Molecular Typing of *Campylobacter jejuni* and *Campylobacter coli*

Shi et al. (7) presented data on the development and application of a PCR-based restriction fragment length polymorphism analysis of a 9.6-kb portion of the lipopolysaccharide gene (LG) cluster of *Campylobacter jejuni* and *Campylobacter coli*. This proposed molecular subtyping system is novel and has the potential of being directly applicable to epidemiology, prevalence, and other studies. I agree that if, as the authors contend, their proposed genotyping scheme has the same molecular basis as the Penner serotyping scheme (5, 6), then *C. jejuni* and *C. coli* isolates previously nontypeable by the Penner scheme are likely to be typeable by the LG scheme.

However, I do not agree that the proposed LG typing scheme is specific for *C. jejuni* and *C. coli* only. While *C. lari* and *C. upsaliensis* are less frequently isolated from clinical specimens than *C. jejuni* and *C. coli*, these organisms have been linked to colitis, bacteremia, spontaneous human abortion, the hemolytic-uremic syndrome, and other illnesses (1, 2, 3). *C. lari* strains are serotypeable by the Penner system (2, 8; A. J. Lastovica, unpublished data), as are *C. upsaliensis* strains (2; Lastovica, unpublished). That some strains of *C. upsaliensis* are serotypeable may reflect the genomic heterogeneity of *C. upsaliensis*, as has been proposed (4).

Since *C. lari* and *C. upsaliensis* are typeable with the Penner system, they also should be typeable by the LG scheme. This question was not adequately addressed, as only a single strain each of *C. lari* and *C. upsaliensis* was analyzed by the LG scheme and no PCR product was obtained (7).

Additional LG analysis on a number of serologically defined *C. lari* and *C. upsaliensis* strains will resolve this question and extend the usefulness of the proposed LG molecular subtyping scheme.

REFERENCES

1. Bourke, B., V. L. Chan, and P. Sherman. 1998. *Campylobacter upsaliensis*: waiting in the wings. Clin. Microbiol. Rev. 11:440–449.
2. Lastovica, A. J., E. Le Roux, and J. L. Penner. 1989. “*Campylobacter upsaliensis*” isolated from blood cultures of pediatric patients. J. Clin. Microbiol. 27:657–659.
3. Lastovica, A. J., and M. B. Skirrow. 2000. Clinical significance of *Campylobacter* and related species other than *C. jejuni* and *C. coli*, p 89–120. In I. Nachamkin and M. J. Blaser (ed.), *Campylobacter*, 2nd ed. American Society for Microbiology, Washington, D.C.
4. Moser, I., B. Rieksneuwöhner, P. Lentzsch, P. Schwerk, and L. H. Wieler. 2001. Genomic heterogeneity and O-antigenic diversity of *Campylobacter upsaliensis* and *Campylobacter helveticus* strains isolated from dogs and cats in Germany. J. Clin. Microbiol. 39:2548–2557.
5. Penner, J. L., and J. N. Hennessey. 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. J. Clin. Microbiol. 12:732–737.
6. Penner, J. L., J. N. Hennessey, and R. V. Congi. 1983. Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. Eur. J. Clin. Microbiol. 2:378–383.
7. Shi, F., Y. Y. Chen, T. M. Wassenaar, W. H. Woods, P. J. Coloe, and B. N. Fry. 2002. Development and application of a new scheme for typing *Campylobacter jejuni* and *Campylobacter coli* by PCR-based restriction fragment length polymorphism analysis. J. Clin. Microbiol. 40:1791–1797.
8. Woodward, D. L., and F. G. Rodgers. 2002. Identification of *Campylobacter* heat-stable and heat-labile antigens by combining the Penner and Lior serotyping schemes. J. Clin. Microbiol. 40:741–745.

Albert J. Lastovica
Department of Medical Microbiology
University of Cape Town
Cape Town, South Africa

Authors' Reply

We are most grateful to Professor Albert Joseph Lastovica for his interest in our recent paper on the development of a novel *Campylobacter* genotyping scheme. Before we comment on the specificity of the LG typing scheme, we should clarify the current molecular understanding of the Penner and LG typing methods.

The molecular basis for the Penner heat-stable antigenic diversity in *C. jejuni* and *C. coli* was thought to be the expression of somatic (O) lipopolysaccharide (LPS) (3, 4, 5, 6, 8, 9, 10). LPS is a major constituent of the outer membrane in gram-negative bacteria and comprises three covalently linked regions: lipid A, core oligosaccharide (inner core and outer core), and O polysaccharide. The variability of the *Campylobacter* LPS outer core and O polysaccharide was thought to contribute to the antigenic basis of the Penner serotyping system. However, recent evidence showed that the high-molecular-weight O polysaccharide is a capsular polysaccharide which is not linked to the LPS molecule of *C. jejuni* and which accounted in some strains for the Penner serotype specificity (2, 13). This evidence also indicated that *C. jejuni* strains carry lipooligosaccharide (LOS) molecules instead of LPS molecules. In addition, Moran et al. (6a) and Oza et al. (7) showed that this LOS is part of the heat-stable antigens, which contribute to passive hemagglutination and direct agglutination.

Part of the LOS biosynthesis gene cluster (*wla*) of *C. jejuni* 81116 has recently been characterized in our laboratory (1). The LOS gene (LG) typing scheme was established by PCR-restriction fragment length polymorphism of the *wla* gene cluster (11). Some of the genes in the same cluster were shown to be involved in protein glycosylation in *C. jejuni* strain 81-176 (12). Therefore, the LG typing scheme should be renamed the LOS/glycosylation typing scheme.

Given the background of both systems, we can now address the question of the specificity of the LG typing system. Although some of the *C. lari* and *C. upsaliensis* strains may share some common surface epitopes (capsule or LOS) and can be typed by the Penner serotyping system, they may still have distinct *wla* gene clusters (different gene elements and organization). Furthermore, the *galE* and *wlaH* genes in the *wla* clusters from *C. lari* and *C. upsaliensis* strains may not be highly similar to those of *C. jejuni* and *C. coli*. Therefore, the primers *galE1* and *wlaH3* would be unable to bind to these genes and would not give a PCR product. However, in all tested *C. jejuni* and *C. coli* strains the *galE* and *wlaH* genes are present and highly conserved and therefore all these strains are 100% typeable with the LG system. We agree with Professor Lastovica's suggestion that additional LG analysis on a number of serologically defined *C. lari* and *C. upsaliensis* strains will help to further confirm the specificity of the LG typing scheme. In addition, genetic analysis of the *wla* gene clusters from *C. lari* and *C. upsaliensis* strains will show why the LG system is specific for *C. jejuni* and *C. coli* strains.

REFERENCES

1. Fry, B. N., V. Korolik, J. A. ten Brinke, M. T. T. Pennings, R. Zalm, B. J. J. Teunis, P. J. Coloe, and B. A. M. van der Zeijst. 1998. The lipopolysaccharide biosynthesis locus of *Campylobacter jejuni* 81116. Microbiology 144: 2049–2061.
2. Karlyshev, A. V., D. Linton, N. A. Gregson, A. J. Lastovica, and B. W. Wren. 2000. Genetic and biochemical evidence of a *Campylobacter jejuni* capsular

- polysaccharide that accounts for Penner serotype specificity. *Mol. Microbiol.* **35**:529–541.
3. **Mandatori, R., and J. L. Penner.** 1989. Structural and antigenic properties of *Campylobacter coli*. *Infect. Immun.* **57**:3506–3511.
 4. **Mills, S. D., W. C. Bradbury, and J. L. Penner.** 1985. Basis for serological heterogeneity of thermostable antigens of *Campylobacter jejuni*. *Infect. Immun.* **50**:284–291.
 5. **Moran, A. P., and J. L. Penner.** 1999. Serotyping of *Campylobacter jejuni* based on heat-stable antigens: relevance, molecular basis and implications in pathogenesis. *J. Appl. Microbiol.* **86**:361–377.
 6. **Moran, A. P., and T. U. Kosunen.** 1989. Serological analysis of the heat-stable antigens involved in serotyping *Campylobacter jejuni* and *Campylobacter coli*. *APMIS* **97**:253–260.
 - 6a. **Moran, A. P., T. U. Kosunen, and M. M. Prendergast.** 2001. Biochemical and serological analysis of the heat-stable antigens involved in serotyping *Campylobacter jejuni/coli*. *Int. J. Med. Microbiol.* **291**(Suppl. 31):72–73.
 7. **Oza, A. N., R. T. Thwaites, D. R. A. Wareing, F. J. Bolton, and J. A. Frost.** 2002. Detection of heat-stable antigens of *Campylobacter jejuni* and *C. coli* by direct agglutination and passive hemagglutination. *J. Clin. Microbiol.* **40**:996–1000.
 8. **Perez-Perez, G. I., and M. J. Blaser.** 1985. Lipopolysaccharide characteristics of pathogenic campylobacters. *Infect. Immun.* **47**:353–359.
 9. **Perez-Perez, G. I., J. A. Hopkins, and M. J. Blaser.** 1985. Antigenic heterogeneity of lipopolysaccharides from *Campylobacter jejuni* and *Campylobacter fetus*. *Infect. Immun.* **48**:528–533.
 10. **Preston, M. A., and J. L. Penner.** 1987. Structural and antigenic properties of lipopolysaccharides from serotype reference strains of *Campylobacter jejuni*. *Infect. Immun.* **55**:1806–1812.
 11. **Shi, F., Y. Y. Chen, T. M. Wassenaar, W. H. Woods, P. J. Coloe, and B. N. Fry.** 2002. Development and application of a new scheme for typing *Campylobacter jejuni* and *Campylobacter coli* by PCR-based restriction fragment length polymorphism analysis. *J. Clin. Microbiol.* **40**:1791–1797.
 12. **Szymanski, C. M., R. Yao, C. P. Ewing, T. J. Trust, and P. Guerry.** 1999. Evidence for a system of general protein glycosylation in *Campylobacter jejuni*. *Mol. Microbiol.* **32**:1022–1030.
 13. **Wren, B. W., D. Linton, N. Dorrell, and A. V. Karlyshev.** 2001. Post genome analysis of *Campylobacter jejuni*. *J. Appl. Microbiol.* **90**:36S–44S.

Feng Shi
Benjamin N. Fry
*Department of Biotechnology and
Environmental Biology
Royal Melbourne Institute of Technology
University
Melbourne, Australia*