

togenicities, because in general such occasional rearrangements are unlikely to affect the virulence of bacterium. Given the rarity of such rearrangements in *M. bovis* BCG, the presently known polymorphic genetic markers seem unsuitable to recognize BCG variants with different reactivities, as suggested by Arya.

REFERENCES

1. Groenen, P. M. A., A. E. Bunschoten, D. van Soolingen, and J. D. A. van Embden. Nature of DNA polymorphism in the direct repeat cluster of *Mycobacterium tuberculosis*; application for strain differentiation by a novel method. Submitted for publication.
2. Hermans, P. W. M., D. van Soolingen, E. M. Bik, P. E. W. de Haas, J. W. Dale, and J. D. A. van Embden. 1991. The insertion element IS987 from *Mycobacterium bovis* BCG is located in a hot spot integration region for insertion elements in *M. tuberculosis* complex strains. *Infect. Immun.* 59:2695-2705.
3. van Soolingen, D., P. E. W. de Haas, P. W. M. Hermans, P. M. A. Groenen, and J. D. A. van Embden. 1993. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* 31:1987-1995.

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Clinical Isolates of *Campylobacter mucosalis*

Figura et al. (2) recently reported on the isolation of *Campylobacter mucosalis* from two children with enteritis by using a filtration technique and incubating in an H₂-enriched microaerobic atmosphere. During a 30-month period, from October 1990 to March 1993, using a similar protocol, the Red Cross Hospital processed 6,111 diarrheic stool specimens from which 1,519 strains of *Campylobacter*, *Helicobacter*, or *Arcobacter* spp. were isolated and identified by recognized criteria (2a).

The prevalence was as follows: 645 (42.5%) *Campylobacter jejuni* subsp. *jejuni* isolates, 337 (22.2%) *Campylobacter upsaliensis* isolates, 187 (12.3%) *Campylobacter* strains dependent on an H₂-enriched microaerobic atmosphere for growth (Oxoid BR 38 gaspak, no catalyst), 159 (10.5%) *C. jejuni* subsp. *doylei* isolates, 100 (6.6%) *Helicobacter fennelliae* isolates, 42 (2.8%) *Campylobacter coli* isolates, 23 (1.5%) *Campylobacter hyointestinalis* isolates, 11 (0.7%) *Helicobacter cinaedi* isolates, 8 (0.5%) *Campylobacter fetus* subsp. *fetus* isolates, 5 (0.35%) *Arcobacter butzleri* isolates, and 1 (0.06%) each of *Campylobacter lari* and *Campylobacter curvus*.

All 187 isolates (185 from stool samples, 1 from a blood culture, and 1 from a gastric biopsy) requiring H₂ were nitrate, reductase, and oxidase positive but negative for urease, catalase, hippurate, and indoxyl acetate. They produced abundant H₂S detectable with lead acetate strips and triple sugar iron agar, and they tolerated 1% glycine but not 1.5% NaCl. These characteristics are identical to those for the isolates described by Figura et al. (2) and for *C. mucosalis* (4, 6), but unfortunately, they are also identical to the description of *Campylobacter concisus* (4, 6). *C. concisus* has been found in the human mouth and, until recently, has only rarely been isolated from human feces. Using H₂-enhanced microaerobic growth conditions, Lauwers et al. (3) isolated 94 strains of *C. concisus* from the stools of enteritis patients. *C. mucosalis* strains can be differentiated from *C. concisus* by growth at 25°C, susceptibility to cephalothin, and the production of "dirty yellow" colonies (4, 5).

Nine of our isolates were determined to be *C. mucosalis* by the above criteria. Molecular techniques were performed by established procedures. The average mol% G+C value

was found to be 38.1, which is the overlap region of the two species (4, 6). DNA-DNA hybridization studies were performed, and the clinical isolates were probed twice, once with the type strain for *C. mucosalis*, NCTC 11000, and again with the type strain for *C. concisus*, NCTC 11485. None of the nine isolates reacted with the *C. mucosalis* probe, while six reacted strongly with the *C. concisus* probe and three did not react with either probe. These three may represent a new species, as preliminary studies of cellular fatty acid profiles and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has indicated.

Differentiation of these two species on the basis of growth temperature, cephalothin sensitivity, and colony color is suspect because of strain variability (2, 6). Figura et al. (2) used the API 20 Campy system for confirmation, but we have not found this system completely satisfactory for typing.

Immunotyping (7), SDS-PAGE (1), and, in particular, DNA hybridization studies (5) all allow excellent discrimination between the two species. Indeed, Roop et al. (5) reported that the DNAs from *C. mucosalis* and *C. concisus* were only 9% complementary. It is premature to base identification of *C. mucosalis*, which has never been isolated from humans before, solely on a very limited number of variable characteristics. Molecular studies must be done for positive identification.

REFERENCES

1. Costas, M., R. J. Owen, and P. J. H. Jackman. 1987. Classification of *Campylobacter sputorum* and allied campylobacters based on numerical analysis of electrophoretic protein patterns. *Syst. Appl. Microbiol.* 9:125-131.
2. Figura, N., P. Guglielmetti, A. Zanchi, N. Partini, D. Armellini, P. F. Bayelli, M. Bugnoli, and S. Verdiani. 1993. Two cases of *Campylobacter mucosalis* enteritis in children. *J. Clin. Microbiol.* 31:727-728.
- 2a. Lastovica, A., and E. L. Roux. Unpublished data.
3. Lauwers, S., T. Devreker, R. Van Etterijck, J. Breynaert, A. Van Zeebroeck, L. Smekens, K. Kersters, and P. Vandamme. 1991. Isolation of *Campylobacter concisus* from human faeces. *Microb. Ecol. Health Dis.* 4(Suppl.):S91.
4. Penner, J. L. 1988. The genus *Campylobacter*: a decade of progress. *Clin. Microbiol. Rev.* 1:157-172.

5. Roop, R. M., II, R. M. Smibert, J. L. Johnson, and N. R. Krieg. 1985. DNA homology studies of the catalase-negative campylobacters and "*Campylobacter fecalis*", an emended description of *Campylobacter sputorum*, and a proposal of the neotype strain of *Campylobacter sputorum*. Can. J. Microbiol. 31:823-831.
6. Vandamme, P., and J. De Lay. 1991. Proposal for a new family, *Campylobacteraceae*. Int. J. Syst. Bacteriol. 41:451-455.
7. Vandamme, P., E. Falsen, B. Pot, B. Hoste, K. Kersters, and J. De Lay. 1989. Identification of EF group 22 campylobacters from gastroenteritis cases as *Campylobacter concisus*. J. Clin. Microbiol. 27:1775-1781.

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Author's Reply

I agree that the problem with atypical or unusual campylobacters is the limited number of tests able to differentiate one species from another. However, I do not think this was a problem in our case. In the two observations we made (1), we could exclude the possibility that strains were *C. concisus* on the basis of the three tests described in the letter by Lastovica et al. Our strains, unlike those of *C. concisus*, were susceptible to cephalothin, produced a dirty yellow pigment, and did not grow at 25°C. Results of the latter test are sometimes variable (3); in our hands, for example, *C. concisus* CCUG 13144 did not grow at 25°C. However, the other two phenotypic characteristics are rather stable (2).

Apparently, Lastovica et al. did not apply these two

distinctive criteria to their H₂-requiring isolates. Thus, it is not surprising that none of the strains they selected as *C. mucosalis* hybridized with *C. mucosalis* NCTC 11000. It would be interesting to see whether those nine strains were susceptible to cephalothin and produced a yellowish pigment.

Microbiologists have been relying on biochemical, tolerance, susceptibility, and colony characteristics of bacteria for almost a century. I believe that the production of a yellowish pigment and susceptibility to cephalothin can adequately differentiate *C. mucosalis* from *C. concisus*. DNA hybridization and other diagnostic tools certainly enable the performance of very accurate assays, but for many researchers, "the phenotypic tests remain the most important diagnostic methods for the identification of bacteria" (3).

In any case, our *C. mucosalis* strains can be requested from the National Collection of Type Cultures, London, United Kingdom, where they have been deposited. They are *C. mucosalis* NCTC 12407 and *C. mucosalis* NCTC 12408.

REFERENCES

1. Figura, N., P. Guglielmetti, A. Zanchi, N. Partini, D. Armellini, P. F. Bayeli, M. Bugnoli, and S. Verdiani. 1993. Two cases of *Campylobacter mucosalis* enteritis in children. J. Clin. Microbiol. 31:727-728.
2. Roop, R. M., II, R. M. Smibert, J. L. Johnson, and N. L. Krieg. 1985. *Campylobacter mucosalis* (Lawson, Leaver, Pettigrew, and Rowland 1981) comb. nov.: emended description. Int. J. Syst. Bacteriol. 35:189-192.
3. Vandamme, P., and H. Goossens. 1992. Taxonomy of *Campylobacter*, *Arcobacter*, and *Helicobacter*. Zentralbl. Bakt. 276:447-472.

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Serologic Diagnosis of Tuberculosis through Assays of Lipoarabinomannan Antigen or Antibody or Lysozyme Level

The coagglutination studies of Sada et al. (5) for simplified diagnosis of tuberculosis, which used *Staphylococcus aureus* Cowan I sensitized with purified immunoglobulin G (IgG) antibody to lipoarabinomannan (LAM), had a sensitivity of 88 and 67% with samples from patients with smear-positive and smear-negative active pulmonary tuberculosis, respectively. For AIDS patients with tuberculosis, sensitivity was 57%. The specificity with samples from 63 control patients with lung diseases other than tuberculosis and with 63 serum samples from a blood bank from persons presumed to be healthy was 100%. The positive and negative predictive values for the LAM antigen assay with samples from patients with sputum-positive active pulmonary tuberculosis were 100 and 97%, respectively (5).

Before the universal acceptance of LAM antigen assays for specific diagnosis of tuberculosis, its specificity would

have to be investigated with samples from *Mycobacterium bovis* BCG vaccinees and patients with other mycobacterial diseases. Apart from global use of BCG as a prophylactic agent incorporated in the WHO Expanded Programme on Immunization, BCG has also been effective in patients with leukemia. In Quebec, leukemia mortality in children below 15 years of age was half as high in those who were vaccinated with BCG as in those who were not, while a British trial found leukemia mortality to be 2.4 per 100,000 in vaccinated subjects versus 4.1 in those who were not (2). The interference in LAM specificity following a recent or an earlier BCG vaccination needs close scrutiny.

Use of IgG antibodies against LAM as a reliable diagnostic marker for tuberculosis on 66 patients with pulmonary, miliary, and pleural tuberculosis in an enzyme-linked immunosorbent assay was 96% specific and 72% sensitive. Nev-