JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1988, p. 584–585
0095-1137/88/030584-02$02.00/0

Vol. 26, No. 3

Yersinia pseudotuberculosis Infection Contracted through Water Contaminated by a Wild Animal

HIROSHI FUKUSHIMA,[1]* MANABU GOMYODA,[1] KANJI SHIOZAWA,[2] SEIJI KANEKO,[3] AND MISAO TSUBOKURA[4]

Public Health Institute of Shimane Prefecture, Nishihamasada, Matsue, Shimane 690-01,[1] Shizuoka Prefectural Institute of Public Health and Environmental Science, 4-27-2 Kita-ando, Shizuoka 420,[2] Tokyo Metropolitan Research Laboratory of Public Health, 3-24-1 Hyakuninchou, Shinjuku-ku, Tokyo 160,[3] and Department of Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori 680,[4] Japan

Received 11 August 1987/Accepted 4 December 1987

We performed epidemiological studies on *Yersinia pseudotuberculosis* in one valley where a 3-year-old boy had been infected with *Y. pseudotuberculosis* serotype 4b in December 1982. *Y. pseudotuberculosis* serotype 4b was isolated from a water sample derived from a mountain stream from which the boy had drunk and from 1 of 41 rats trapped in the upper part of this stream in December 1986. The restriction endonuclease patterns of the plasmids in these isolates showed the rat and patient isolates to be identical but distinct from the water isolate. These data suggest the potential for transmission of *Y. pseudotuberculosis* through water contaminated by nondomesticated animals carrying this species.

Yersinia pseudotuberculosis causes sporadic and epidemic infection in humans and has a wide distribution in both wild and domestic animals. Rodents and birds are natural reservoirs (9). We reported that the main source of infection to humans may be mountain water contaminated by wild animals (3). Although there are reports on the isolation of this bacteria from well water (1), rats (6), and hares (11), the link between wild animals harboring the bacteria, contamination of water, and human infection in the ecology of *Y. pseudotuberculosis* is unknown. We have now obtained strong evidence for the transmission of the organism to a child who drank water from a mountain stream containing trapped animals.

A 3-year-old boy was found to be infected with *Y. pseudotuberculosis* serotype 4b on 13 December 1982. There were 10^6 *Y. pseudotuberculosis* serotype 4b cells per g of feces on the clinical day 8 of infection. The clinical manifestations were fever (39°C), abdominal pain, diarrhea, rash, pharyngitis, and joint pain. However, no one in his family was infected with this organism in 1982 or 1986. From October 1986 to May 1987 in Hirata City, Shimane, Japan, 40 samples of water were collected every week from a water tank supplied by a mountain stream. A total of 46 small wild animals, including 41 rats (23 *Apodemus speciosus*, 15 *Apodemus argenteus*, and 3 *Microtus montebelli*) and 5 moles (*Urotrichus talpoides*) were trapped in the upper part of this stream. The stomach, duodenum, jejunum, ileum, cecum, colon, rectum, and mesenteric lymph nodes from each animal were isolated. The number of *Yersinia* cells in the water samples and the cecal contents of these animals was determined (in CFU) at 32°C for 24 and 48 h, using plates of VYE agar (2) and Irgasan-novobiocin agar, in which 2.5 mg of novobiocin was added to 1 liter of the yersinia selective agar base (Difco Laboratories, Detroit, Mich.). A 1-liter water sample was passed through a membrane filter (45-μm pore size), and this filter was suspended in 5 ml of PMP broth (4). A 0.5-ml sample of the suspension was mixed with 0.5 ml of 0.72% KOH in 0.5% NaCl for 30 s, and 0.1 ml of this was spread onto Irgasan-novobiocin and VYE agar plates. The remaining water suspension and suspensions of intestinal contents in 0.067 M phosphate buffer solution (10 ml) and of mesenteric lymph nodes in PMP broth (10 ml) were incubated at 4°C for 3 weeks. Then the suspensions were subcultured on Irgasan-novobiocin and VYE agar plates, using KOH after-enrichment treatment. Identification of the isolates was as described previously (11).

Y. pseudotuberculosis serotype 4b was isolated from one sample of water collected on 15 December 1986 and from the jejunum, ileum, cecum, colon, rectum, and mesenteric lymph nodes of one (4.3%) *A. speciosus* trapped on 26 December 1986. These organisms were found at a concentration of 1 cell per 10 ml of water and 5×10^4 cells per g of cecal contents from the rat. Serotype 5a was isolated from the cecum of one (33.3%) *M. montebelli* and serotype 6 was isolated from the mesenteric lymph nodes of one (20.0%) *U. talpoides* on 9 February 1987 by postenrichment culture. These four isolates and the isolate from the 3-year-old boy were tested for melibiose fermentation. Autoagglutination at 37°C was determined by the method of Laird and Cavanaugh (8), modified by using tryptic soy broth. Pyrazinamidase activity was determined by the method of Kandolo and Wauters (5). The presence of plasmid DNA was determined by the method by Kaneko and Maruyama (7). The three serotype 4b strains showed melibiose fermentation, positive reactions of virulence-associated phenotypic markers such as autoagglutination and pyrazinamidase, and the presence of a 42-megadalton plasmid. Serotype 5a and 6 strains were melibiose nonfermenting, showed negative autoagglutination and pyrazinamidase reactions, and lacked the virulence plasmid. Serotype 6, however, harbored two different-sized plasmids of 40 and 50 megadaltons. This study may be the first evidence that the same serotype of *Y. pseudotuberculosis* was present in a patient, in drinking water, and in a wild rat trapped at the same point in the same season (December) and that melibiose-nonfermenting avirulent *Y. pseudotuberculosis* strains are present in wild animals.

Although a serological study of isolates suggested that *Y. pseudotuberculosis* is generally transmitted to humans via water contaminated by bacteria in feces from infected wild

* Corresponding author.

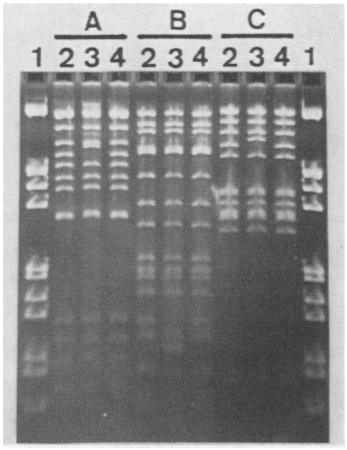

FIG. 1. *Bam*HI (A), *Hin*dIII (B), and *Eco*RI (C) digestion patterns of plasmid DNA from strains of *Y. pseudotuberculosis* serotype 4b isolated from a patient, water, and a rat. Lanes: 1, molecular size markers (lambda DNA cut with *Eco*RI plus *Hin*dIII) (from top to bottom, 13.8, 3.35, 3.23, 2.78, 2.29, 1.31, 1.24, 1.03, 0.89, 0.62, 0.54, and 0.37 megadaltons); 2, Pa1345 (patient); 3, W134 (water); 4, R148 (rat).

animals such as rats and hares, the plasmid analysis data do not entirely solidify the link between the rat and patient isolates. Figure 1 shows a comparison among the restriction endonuclease patterns of the plasmids isolated from three strains of serotype 4b. Plasmid DNA isolated from serotype 4b was digested with restriction endonucleases *Bam*HI, *Hin*dIII, and *Eco*RI and subjected to 0.7% agarose gel electrophoresis (10). The plasmid DNA from the isolates from the boy and the rat gave a similar restriction fragment pattern, but the plasmid DNA from isolate from the water gave one fragment of distinct size in each restriction fragment pattern obtained by *Bam*HI, *Hin*dIII, and *Eco*RI digestion. Although the isolate from the patient was obtained in a different year from that in which the water and rat isolates were obtained, the identical plasmid profile of the rat and patient isolates strongly suggests the potential for transmission through water contaminated by a wild animal. The distinctly different plasmid profiles of the rat isolate and the water isolate suggest that the source of the contamination is other wild animals, including rats, rather than water. The different plasmid profiles of *Y. pseudotuberculosis* serotype 4b may be pertinent for a future epidemiological study.

We thank M. Ohara, Kyushu University, for reading the manuscript.

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