Letter to the Editors

Differentiation of enteropathogenic campylobacter

The identification of campylobacters is a topic recently discussed in your columns (May issue, page 509; June issue, page 603). One of the problems facing clinical microbiologists is the lack of suitable tests for distinguishing between strains of the 'thermophilic' group of campylobacters that cause acute enterocolitis. Véron and Chatelain¹ recognised the organisms *Vibrio jejuni*² and *Vibrio coli*,³ described in 1931 and 1948 respectively, in their classification of the genus *Campylobacter*, which became *Campylobacter jejuni* and *C. coli*, names recently accepted by an international committee.⁴ However, since their distinguishing features were unclear, Smibert,⁵ in the United States, grouped them together as *C. fetus* subsp. *jejuni*.

During the past three years we have examined some 1200 campylobacter strains and now recognise groupings that conform to the Institut Pasteur's *C. jejuni* and *C. coli* type strains,⁶ although many strains had intermediate characteristics; we also found a nalidixic-acid-resistant group of thermophilic campylobacters (NARTC), as we mentioned in our letter published in your June issue, page 603. The *jejuni/coli* groupings were based mainly on tolerance to tri-phenyltetrazolium chloride (TTC) and growth at 30.5°C and 45.5°C, finely balanced tests that are unsuitable for routine use. Recently, however, by the use of two simple tests we found that it was possible to distinguish not only *C. jejuni* from *C. coli*, but two biotypes of *C. jejuni*.

Hippurate hydrolysis This is a modification of the test described by Hwang and Ederer⁷ and used by Harvey⁸ to distinguish *C. fetus* from *C. jejuni*. It detects

glycine, which is an end-product of the hydrolysis of hippurate. Strains were grown on blood agar for 18 hours at 37°C in an atmosphere containing 5.7% O₂ and 10% CO₂. A 2 mm loopful of growth (amount not critical) was suspended in 2 ml of sterile distilled water; 0.5 ml sodium hippurate (5% soln) was added and the mixture was incubated for 2 hours in a water bath set at 37°C. 1 ml ninhydrin solution (3.5 g ninhydrin in 100 ml of a 1:1 mixture of acetone and butanol) was added, and the test was left for a further 2 hours at room temperature. The development of a purple colour indicated the presence of glycine, ie, a positive test.

H₂S production in iron medium A large loopful of overnight growth, prepared as above, was inoculated as a lump into nutrient broth (Oxoid No. 2) containing 0.12% agar and 0.05% each of FeSO₄·7H₂O, sodium metabisulphite, and sodium pyruvate. The test was left at room temperature for 4 hours and then examined for blackening around the lump of growth (positive).

Having applied these tests to 126 campylobacter strains that had previously been categorised according to our former methods,⁶ we were able to construct a simple schema, suitable for routine use, for the identification of campylobacters of intestinal origin (Table). Many of our 'intermediate' strains were H₂S positive in the new test, and since these were all hippurate positive we have designated them *C. jejuni* biotype 2. We hope to present a full report after we have tested the rest of our collection of strains.

MB SKIRROW

J BENJAMIN

Worcester Royal Infirmary,
Castle Street Branch,
Worcester WR1 3AS

References

- ¹ Véron M, Chatelain R. Taxonomic study of the genus *Campylobacter* Sebald and Véron and designation of the neotype strain for the type species *Campylobacter fetus* (Smith and Taylor) Sebald and Véron. *Int J Syst Bacteriol* 1973;23: 122-34.
- ² Jones FS, Orcutt M, Little RB. Vibrios (*Vibrio jejuni* n.sp.) associated with intestinal disorders of cows and calves. *J Exper Med* 1931;53:853-64.
- ³ Doyle LP. The etiology of swine dysentery. *Amer J Vet Res* 1948;9:50-1.
- ⁴ Approved lists of bacterial names. *Int J Syst Bacteriol* 1980;30:225-420.
- ⁵ Smibert RM. *Campylobacter*. In: *Bergey's Manual of Determinative Bacteriology*. 8th edn. Baltimore: Williams and Wilkins, 1974:207-11.
- ⁶ Skirrow MB, Benjamin J. '1001' Campylobacters: cultural characteristics of intestinal campylobacters from man and animals. *J Hyg Camb* in press.
- ⁷ Hwang M, Ederer GM. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. *J Clin Microbiol* 1975;11:114-5.
- ⁸ Harvey SM. Hippurate hydrolysis by *Campylobacter fetus*. *J Clin Microbiol* 1980;11:435-7.

	<i>C. fetus</i>	<i>C. jejuni</i> biotype 1*	<i>C. jejuni</i> biotype 2	<i>C. coli</i> †	NARTC‡
Growth at 25°C	+	-	-	-	-
Growth at 43°C	-	+	+	+	+
Nalidixic acid (30 µg disc)	R	S	S	S	R
Hippurate hydrolysis	-	+	+	-	-
H ₂ S in iron medium	-	-	+	-	+

R = resistant; S = sensitive

*Includes Institut Pasteur *C. jejuni* type-strain and NCTC 11168

†Includes Institut Pasteur *C. coli* type-strain and NCTC 11353

‡Includes NCTC 11352