JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1995, p. 742–744 Vol. 33, No. 3
0095-1137/95/$04.00+0

A Chemically Defined Liquid Medium That Supports Primary Isolation of *Rochalimaea* (*Bartonella*) *henselae* from Blood and Tissue Specimens

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Received 9 June 1994/Returned for modification 11 August 1994/Accepted 12 December 1994

***Rochalimaea* (*Bartonella*) *henselae* is a fastidious, slowly growing, gram-negative bacillus that is an etiologic agent of bacillary angiomatosis, cat scratch disease, and related syndromes. Accumulation of direct microbiologic evidence of the relationship between the organism and the syndromes compatible with cat scratch disease has been hindered by the difficulties in the primary isolation of the organism from infected tissue specimens. A chemically defined liquid medium was developed to support the growth of *Rochalimaea* species to facilitate study of the organism. This medium was also used successfully to isolate *R. henselae* from clinical specimens from infected patients and a domestic cat. Recovery of *R. henselae* in this was more successful than when recovery was attempted on solid agar. This cell-free, extract-free, defined medium additionally supported the growth of *Rochalimaea quintana* and *Afipia felis*.**

Rochalimaea henselae is a human pathogen responsible for a wide spectrum of granulomatous and vasculoproliferative diseases in human immunodeficiency virus type 1-infected and nonimmunocompromised patients (2, 4, 5, 7, 9). It has been identified in tissue specimens from patients with bacillary angiomatosis and peliosis hepatis (3, 5, 7, 9) and has been isolated from the blood of patients with persistent bacteremia (4, 7), stellate neuroretinitis and chronic fatigue-like syndromes (10), and adenitis (2). Serologic and microbiologic evidence suggests a potential role of *R. henselae* in some cases of aseptic meningitis (4, 10). To date diagnoses have been based on clinical syndromes and serologic evidence of antibodies associated with infection by indirect fluorescent-antibody (IFA) testing, without the isolation of a microbiologic isolate in most clinical settings. Seronegative disease (10) and cross-reactivity between *R. henselae* and *Rochalimaea quintana* antigens from patient sera in the IFA assay have been demonstrated to occur, supporting the benefit of establishing a microbiologic diagnosis.

Primary isolation of *R. henselae* has been reported with a variety of solid media, including sheep (2, 4), rabbit (9), and human (2, 4) blood-based agars and chocolate and charcoal-yeast extract (CYE) agar (2, 4). However, the duration of incubation necessary for the recovery of *R. henselae* from these solid agars and the potential for contamination with environmental fungi during these long incubations have hindered the isolation of the organism in most clinical laboratories. Growth of *R. henselae* has been demonstrated in Haemophilus Test Medium (HTM) broth (BBL, Becton-Dickinson, Cockeysville, Md.) (2, 9a), blood culture media including BACTEC bottles (2, 2a, 4), and noncommercially available media (1a, 8), although primary isolation of the organism has not been demonstrated with either of these liquid-phase media. We report the primary isolation of *R. henselae* from specimens from three previously described patients presenting with clinical disease and from an asymptomatic domestic cat using a fully defined liquid medium based on a modified tissue culture medium supplemented with hemin. This liquid medium yielded rapid growth, with the organism being demonstrated following incubation of the medium with a clinical specimen within 10 days. This medium may aid clinical laboratories in the isolation of *R. henselae* and allow further characterization of the antigens produced by this organism. (The data discussed here were presented in part at the 94th General Meeting of the American Society for Microbiology.)

Patients. Patients with suspected *R. henselae*-related diseases were referred to the Infectious Diseases Clinic at Wilford Hall Medical Center. Following an interview and a physical examination, laboratory data tailored to the differential diagnosis of each patient were collected. When it was clinically indicated, an excisional biopsy specimen of inflamed lymph nodes was obtained after informed consent was obtained. The biopsied material was then submitted for histopathologic examination and microbiologic culture. In those instances in which a tissue biopsy could not be performed, blood was obtained for culture in Isolator blood culture tubes (Wampole, Cranbury, N.J.). Lastly, serum was obtained and was sent to the Viral and Rickettsial Zoonoses Branch of the Centers for Disease Control and Preventions (CDC) assessment of anti-*Rochalimaea* activity by IFA screening (11).

Isolation of *R. henselae*. Clinical specimens were quickly plated by using culture conditions that have been described in detail elsewhere (2). Briefly, patient materials were plated onto solid-phase agar, including CDC anaerobic blood agar and chocolate buffered CYE agar (BBL, Becton-Dickinson, Cockeysville, Md.), and were inoculated directly into cooked meat, HTM broth (Becton-Dickinson), and the *R. henselae* isolation medium described below. Plates were sealed in individual gas-permeable polypropylene bags (Fisher Scientific, Pittsburgh, Pa.), and all materials were incubated in a humid 5% CO_2 environment at 35°C for up to 30 days. Preliminary identification was based on colony morphology, Gram staining charac-

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teristics, and oxidase and catalase testing. Identification was made by gas chromatographic analysis of the whole-cell fatty acid content (Hewlett-Packard 5890 gas chromatograph with Microbial Identification System software, version 3.0; Microbial ID Inc., Newark, Del.) and was confirmed by analysis of restriction fragment length polymorphisms after PCR amplification of the citrate synthase gene performed at CDC (1).

Medium preparation. *R. henselae* isolation medium was prepared with RPMI 1640 (Mediatech; Fisher Scientific) and 1% each L-glutamine (Sigma Chemical Co., St. Louis, Mo.), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer (Sigma), sodium pyruvate (Sigma), and nonessential amino acids (Sigma). Hemin (Sigma) was solubilized in 0.01 N sodium hydroxide and was added to the modified RPMI 1640 formula at 15 mg/liter of broth. The broth pH was adjusted to 7.0 with acetic acid, and the broth was filter sterilized by serial filtration through a 0.2-μm-pore-size filter (Corning, Corning, N.Y.). Aliquots of 2 ml were used for primary isolation of inoculated specimens.

R. henselae isolation medium was used in parallel with other media in the isolation of the organism from three patients who presented to Wilford Hall Medical Center and one domestic cat that appeared to be healthy. These patients and the cat are described in detail elsewhere (10). All *R. henselae*-infected patients were human immunodeficiency virus type 1 seronegative. The patients presented with unique manifestations including neuroretinitis (7), adenitis (7), and a chronic fatigue-like syndrome manifested by prolonged fatigue, diffuse symmetric adenopathy, and low-grade fevers to 100.5°F (38°C) (7). The cat was the family pet of the patients with neuroretinitis. Primary isolation of the organism was successful with the broth, with preliminary microbial identification made after transferring the isolates to solid-phase media. The organism obtained from the cat was confirmed to be *R. henselae*. Growth was demonstrated to be more rapid in broth medium than on solid agar, with isolation occurring between 10 and 16 days after inoculation. The growth-promoting properties of this medium were further demonstrated by performing kinetic growth studies on two clinical isolates of *R. henselae* (strains KL and AM) (2) (Fig. 1). To demonstrate the potential usefulness of this medium for the isolation of organisms from patients with bacillary angiomatosis and cat scratch disease, kinetic growth studies were performed with *R. quintana* ATCC VR 358 and *Afipia felis* BV (Armed Forces Institute of Pathology, Washington, D.C.) as well. All four organisms demonstrated rapid growth over a 7-day period and were easily subcultured from the isolation medium onto CDC anaerobic blood agar (BBL) with exuberant lawns. While not indicating primary isolation of *A. felis* and *R. quintana*, the *R. henselae* isolation medium clearly supports the growth of these organisms.

Diseases associated with *R. henselae* range from persistent bacteremia to vascular endothelial proliferative processes to granulomatous diseases. Significant evidence has demonstrated the role of this organism as an etiologic agent in cat scratch disease, a commonly encountered syndrome (1, 2, 6, 11). However, despite the serologic and genetic data presented to date (1, 6, 11), primary isolation of *R. henselae* has been difficult. The primary isolation of *R. henselae* has been reported on a variety of different cellular and acellular media. Isolation has been performed by a mammalian cell monolayer coculture method (3) and with a variety of acellular solid agars. These methods may be time-consuming and cumbersome, while potential isolates can be lost because of environmental contamination resulting from the excessively long incubation periods

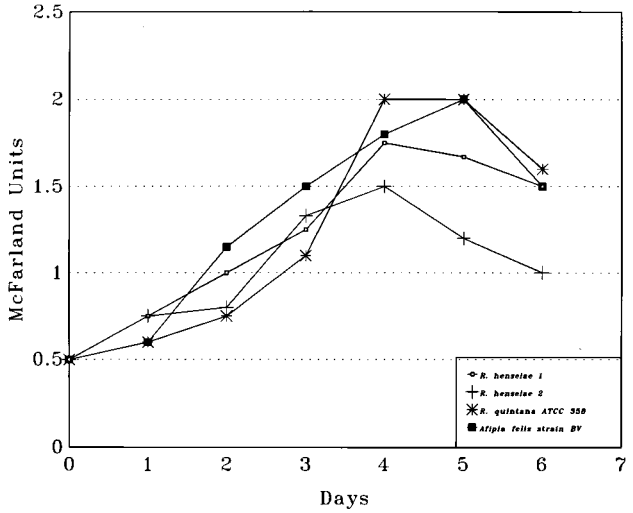

FIG. 1. Growth curves for two clinical strains of *R. henselae* (strains KL and AM), *R. quintana* ATCC VR 358, and *Afipia felis* BV in *Rochalimaea* isolation medium. Isolates were inoculated into 2 ml of medium, and the mixture was vortexed to uniformly suspend the bacteria to match the turbidity of a 0.5 McFarland standard. Readings were obtained every 24 h until the plateau phase of growth was achieved. Growth and viability were determined by subculture of the *Rochalimaea* isolation medium onto CDC anaerobic blood plates (BBL) on days 0, 3, 5, and 7 and incubating the organisms at 35°C in a 5% CO_2 atmosphere. Scale: 0.5 McFarland corresponds to 10^4 CFU/ml for *R. henselae* and between 10^4 and 5×10^5 CFU/ml for *A. felis* and *R. quintana*; 1 McFarland corresponds to 10^5 CFU/ml and 2 McFarlands correspond to $>10^5$ CFU/ml for all three organisms.

in humid environments. In this brief report we described the development of a completely defined liquid medium that was useful for the primary isolation of *R. henselae* from clinical specimens (blood and tissue). This liquid medium does not contain the uncharacterized cell lysate products or extract materials used in previous studies (8). Additionally, all components of this medium are dialyzable and will allow the simple isolation of *Rochalimaea* antigens for use as potential immunodiagnostic materials and skin test reagents. This simple-to-prepare medium may provide clinical microbiology laboratories with additional options for the primary isolation of *R. henselae* and will also support the growth of other putative pathogens including *R. quintana* and *A. felis.*

REFERENCES

1. **Anderson, B., C. Kelly, R. Therlkel, and K. Edwards.** 1993. Detection of *Rochalimaea henselae* in cat-scratch disease skin test antigens. J. Infect. Dis. **168:**1034–1036.
1a. **Cockerell, J. J., P. R. Bergstresser, C., Myrie-Williams, and P. M. Tierno.** 1990. Bacillary epithelioid angiomatosis occurring in an immunocompetent adult. Arch. Dermatol. **126:**787–790.
2. **Daly, J. S., M. G. Worthington, D. J. Brenner, C. W. Moss, D. G. Hollis, R. S. Weyant, A. G. Seigerwalt, R. E. Weaver, M. I. Daneshvar, and S. O'Connor.** 1993. *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. J. Clin. Microbiol. **31:**872–881.
2a. **Dolan, M. J., M. T. Wong, R. L. Regnery, J. H. Jorgensen, M. Garcia, J. Peters, and D. Drehner.** 1993. Syndrome of *Rochalimaea henselae* adenitis suggesting cat scratch disease. Ann. Intern. Med. **118:**331–336.
3. **Koehler, J. E., F. D. Quinn, T. G. Berger, P. E. LeBoit, and J. W. Tappero.** 1992. Isolation of *Rochalimaea* species from cutaneous and osseous lesions of bacillary angiomatosis. N. Engl. J. Med. **327:**1625–1631.
4. **Lucey, D., M. J. Dolan, C. W. Moss, M. Garcia, D. G. Hollis, S. Wegner, G. Morgan, R. Almeida, D. Leong, K. S. Greisen, D. F. Welch, and L. N. Slater.** 1992. Relapsing illness due to *Rochalimaea henselae* in immunocompetent hosts: implications for therapy and new epidemiological associations. Clin. Infect. Dis. **14:**683–688.
5. **Perkocha, L. A., S. M. Geagham, T. S. Yen, S. L. Nishimura, S. P. Chan, R. Garcia-Kennedy, G. Honda, A. C. Stoloff, H. Z. Klein, R. L. Goldman, S. Van**

Meter, L. D. Ferrel, and P. E. LeBoit. 1990. Clinical and pathological features of bacillary peliosis hepatis in association with human immunodeficiency virus infection. N. Engl. J. Med. **323:**1581–1586.

6. **Regnery, R. L., J. G. Olson, B. A. Perkins, and W. Bibb.** 1992. Serologic response to *Rochalimaea henselae* antigen in suspected cat scratch disease. Lancet **339:**1443–1445.
7. **Relman, D. A., J. S. Loutit, T. M. Schmidt, S. Falkow, and L. S. Tompkins.** 1990. The agent of bacillary angiomatosis: an approach to the identification of uncultured pathogens. N. Engl. J. Med. **323:**1573–1580.
8. **Schwartzman, W. A., C. A. Nesbit, and E. J. Baron.** 1993. Development and evaluation of blood-free medium for determining growth curves and optimizing growth of *Rochalimaea henselae*. J. Clin. Microbiol. **31:**1882–1885.
9. **Slater, L. N., D. F. Welch, D. Hensel, and D. W. Coody.** 1990. A newly recognized fastidious gram-negative pathogen as a cause of fever and bacteremia. N. Engl. J. Med. **323:**1587–1593.

9a. **Welch, D. F., D. A. Pickett, L. N. Slater, A. G. Seigerwalt, and D. J. Brenner.** 1992. *Rochalimaea henselae* sp. nov., a cause of septicemia, bacillary angiomatosis, and parenchymal bacillary peliosis. J. Clin. Microbiol. **30:**275–280.

10. **Wong, M., C. Lattuada, Jr., E. Mokulis, R. LaBarre, A. McRae, J. Reed, S. Smith, R. Regnery, and M. Dolan.** 1994. The clinical spectrum of disease associated with *Rochalimaea henselae* also includes ocular disease, chronic fatigue syndrome, and seronegative infection in immunocompetent patients, and stellate granulomatous disease in AIDS, abstr. C-462, p. 572. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
11. **Zangwill, K. M., D. H. Hamilton, B. A. Perkins, R. L. Regnery, B. D. Plikaytis, J. L. Hadler, M. L. Cartter, and J. D. Wegner.** 1993. Cat scratch disease in Connecticut: epidemiology, risk factors and evaluation of a new diagnostic test. N. Engl. J. Med. **329:**8–13.