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Campylobacter cinaedi Is Normal Intestinal Flora in Hamsters

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During the course of studies to reproduce proliferative enteritis in hamsters, *Campylobacter cinaedi* was recovered from the feces of the majority of healthy hamsters obtained from two commercial sources. The organisms were cultured by using filtration, a nonselective medium, and a microaerophilic atmosphere containing hydrogen. Isolation was hindered by the fastidious nature of *C. cinaedi* and by the presence of other *Campylobacter* species in the hamster intestine. All hamster *C. cinaedi* isolates were phenotypically similar to *C. cinaedi* ATCC 35683. Comparison of whole-cell protein profiles of one hamster isolate with a reference strain of *C. cinaedi* by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting with *C. cinaedi*-specific rabbit antiserum supported the phenotypic identification of these isolates. Hamsters may be an animal reservoir for human *C. cinaedi* infections.

Campylobacter cinaedi (10), previously known as *Campylobacter*-like organism type 1 (3), has been isolated from homosexual men with enteritis, proctocolitis, and asymptomatic rectal infection (6, 9). It has also been described as a cause of fever and acquired immunodeficiency syndrome-related complex (2, 7–9). *C. cinaedi* has been isolated almost exclusively from homosexual men and, unlike other recognized *Campylobacter* species, an animal reservoir for *C. cinaedi* has not been established. In previous studies originally undertaken to use the hamster as a model for proliferative enteritis, an intestinal disease of pigs (C. J. Gebhart et al., manuscript in preparation), we isolated *C. cinaedi* from the fecal floras of healthy hamsters. Hamsters are thus a natural animal reservoir for *C. cinaedi* and may serve as a source for introduction of the organism into the human population.

We used Syrian hamsters from 3 to 25 weeks of age which were obtained on six separate occasions from two commercial sources (Harlan Sprague Dawley, Inc., Indianapolis, Ind., and Charles River Breeding Laboratories, Inc., Wilmington, Mass.). Hamsters from each source were housed separately. Fresh fecal pellets were collected from each of the hamsters on arrival as well as from the shipping container and emulsified in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) containing 1% KNO_3 and 0.15% agar. The suspension was passed through 0.8-μm (Millipore Corp., Bedford, Mass.) or 0.65-μm (Sartorious, Inc., Hayward, Calif.) filters directly onto nonselective Mueller-Hinton agar plates containing 5% sheep blood. Plates were incubated at 37°C in a microaerophilic atmosphere achieved by evacuating an anaerobic jar (GasPak 150; BBL Microbiology Systems, Cockeysville, Md.) twice to approximately 400 mm Hg (ca. 53.33 kPa) and refilling it each time with a gas mixture of 10% hydrogen–10% carbon dioxide–80% nitrogen. Plates were examined every 48 h for 6 days for colonies with *Campylobacter*-like morphology. Several colonies were picked from each plate and subcultured for identification.

A broth dilution technique was used to purify multiple colony types. Colonies were grown in Mueller-Hinton semi-solid broth with 1% KNO_3, 0.15% agar, and 5% sheep blood for 24 to 48 h, mixed, and serially diluted 10-fold into 5 to 10 more Mueller-Hinton broths. Samples from several high dilutions (10^7 to 10^9) were plated and incubated as described above. Individual colonies from each plate were then tested for phenotypic characteristics by previously published methods (1, 3, 4).

Whole-cell extracts of a human *C. cinaedi* strain (1753) and a representative hamster isolate (strain 1193) were prepared from 48-h cultures (3a). The cells were solubilized in 1:1 proportions of 2× sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer and were heated at 100°C for 3 min. Protein content was estimated by using the BCA protein assay (Pierce Chemical Co., Rockford, Ill.). Samples containing 25 μg of protein were electrophoresed through 12.5% sodium dodecyl sulfate-polyacrylamide gels in the discontinuous buffer system of Laemmli (5). Gels were stained with 0.25% Coomassie brilliant blue (Sigma Chemical Co., St. Louis, Mo.). For Western (immuno-) blotting, proteins were transferred from unstained gels to 0.45-μm nitrocellulose paper (Millipore Corp.), using the method described by Towbin (11). The nitrocellulose papers were blocked in buffer composed of 0.15 M NaCl, 5 mM tetrasodium EDTA, 10 mM TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], 0.25% gelatin, and 0.1% Tween 20 (pH 7.3). Rabbit anti-*C. cinaedi* antiserum diluted 1:500 was then incubated with the nitrocellulose papers at 37°C for 1 h, followed by washing and incubation with ^{125}I-labeled staphylococcal protein A (New England Nuclear Corp., Boston, Mass.) at 22°C for 1 h. After a final wash, the blots were exposed to Cronex MRF 32 film (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) with light-intensifying screens for 24 h at −70°C.

C. cinaedi was isolated from 54 (75%) of 72 healthy hamsters. Isolates were phenotypically similar to the reference strain, *C. cinaedi* ATCC 35683 (Table 1). They were gram negative and spiral-shaped and demonstrated typical *Campylobacter* corkscrew motility in broth culture. Colonies were small (<1 mm), nonhemolytic, and gray to gray-white, with a flat, irregular to nondiscrete, watery morphology. Electron microscopy revealed single polar flagella. The organisms were oxidase and catalase positive and grew at 37°C but not at 25 or 42°C. They were susceptible to nalidixic

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TABLE 1. Characteristics of human and hamster *C. cinaedi* isolates[a]

Characteristic	Human *C. cinaedi* ATCC 35683	Hamster *C. cinaedi* 1193
Catalase	+	+
Nalidixic acid (30-μg disk)	S	S
Cephalothin (30-μg disk)	I	I
42°C Growth	−	−
25°C Growth	−	−
Anaerobic growth	−	−
H_2S (TSI)	−	−
H_2S (Pb acetate)	$+^w$	$+^w$
0.04% Triphenyltetrazolium chloride	+	+
1.0% Glycine	$+^w$	+
2.0% NaCl	−	−
Nitrate reduction	+	+
Nitrite reduction	−	−
Hippurate hydrolysis	−	−
Urease	−	−

[a] Abbreviations: S, Susceptible; I, intermediate; w, weak reaction.

acid (30-μg disk) and intermediately susceptible to cephalothin (30-μg disk) by the agar diffusion method. Isolates did not grow aerobically or anaerobically at 37°C and did not grow in the presence of 2% NaCl but grew in the presence of 1.0% glycine and 0.04% triphenyltetrazolium chloride. They did not hydrolyze hippurate or produce urease. They did not produce H_2S in triple-sugar iron slants but produced H_2S on lead acetate paper. They reduced nitrate to nitrite but did not reduce nitrite.

Our phenotypic identification was supported by the results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. The protein profile of a representative *C. cinaedi* hamster isolate was similar to that of the reference *C. cinaedi* strain and was distinct from the protein profiles of *C. fennelliae* and a hamster isolate of *C. jejuni* (Fig. 1A). In addition, by Western blot, the isolate reacted with anti-*C. cinaedi* antiserum in a pattern similar to that of the reference *C. cinaedi* strain (Fig. 1B).

To recover a variety of *Campylobacter* species, we used filtration of feces onto nonselective media and incubation at 37°C instead of the more selective methods which favor primarily recovery of *C. jejuni*. We also included hydrogen in the microaerophilic system, which appeared to enhance growth of some of the more fastidious *Campylobacter* species on primary isolation. Plates were incubated for 6 days, with the hydrogen atmosphere replenished at 48- to 72-h intervals, to ensure detection of slowly growing species. Furthermore, with the broth dilution purification technique, we were able to separate *C. cinaedi* from other fastidious *Campylobacter*-like organisms present in the cultures. We believe that use of these methods for the isolation and purification of *Campylobacter* species will expand our awareness of the diversity of animal reservoirs and human infections associated with these organisms.

The unique epidemiologic association of *C. cinaedi* with homosexual men has never been satisfactorily explained. Previously, sexual transmission of *C. cinaedi* among homosexual men has been inferred because the infection was most common in men with multiple sexual partners (9), was associated with specific sexual practices that involved oro-anal contact (9), and because a marked decline in prevalence occurred in concert with changing sexual practices adopted by homosexual men in the acquired immunodeficiency syndrome era (A. M. Rompalo, C. B. Price, P. L. Roberts, and W. E. Stamm, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 819, 1985). On the basis of similar evidence, *C. jejuni* has also been considered a sexually transmitted disease among homosexual men (9), presumably after introduction of the organism into this population from the usual animal reservoirs. Hamsters are the first animal species described from which *C. cinaedi* has been isolated and thus may be a reservoir for human *C. cinaedi* infection.

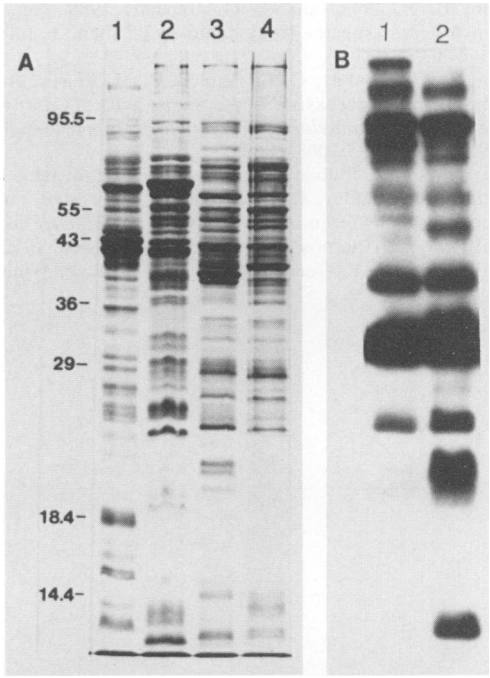

FIG. 1. (A) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of whole-cell preparations from *Campylobacter* isolates. Lanes: 1, *C. jejuni* isolated from a hamster; 2, *C. fennelliae* 1613; 3, human *C. cinaedi* 1753; 4, hamster *C. cinaedi* 1193. (B) Western blots. Lanes: 1, hamster *C. cinaedi* 1193; 2, a human reference strain, *C. cinaedi* 1753. Proteins were reacted with *C. cinaedi*-specific rabbit antiserum (1:500 dilution).

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