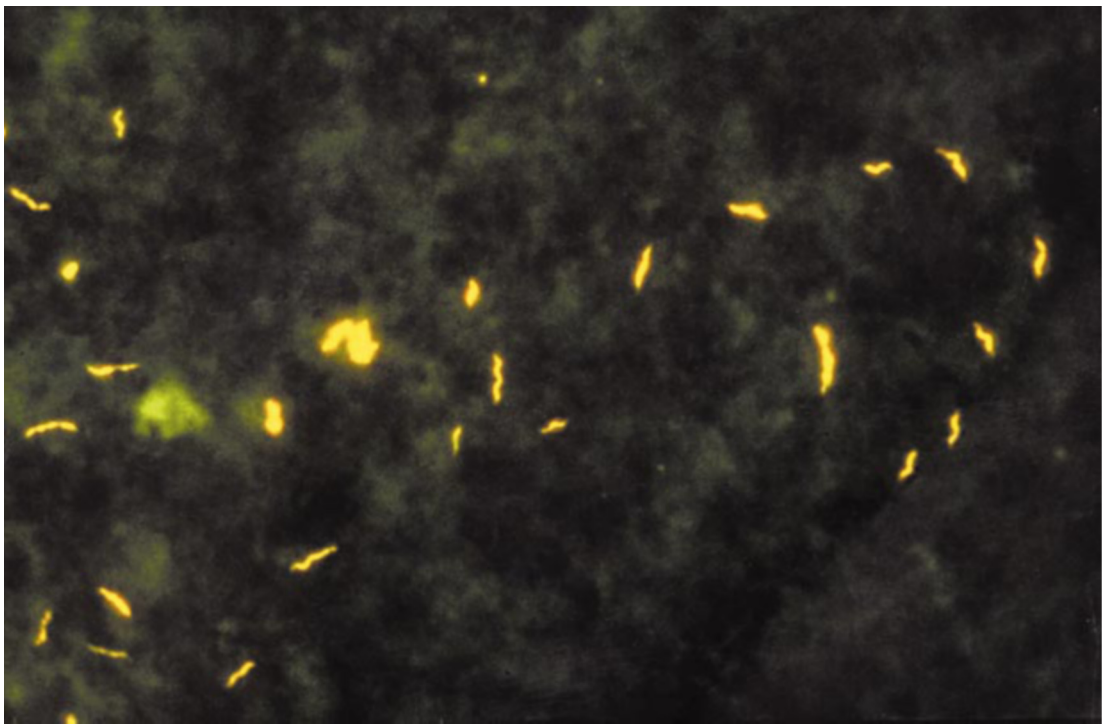

Figure 2 Acridine orange stain on a positive blood culture taken 3 days after the patient was admitted for the second time.

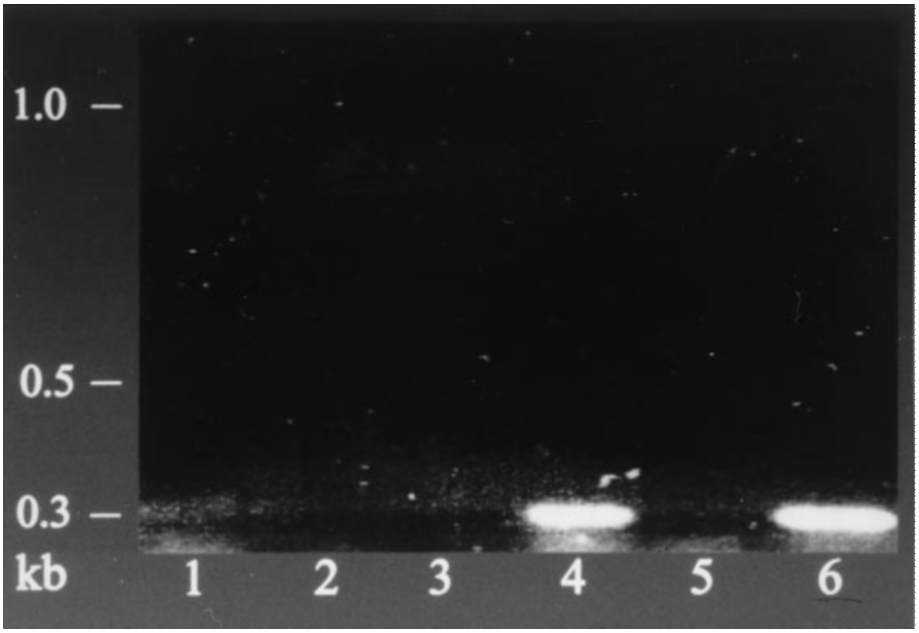

Figure 3 PCR of a 292-bp fragment of the 16s rRNA gene: using a universal (forward) and a *Helicobacter*-specific (reverse) primer PCR was performed with genomic DNA of *Escherichia coli* (lane 1), *Campylobacter jejuni* (lane 2), *Campylobacter coli* (lane 3), *Helicobacter pylori* (lane 4), no DNA (lane 5) and the patient's isolate (lane 6).

Originally reported from homosexual men with proctitis in 1985, systemic disease in HIV patients caused by *H. cinaedi* was first recognized some 5 years ago [3–5]. Its frequency, however, remains underestimated. The organism is typically isolated from blood cultures. Although *H. cinaedi* is clearly Gram-negative, organisms may be missed on Gram stains of blood cultures because of high background. Thus an acridine orange stain on positive blood culture bottles, in which no organisms can be found on Gram stain, should be implemented as a standard procedure in all clinical laboratories using an automated blood culture system. Rapid species identification of *H. cinaedi* can be achieved using amplification and sequencing of a 292-bp product of the 16 S rRNA gene.

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