Arthritis Caused by *Haemophilus paraphrophilus* and Isolation of the Organism by Using an Improved Culture Protocol

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A case of bacterial arthritis caused by *Haemophilus paraphrophilus* is presented. This is the first reported case of arthritis caused by this organism. Culturing joint fluid in blood culture bottles provides for bacteria that are slow growing or present in small numbers.

*Haemophilus influenza* is a main cause of bacterial arthritis in early childhood (13, 20), and it has some importance in the etiology of purulent arthritis in adults (7). It has been suggested that this organism may not always be easy to recover from synovial fluid (SF), since the proportion of bacteriologically confirmed purulent arthritis is greatest precisely in that same age group (6 months to 4 years) (2, 16). In fact, small gram-negative rods have occasionally been observed on direct staining of culture-negative SF samples (4). In contrast, with the exception of two isolations of *H. parainfluenza* and one of *H. paraphrophilus* from SF (3, 11, 12), the more reticent members of the genus have not so far been implicated in the etiology of purulent arthritis (1). In this paper, we show that *H. paraphrophilus* also can cause purulent arthritis, and we propose the possibility that a reason that this event has not been described before could be that standard culture methods for SF specimens make insufficient provisions for the particular atmospheric demands and slow rate of growth of *H. paraphrophilus*.

The patient was a 15-year-old boy who had previously been healthy. On 8 June 1986, he visited a general practitioner because his left knee had been swollen and painful for 2 days. He had no symptoms or signs of infectious disease, and there was no preceding trauma to the knee. A large amount of clear, thin, yellowish fluid was aspirated. The protein concentration was 45 g/liter; routine agar plate bacterial cultures were negative; direct staining and leukocyte count were not done. The following parameters were determined: peripheral leukocyte count, 7,400/mm³; C-reactive protein (CRP), 98 mg/liter; erythrocyte sedimentation rate (ESR), 61 mm/h; and rheumatoid factor, negative. He was treated with anti-inflammatory drugs.

Five days later, the knee swelling recurred, and the patient was seen as an outpatient at Rheumatism Foundation Hospital. He had no fever or other symptoms except restriction of movement of the swollen left knee. Arthrocentesis yielded 110 ml of turbid, greenish SF. The SF protein content was 61 g/liter, and the leukocyte count was 63,600/mm³ with 71% polymorphonuclear neutrophils. Radiologically, the knee was normal. The following parameters were determined: peripheral leukocyte count, 8,300/mm³; CRP, 85 mg/liter; ESR, 81 mm/h; and rheumatoid factor, negative. The knee was immobilized, and the anti-inflammatory treatment was continued.

Staining of the SF sample showed fairly abundant leukocytes but no bacteria. The sample was cultured aerobically on sheep blood agar and chocolate agar (Gibco Ltd, Paisley, Scotland) incubated in 5% CO₂ at 35°C and cultured anaerobically on brucella blood agar (Tammer-Tutka, Tampere, Finland) incubated in a GasPak jar (BBL Microbiology Systems, Cockeysville, Md.). Plates were discarded as negative after 3 days. In addition, SF was injected in bulk into blood culture bottles (Hemobact Aerobe and Hemobact Anaerobe, Orion Diagnostica, Espoo, Finland) as previously described (19). Subcultures of the broth on solid media were performed as described above for primary SF culture on the day after inoculation and after 1 week. The first subcultures were negative too. However, the second subcultures, performed on day 6, 4 days later, yielded growth of a small gram-negative rod from both bottles. On first passage, it was completely CO₂ dependent and grew aerobically only on chocolate agar; later the absolute CO₂ dependency was lost. It was identified biochemically as *H. paraphrophilus* (8) by the RapID NH system (Innovative Diagnostic Systems, Inc., Atlanta, Ga.) and supplementary reactions from various API panels (API System S.A., Montalieu Vercieu, France). The isolate was nonhemolytic, oxidase positive, and catalase negative. It displayed satellite growth and was V-factor dependent but produced porphyrin and was X-factor independent (8). It fermented glucose, lactose, and sucrose. It was negative for ornithine, lysine, arginine decarboxylase, and urease, and indole was not produced. The identification was subsequently confirmed by R. E. Weaver, Clinical Laboratory Branch, Centers for Disease Control, Atlanta, Ga. The organism was susceptible to cephahlin, cefuroxime, cefotaxime, ceftazidime, ampicillin, piperacillin, tetracycline, and chloramphenicol but was resistant to erythromycin, clindamycin, and vancomycin by disk diffusion assay.

When bacterial growth was detected, the patient was called to Lahti Central Hospital (June 23). The left knee was still swollen and the patient was subfebrile. The following parameters were determined: peripheral leukocyte count, 6,700/mm³; neutrophilia, none; CRP, 73 mg/liter; ESR, 92 mm/h; ASO-titer, 600 IU; and rheumatoid factor and antinuclear antibodies, as well as titers of antibodies against *Salmonella*, *Yersinia*, and *Campylobacter* antigens and *Neisseria gonorrhoeae* antigens, negative. The initial antibiotic treatment was intravenous cefuroxime (750 mg every 8 h) in accordance with the sensitivity results. The knee was also treated for 5 days with daily lavations supplemented by

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intra-articular instillation of neomycin and bacitracin. After 6 days on cefuroxime, oral cephalixin (500 mg every 8 h) for 4 weeks was substituted. The patient was discharged after 10 days with the left knee still slightly swollen (ESR, 36 mm/h; and CRP, <10 mg/liter). Six months later, he was completely well, and all laboratory values were within the normal range.

H. paraphrophilus is by no means a rare microbe: in the throat flora it is about as prevalent as H. influenzae (10), and it can also be isolated from dental plaques (9). Conversely, there is no doubt that the isolation of H. paraphrophilus from blood, cerebrospinal fluid, or SF is indeed a rare event (1). In a Swedish survey of infections caused by Haemophilus species other than H. influenzae, none was isolated in 13 years in 2 districts with a combined population of 817,900 inhabitants (18). But since the culture conditions employed are not provided, it is not possible to unconditionally equate lack of recovery with absence of the microbe. By the supplementary use of blood culture bottles, we have on two occasions been able to isolate slow-growing and fastidious organisms from SF in spite of negative results by conventional methods of isolation in previous attempts (5, 15). Also, 20% of ordinary pathogens would have been missed without this strategy, and 40% of SF specimens from patients on antibiotics would have been false-negatives (19).

Isolations of the less well-known Haemophilus species from throat swab specimens have mostly been accomplished by methods designed specifically to provide ideal growth conditions for them. Protocols for the optimal isolation of a priori-specified pathogens (e.g., Campylobacter sp.) have been developed and refined in recent years. In contrast, progress in the field of general isolation system improvement has been largely confined to blood cultures. Thus far, we have only adapted the basic principles from that field for SF culture, i.e., the use of a large volume of inoculum to account for a potentially low number of infective units, a large volume of rich medium under a suitable atmosphere to dilute out potential growth inhibitory and permit growth of fastidious organisms, and repeated subcultures to make provision for slow-growing ones. Improvement of ascites fluid culture results by the same strategy has been reported recently (14). Removal of antibiotics (6) and lysing of leukocytes to liberate intracellular organisms (17) might improve the SF isolation rate still further.

Finally, the questions must be asked whether our isolate might be simply a contaminant, and whether the arthritis of the patient was not, after all, of infectious origin. Since no bacteria were found by direct staining, and we have no corroborating serological data to offer, that possibility is difficult to rule out with complete confidence. But we think it can be ruled out for three reasons. First, while common in throat flora, H. paraphrophilus does not inhabit the skin. Second, no reference strain of the bacterium was kept in the laboratory at the time of the isolation. Last, if contamination by respiratory secretions had occurred, it would be unexpected that this fastidious and slow-growing organism would be the only one to appear. Since the clinical events are compatible with a diagnosis of arthritis caused by a Haemophilus sp., we consider our isolate to be genuine and believe that it confirms our point that careful culture of SF samples occasionally brings unexpected rewards for both clinicians and microbiologists.

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LITERATURE CITED