JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1994, p. 721–724
0095-1137/94/$04.00+0

Vol. 32, No. 3

Campylobacter fetus Diarrhea in a Hutterite Colony: Epidemiological Observations and Typing of the Causative Organism

R. P. RENNIE,[1]* D. STRONG,[2] D. E. TAYLOR,[1] S. M. SALAMA,[1] C. DAVIDSON,[3] AND H. TABOR[3]

Departments of Medical Microbiology and Infectious Diseases, University of Alberta and University of Alberta Hospitals,[1] Epidemiology and Communicable Diseases, Division of Public Health, Alberta Health,[2] and Provincial Laboratory of Public Health,[3] Edmonton, Alberta, Canada

Received 2 September 1993/Returned for modification 11 October 1993/Accepted 20 December 1993

Following a case of *Campylobacter fetus* sepsis and meningitis in a 4-month-old female member of a Hutterite colony, an epidemiological investigation revealed at least 18 cases of diarrhea in other members of the colony. *C. fetus* was isolated from 7 of 15 fecal samples submitted from affected persons. A case control study suggested that persons who worked in the abattoir were 2.03 times more likely to have had diarrhea, but none of the risk factors studied were significant. The epicurve of the outbreak was inconclusive as to the likely mode of spread of *C. fetus*. All of the *C. fetus* strains isolated from the blood of the infant and from the fecal samples were the same by biochemical and antibiotic susceptibility tests. Pulsed-field gel electrophoresis showed that all isolates produced identical restriction endonuclease patterns and differed from other nonepidemiologically related strains of *C. fetus*.

Campylobacter fetus, the type species of the genus *Campylobacter*, is a well-known cause of abortion and sterility in domestic cattle. The first reported human case occurred in 1947 when a spontaneous abortion in a pregnant female was attributed to a *C. fetus* bacteremia (24). *C. fetus* diarrhea was not consistently reported until the early 1970s, when better selective media and culture techniques were developed (4, 6).

C. fetus infection during pregnancy presents most often with diarrhea in the mother followed by a relatively rapid onset of sepsis and/or meningitis in the neonate 12 to 24 h postpartum (3, 7, 13, 16, 23). Diarrhea caused by *C. fetus* in young infants and children is not usually documented.

While outbreaks of diarrhea associated with *C. jejuni* are well known to occur at rates equivalent to or greater than *Salmonella* infections (20), outbreaks of *C. fetus* diarrhea are rare. Klein et al. (12) reported a mixed outbreak of *C. jejuni* and *C. fetus* diarrhea following consumption of raw milk at a common meal. Although the milk from the meal was not cultured, it was epidemiologically associated with the outbreak. Since laboratory methods have made it possible to clearly differentiate between *C. fetus* and what used to be called *C. fetus* subsp. *jejuni* (21), most laboratories should be capable of isolating *C. fetus* from feces by using any of the commonly employed *Campylobacter* isolation media—for example, Skirrow's, Preston blood-free, or *Campylobacter* Selective Medium (4, 8, 11, 18, 22)—unless incubation of samples is performed only at 42°C, since *C. fetus* grows poorly or not at all at this elevated temperature.

We report here an investigation of an outbreak of *C. fetus* diarrhea in a Hutterite colony in Alberta. The investigation followed the identification of a case of sepsis with signs of meningitis in a 4-month-old female member of the colony. Identification and characterization of the causative organism were performed by standard techniques and pulsed-field gel electrophoresis (PFGE).

* Corresponding author. Mailing address: Clinical Microbiology, University of Alberta Hospitals, WMC 2B3.08, 8440 112 St., Edmonton, Alberta, Canada T6G 2B7. Phone: (403) 492-4461. Fax: (403) 492-3864.

MATERIALS AND METHODS

Outbreak investigation. In October 1992, a 4-month-old female member of a Hutterite colony presented to a regional hospital in central Alberta with signs and symptoms of sepsis with possible meningitis. She was born prematurely at 29 weeks gestation, was managed initially for respiratory difficulties and feeding problems, and was diagnosed shortly after birth with a possible allergy to cow's milk which was alleviated by feeding a commercial soy-based milk preparation. She remained in reasonable health until an episode of diarrhea which rapidly progressed to her clinical presentation of sepsis. A cerebrospinal fluid collected at the regional hospital had 780 leukocytes (72% polymorphonuclear leukocytes) with a glucose level of 3.6 mmol/liter and a protein level of 2.3 g/liter; there was no growth on culture. An organism resembling *C. fetus* was isolated from blood cultures taken at the regional hospital and incubated at 35°C; feces were not cultured. The infant was started on cefotaxime and gentamicin and was subsequently transferred to the University of Alberta Hospitals, where these antibiotics were continued for a total of 3 weeks. Additional management for nutritional rickets and chronic bilateral lung changes was instituted, and the child was discharged home early in November.

An outbreak investigation was initiated by one of us (D.S.) following the isolation of *C. fetus* from the blood of the infant, together with the reporting of several cases of diarrhea in the Hutterite colony. Additional cases were identified following interviews of colony leaders and members of the hospitalized infant's family. The various facilities and food preparation methods were examined, and feces, raw milk, and cheese were collected. A case control study using a self-administered questionnaire was initiated to search for possible sources of the outbreak. A case was defined as any instance of loose watery stools, abdominal cramps, or vomiting in an individual in September and October. Any colony member not meeting the

case definition was used as a control. The responses for all cases and controls were used to calculate relative risk ratios for each of the risk factors studied. Data were analyzed statistically with Epi Info (version 5.01b; U.S. Department of Health and Human Services).

Isolation and identification of the organisms. The initial blood culture isolate was referred to the Provincial Laboratory of Public Health, Edmonton, for identification. Submitted specimens of feces were cultured on modified charcoal cefoperazone deoxycholate agar (4) and incubated microaerophically at 35°C for 5 days. Samples of raw milk and soft cheese were processed by the environmental microbiology section of the Provincial Laboratory by standard methods (14) and were cultured on the same *Campylobacter* isolation medium. Isolates with typical greyish colonies were identified as *C. fetus* by the following procedures: Gram stain, oxidase using the tetramethyl indophenyl oxidase reagent (9), catalase, motility, hydrogen sulfide (H_2S) in triple sugar iron agar with lead acetate strips (22), hippurate hydrolysis (10), indoxyl acetate reactivity, growth on media containing 1% glycine or 1.5% NaCl, and the ability to grow at 25 and 35°C and poorly or not at all at 42°C (18).

Antimicrobial susceptibilities were performed by disk diffusion and by broth microdilution in Mueller-Hinton medium under microaerophilic conditions at 35°C for 48 h (2, 15). The isolates were tested against gentamicin, tobramycin, cefotaxime, chloramphenicol, erythromycin, imipenem, ciprofloxacin, cephalothin, cefuroxime, ceftazidime, and nalidixic acid.

PFGE. Overnight cultures of bacterial cells of the clinical isolates of *C. fetus* (UA1002, 1070 to 1075, and 1076), five unrelated *C. fetus* strains (UA777, 778, 779, 881, and 882) and the type strain UA60 (ATCC 27374) were harvested and suspended in 1 to 2 ml of 10 mm Tris-EDTA (TE) (British Drug Houses, Vancouver, British Columbia, Canada) to give a McFarland no. 8 turbidity. Low-melting-point agarose blocks were prepared as previously described (25).

DNA inserts of 1- to 2-mm thickness were cut and washed three times in TE buffer containing 1 mM phenylmethylsulfonyl fluoride (Sigma, St. Louis, Mo.) and then washed three times for 15 min each time in TE buffer only. Before enzyme digestion, the TE buffer was removed and replaced by 100 μl of a fresh preparation of the appropriate digestion buffer and DNA inserts were incubated at room temperature for 30 min, after which the buffer was replaced with 100 μl of fresh digestion buffer and 10 to 20 U of the restriction endonuclease was added. The mixture was incubated at 37°C for 18 to 24 h. The restriction endonucleases *Sal*I and *Sma*I (Boehringer GmbH, Mannheim, Germany) were used. After incubation, the DNA inserts were washed in TE buffer and loaded into 1% agarose (GIBCO BRL, Gaithersburg, Md.) gel wells, and the contour-clamped homogeneous electric field system (LKB Instruments, Brauma, Sweden) of PFGE was used to separate the DNA fragments (25).

RESULTS

Outbreak investigation. Preliminary observations showed that all meals for the colony were prepared in a single kitchen. Cooks and helpers were assigned on a weekly basis, and none had been ill before or during the outbreak. Adult members ate meals in a communal dining area with males and females seated in a fixed arrangement on opposite sides of the room. All but one table had at least one adult ill with diarrhea during September or October.

Younger children ate in an adjacent room except for the 4-month-old infant with septicemia and meningitis, who was cared for by her mother and grandmother in a communal home. The infant was fed exclusively with commercial formula. Each of four communal homes in the colony had at least three persons ill during September or October.

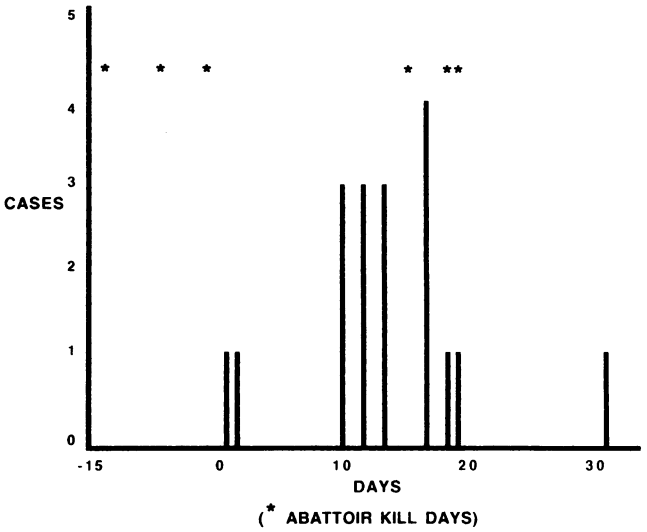

FIG. 1. Epicurve of diarrhea onset in members of the Hutterite colony. The three kill days prior to the first case of diarrhea were 4, 9, and 16 September, Day 0 (the date of onset of the first case of diarrhea) is 18 September.

Members of the colony drank raw milk from their own dairy cows. This milk was used also to make soft cheese and yogurt for use only in the colony. Raw cheese had been consumed intermittently during August and September.

Unchlorinated drinking water was obtained from a well located on high ground; regular testing had not shown any contamination. Dairy and chicken barns were located several hundred feet from the well.

Chickens, ducks, pigs, and cattle raised on the colony were used locally and for commercial sale. Both men and women participated in the slaughtering process, and all were at risk for fecal contamination. The kitchens and slaughterhouse were clean when examined, and all refrigerators and freezers were at proper cooling temperatures.

The case control questionnaire identified 18 of 90 members of the colony having diarrhea and other associated symptoms in September or October. Fever was observed in nine persons, cramps and general malaise were observed in eight persons, nausea was observed in three persons, and vomiting was observed in two persons. None of the control individuals reported any of these symptoms. The mean age was 21.8 years for cases and 21.3 years for controls. There was no gender difference between cases and controls ($\chi^2 = 1.1, P < 0.3$). The relative risk factors examined included consumption of raw milk and soft cheese made from the milk and work in the abattoir. Persons who worked in the abattoir were 2.03 times more likely to have met the case definition, but none of the risk factors investigated produced a statistically significant relative risk.

The epicurve for ill persons and the kill dates in the abattoir are shown in Fig. 1. The first two individuals who reported having diarrhea on 18 and 19 September (Fig. 1, Day 0) were negative for *C. fetus* when their stools were cultured. The mother of the infant with septicemia reported having diarrhea on 1 October, 8 days before the onset of illness in the infant.

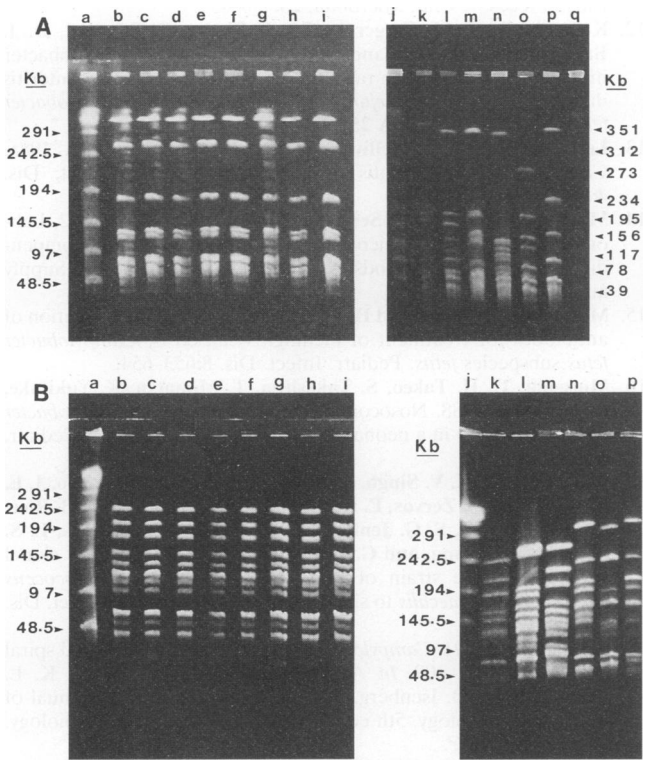

FIG. 2. PFGE of the chromosomal DNA of the clinical isolates and a set of control *C. fetus* strains digested with the restriction endonucleases *Sma*I (A) and *Sal*I (B). These gels were subjected to electrophoresis for 24 h and 175 V at 8°C with pulse times of 20 to 30 s in a 1% agarose gel. Bacteriophage concatemers (lanes a, j, and q) were used to determine the fragment sizes. Lanes b to i, clinical isolates (b, UA1002; c, UA1070; d, UA1071; e, UA1072; f, UA1073; g, UA1074; h, UA1075; and i, UA1076). Lanes k to p, control strains (k, UA777; l, UA778; m, UA779; n, UA781; o, UA782; and p, UA60 [ATCC 27374].)

Identification and typing of the organism. A total of 15 feces samples were submitted from the 18 persons who reported having diarrhea during September or October; 7 of these grew *C. fetus* in addition to the blood culture isolate from the infant. Feces samples were not obtained from persons not reporting diarrhea, and blood cultures were not collected from any members of the colony other than the septicemic infant because of the retrospective nature of the outbreak investigation. *C. fetus* was not isolated from any of the other samples submitted for culture, including the raw milk and cheese. The isolates were motile, gram-negative curved bacilli, which were oxidase and catalase positive, H_2S and hippurate negative, and indoxyl acetate positive; they all grew on media containing 1% glycine but not 1.5% NaCl, and all isolates grew well at 25 and 35°C but poorly at 42°C. All isolates were susceptible to gentamicin, tobramycin, cefotaxime, imipenem, chloramphenicol, erythromycin, and ciprofloxacin and resistant to cephalothin, cefuroxime, ceftazidime, and nalidixic acid.

All eight isolates produced identical PFGE patterns after digestion with restriction endonucleases *Sma*I and *Sal*I (Fig. 2). DNA of the type strain ATCC 27374 produced a pattern identical to that of the clinical isolates when digested with *Sma*I; however, when the DNA was digested with *Sal*I the pattern was different. DNA of other *C. fetus* control strains produced different patterns for different enzymes. The size of the genomic DNA of the clinical isolates was determined to be 1,130 kb.

DISCUSSION

This outbreak might have gone unrecognized but for the case of septicemia and meningitis in the 4-month-old infant which did not fit the earlier described pattern of *C. fetus* invasive infection; that is, an early presentation of illness in the neonate, usually within 24 h of delivery (3, 7, 16).

The outbreak investigation showed that while cleanliness was clearly followed in the colony, such practices as the consumption of raw milk and milk products or work in an abattoir may present risks to members of the group, although these risks were not statistically significant in the current study.

Information from the case control investigation suggested that the infant may have acquired the *C. fetus* secondarily from a caregiver (i.e., her mother). The infant had only received commercial formula since shortly after birth. The mother was the first culture-positive fecal case. Although the source of her infection was not identified, she may have acquired the organism following her participation in slaughtering on 9 September. However, she also drank raw milk and ate cheese with most meals. The first two persons who met the case definition, but who were culture negative, also drank milk, ate cheese, and helped slaughter animals on 9 September. Since the investigation was not initiated until the end of October, it is not surprising that some ill persons may have cleared the *C. fetus* from their gastrointestinal tracts by the time the samples were collected. It is of interest, however, that seven persons still carried the organism in their intestinal tract for at least 4 to 6 weeks.

The failure to find a statistically significant relative risk factor may be due to the small number of cases. None of the risk factors studied could account for more than 61% of the cases. The prolonged duration of the outbreak does not indicate a single point source, as the epicurve is more consistent with a continuing source of the organism. It is possible that at least some cases resulted from the consumption of raw milk or cheese or from working in the abattoir. Evidence for all these modes of transmission were lacking, in part because it was difficult to quantify the risk of direct exposure to fecal material during slaughtering or to consumption of contaminated milk. *C. fetus* has rarely been isolated from milk or cheese, especially when the epidemiological investigation was conducted so long after the outbreak apparently began (12).

The identification and susceptibility of these strains are consistent with previous reports of *C. fetus* strains. Although it is often suggested that this species does not grow at 42°C, in at least two cases (the outbreak described here and the one described in reference 3) growth at 42°C, although poor, was evident. Strains in the current study appear to be different from those identified by Klein et al. (12). Those authors observed that the *C. fetus* isolates from affected persons were susceptible to cephalothin, whereas our isolates were all resistant to cephalothin, which is the pattern usually attributed to *C. fetus*. Also, they reported that their isolates grew well at 42°C, whereas our isolates grew poorly at the elevated temperature. We are unable to reconcile these differences.

PFGE has been used for epidemiological separation of bacterial strains (1, 17, 25) and was successfully applied here to differentiate the outbreak organisms from other *C. fetus* strains (19). It was necessary to use more than one enzyme to show clearly that all these strains, including the isolate from the case of septicemia and meningitis, were the same as each other but different from other strains of *C. fetus*. In addition, the DNA

genome size (i.e., 1,130 kb) of all the clinical isolates was within previously published values for *C. fetus* (19). This differs from that for *C. jejuni* and *C. coli* (5, 19, 25).

ACKNOWLEDGMENTS

This study was supported in part by funding from the Canadian Bacterial Diseases Network (Canadian Networks of Centres of Excellence Program). D.E.T. is a Medical Scientist of the Alberta Heritage Foundation for Medical Research.

We thank W. L. Albritton for constructive criticism of the manuscript.

REFERENCES

1. **Anderson, D. J., J. S. Kuhns, M. L. Vasil, D. N. Gerding, and E. N. Janoff.** 1991. DNA fingerprinting by pulsed field gel electrophoresis and ribotyping to distinguish *Pseudomonas cepacia* isolates from a nosocomial outbreak. J. Clin. Microbiol. **29:**648–649.
2. **Bauer, A. W., M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. **45:**493–496.
3. **Bingham, W. T., A. Chan, R. Rennie, and K. E. Williams.** 1992. Neonatal *Campylobacter fetus* meningitis: a report of an unusual case. Clin. Pediatr. **31:**255–256.
4. **Bolton, F. J., D. N. Hutchinson, and D. Coates.** 1984. Blood-free selective medium for the isolation of *Campylobacter jejuni* from feces. J. Clin. Microbiol. **19:**169–171.
5. **Chang, N., and D. E. Taylor.** 1990. Use of pulsed-field agarose gel electrophoresis to size genomes of *Campylobacter* species and to construct a *Sal*I map of *Campylobacter jejuni* UA580. J. Bacteriol. **172:**5211–5217.
6. **Dekeyser, P., M. Gossuin-Detrain, J. P. Butzler, and J. Sternon.** 1972. Acute enteritis due to related vibrios: first positive stool cultures. J. Infect. Dis. **125:**390–392.
7. **Forbes, J. C., and D. W. Scheifele.** 1987. Early onset campylobacter sepsis in a neonate. Pediatr. Infect. Dis. **6:**494.
8. **Gun-Munro, J., R. P. Rennie, J. H. Thornley, H. L. Richardson, D. Hodge, and J. Lynch.** 1987. Laboratory and clinical evaluation of isolation media for *Campylobacter jejuni*. J. Clin. Microbiol. **25:** 2274–2277.
9. **Hendrickson, D. A., and M. M. Krenz.** 1991. Reagents and stains, p. 1289–1314. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
10. **Hwang, M. N., and G. M. Ederer.** 1975. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. J. Clin. Microbiol. **1:**114–115.
11. **Karmali, M. A., A. E. Simor, M. Roscoe, P. C. Fleming, S. S. Smith, and J. Lane.** 1986. Evaluation of a blood-free, charcoal based, selective medium for the isolation of *Campylobacter* organisms in feces. J. Clin. Microbiol. **23:**456–459.
12. **Klein, B. S., J. M. Vergeront, M. J. Blaser, P. Edmond, D. J. Brenner, D. Janssen, and J. P. Davis.** 1986. Campylobacter infection associated with raw milk. An outbreak of gastroenteritis due to *Campylobacter jejuni* and thermotolerant *Campylobacter fetus* subsp. *fetus*. JAMA **255:**361–364.
13. **Lee, M. M., R. C. Nelliver, and L. J. La Scolea, Jr.** 1985. Campylobacter meningitis in childhood. Pediatr. Infect. Dis. **4:**544–547.
14. **Minister of Supply and Services, Canada.** September 1992. Laboratory procedure of microbiological analysis of food. Compendium of analytical methods, vol. 3, MFLP 46. Ministry of Supply and Services, Canada.
15. **Morooka, T., T. Oda, and H. Shigeoka.** 1989. In vitro evaluation of antibiotics for treatment of meningitis caused by *Campylobacter fetus* subspecies *fetus*. Pediatr. Infect. Dis. **8:**653–654.
16. **Morooka, T., H. Takeo, S. Takeshita, T. Mimatsu, K. Yukitake, and T. Oda.** 1988. Nosocomial meningitis due to *Campylobacter fetus* subsp. *fetus* in a neonatal intensive care unit. Eur. J. Pediatr. **148:**89–90.
17. **Murray, B. E., K. V. Singh, S. M. Markowitz, H. A. Lapardo, J. E. Patterson, M. J. Zervos, E. Rubeglio, G. M. Eliopoulos, L. B. Rice, F. W. Goldstein, S. G. Jenkins, G. M. Caputo, R. Masuas, L. S. Moore, E. S. Wang, and G. Weinstock.** 1991. Evidence for clonal spread of single strain of β-lactamase-producing *Enterococcus* (*Streptococcus*) *faecalis* to six hospitals in five states. J. Infect. Dis. **163:**780–785.
18. **Penner, J. L.** 1991. *Campylobacter*, *Helicobacter*, and related spiral bacteria, p. 402–409. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
19. **Salama, S. M., M. M. Garcia, and D. E. Taylor.** 1992. Differentiation of the subspecies of *Campylobacter fetus* by genomic sizing. Int. J. Syst. Bacteriol. **42:**446–450.
20. **Skirrow, M. B.** 1989. Campylobacter perspectives. PHLS Microbiol. Digest **6:**113–117.
21. **Smibert, R. M.** 1978. The genus *Campylobacter*. Annu. Rev. Microbiol. **32:**673–709.
22. **Smibert, R. M.** 1981. The genus *Campylobacter*, p. 609–617. *In* M. P. Starr (ed.), The prokaryotes. A handbook on habitats, isolation and identification of bacteria. Springer-Verlag, New York.
23. **Torphy, D. E., and W. W. Bond.** 1979. *Campylobacter fetus* infections in children. Pediatrics **64:**898–903.
24. **Vinzent, R., J. Dumas, and N. Picard.** 1947. Septicemia grave au cours de la grossesse due a un vibrion. Avortement consecutif. Bull. Acad. Natl. Med. **131:**90.
25. **Yan, W., N. Chang, and D. E. Taylor.** 1991. Pulsed-field gel electrophoresis of *Campylobacter jejuni* and *Campylobacter coli* genomic DNA and its epidemiologic application. J. Infect. Dis. **163:**1068–1072.