International Committee on Systematic Bacteriology

Subcommittee on the taxonomy of *Campylobacter* and related bacteria

Minutes of the meetings, 13 and 16 September 1999, Baltimore, USA

Session 1. Closed meeting

Minute 1. Call to order. The Chairman, P. Vandamme, called the meeting to order at 15:50 on 13 September 1999 in the St George Room at the Renaissance Harbourplace Hotel in Baltimore, USA.

Minute 2. Record of attendance. The members present were P. Vandamme (Chairman), F. E. Dewhirst, A. J. Lastovica, F. Mégraud, S. L. W. On (Secretary), R. J. Owen and I. V. Wesley. Apologies for absence were received from J.-P. Butzler, J. G. Fox, A. Lee and T. J. Trust. New members A. J. Lastovica and I. V. Wesley were welcomed to their first meeting.

Minute 3. Agenda. The proposed agenda was approved.

Minute 4. Minutes of the previous (1998) meeting. The minutes of the meeting held in Budapest, Hungary, were approved.

Minute 5. Matters concerning membership. J. B. Ursing had resigned from the subcommittee. The subcommittee recognizes the excellent work performed by Dr Ursing on its behalf and thanks him for his valuable contributions. The present members of the subcommittee are as follows: J.-P. Butzler (Belgium), F. E. Dewhirst (USA), J. G. Fox (USA), A. J. Lastovica (South Africa), A. Lee (Australia), F. Mégraud (France), S. L. W. On (Secretary) (Denmark), R. J. Owen (UK), T. J. Trust (Canada), P. Vandamme (Chairman) (Belgium) and I. V. Wesley (USA).

Minute 6. Recent taxonomic developments within the genus *Campylobacter* and related bacteria. An overview of recent taxonomic changes in *Campylobacter* and related bacteria was given by P. Vandamme. The genus *Sulfurospirillum* was to be included in the family *Campylobacteraceae* in the forthcoming edition of *Bergey's Manual of Systematic Bacteriology*. A formal proposal to this effect by submission to the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM) was expected. Two new *Sulfurospirillum* species, *Sulfurospirillum barnesii* and *Sulfurospirillum arsenophilum*, had been formally described [Stolz *et al.* (1999). *Int J Syst Bacteriol* **49**, 1177–1180], and the descriptions of two candidate *Helicobacter* species, 'Candidatus *H. bovis*' and 'Candidatus *H. suis*' were in press at the time of the meeting. Details of the latter two taxa were subsequently published by De Groote *et al.* (1999) (*Int J Syst Bacteriol* **49**, 1707–1715, and *Int J Syst Bacteriol* **49**, 1769–1777, respectively). It was noted that three putative new taxa ('*H. suncus*', and two unnamed taxa from cotton-top tamarins and IL-10-deficient mice, respectively) had been described in journals other than the IJSEM and, at the time of writing, had yet to be validated.

Minute 7. Minimal standards for the description of new *Helicobacter* species. F. E. Dewhirst reported that a paper

describing the minimal standards for the description of new *Helicobacter* spp. had now been submitted on behalf of the subcommittee to the IJSEM. The paper took into consideration comments and contributions from other members of the subcommittee, as described in the minutes of the previous subcommittee meeting. The paper was presently under review. F. E. Dewhirst agreed to circulate copies of the paper to other subcommittee members.

Minute 8. Divergence of 16S rRNA genes in *Campylobacter* and *Helicobacter* species. S. L. W. On reported on recent research demonstrating significant variation of the 16S rRNA genes among strains of *C. hyointestinalis* and *H. cinaedi*. Details of the variability of the 16S rRNA genes of *C. hyointestinalis* subsp. *hyointestinalis* and *C. hyointestinalis* subsp. *lawsonii* are published [Harrington & On (1999). *Int J Syst Bacteriol* **49**, 1171–1175]. Briefly, sequence similarities between strains ranged from 95.7 to 100%. These values are considerably more diverse than those considered to be in the normal range for a single species, particularly when current guidelines suggest that similarity values lower than 97% indicate distinct species. The proximity of a forthcoming *Campylobacter* species, *C. lanienae*, to the *C. hyointestinalis* subspecies was discussed and it was suggested that the taxonomic position of this new species may also require closer scrutiny. A similar situation for *H. cinaedi* was described (where infraspecific 16S rDNA sequence similarities range from 96.5 to 100%). These results have now been submitted for publication (by authors including P. Vandamme and S. L. W. On) in an international journal. These reports were made in view of the extensive use of 16S rRNA gene sequence comparisons to delineate potential new species belonging to the ϵ subclass of the *Proteobacteria*, in order to emphasize the need for cautious interpretation of such data. F. E. Dewhirst remarked that the use of gene sequences such as *recA* could be used as alternatives to the 16S rRNA gene to investigate phylogenetic relationships between these species. In addition, RFLP analyses of the 23S rRNA gene could possibly resolve conflicts arising from highly divergent 16S rRNA genes of certain species.

Minute 9. Nomenclatural issues. Discussions concerning the nomenclature of *C. sputorum* bv. *fecalis*, *C. fetus* subsp. *venerealis* and *C. hyoilei* had been undertaken by the subcommittee at its last meeting (Budapest, Hungary, 2 September 1998). It was further remarked that the infra-subspecific (biovar) designations of *C. sputorum* had been amended in 1998, and further noted during the minutes of the previous subcommittee meeting. The subcommittee agreed to (i) endorse the conservation of the subspecies epithet *C. fetus* subsp. *venerealis*, and the revised biovar nomenclature of *C. sputorum*; and (ii) discourage the use of the name *C. hyoilei* in favour of *C. coli* by publishing recommendations in IJSEM in due time.

Discussions on the nomenclature and taxonomic status of '*Flexispira rappini*', '*Gastrospirillum*' spp. and '*Helicobacter heilmannii*' were also undertaken.

(i) '*Flexispira rappini*'. This name was originally given to strains from ovine abortions and canine diarrhoea that resembled *Campylobacter* spp. in their common growth requirements and biochemical characteristics but that showed a distinctive ultrastructure. Strains had subsequently been isolated from human diarrhoea. The name had never been validly published. Subsequent 16S rRNA gene sequence analysis had established that '*F. rappini*' strains belonged to the genus *Helicobacter*, but that the distinctive ultrastructure was not the property of a single taxon. Both *H. bilis* and *H. trogonum* demonstrated the same cell morphology. F. E. Dewhirst had submitted a manuscript to IJSEM describing nine phylogenetically distinct groups (including *H. bilis* and *H. trogonum*), each with the ultrastructural characteristics attributed to '*F. rappini*'. Therefore, the name '*F. rappini*' at present refers to a morphotype that represents a taxonomic complex of multiple species.

(ii) '*Gastrospirillum*' spp. and '*Helicobacter heilmannii*'. The synonymy between these two frequently used but colloquial names is well recognized. Unculturable bacteria found in the human gastric mucosa that are morphologically distinct from *H. pylori* in having a larger and more tightly coiled helical cell body have been referred to as both '*Gastrospirillum hominis*' and '*Helicobacter heilmannii*'. 16S rRNA gene sequence analysis shows two phylogenetically distinct variants in human gastric tissue. Similar unculturable bacteria have been found in pigs and a range of exotic carnivores, where they are commonly referred to as '*Gastrospirillum*' spp. Both *H. felis* and *H. bizzozeronii* also show the distinctive cell morphology common to the 'gastrospirilla'. Recent studies have indicated that the so-called type 1 variant of the human '*G. hominis*/*Helicobacter heilmannii*' organism may represent the same species as the pig organism, recently named '*Candidatus H. suis*'. At present, strains showing this phylogenetic affiliation have not been cultured. The '*G. hominis*/*Helicobacter heilmannii*' type 2 variant demonstrated a high sequence similarity with several species isolated from the gastric mucosa of dogs and/or cats, namely *H. bizzozeronii*, *H. felis* and *H. salomonis*. Furthermore, a cultured human strain of the '*H. heilmannii*' type 2 variant has been identified as one of *H. bizzozeronii*. It was suggested that the subcommittee prepares a report and/or recommendations concerning the nomenclature of '*Gastrospirillum*' spp. and '*Helicobacter heilmannii*' to ensure the wider scientific community are correctly oriented as to the present taxonomic status and possible zoonotic significance of these bacteria. P. Vandamme volunteered to initiate a paper on this subject at the end of 1999.

As a final issue concerning nomenclature, the subcommittee agreed to recommend that the same specific epithets used for new *Campylobacter* species are not used for *Helicobacter* species, to avoid confusion.

Minute 10. Other topics. (a) Matters arising from the previous meeting. Minute 6: publication of new taxa in journals other than the IJSEM. The letter to key publishers requesting clarification of their policy in this regard had not yet been sent. P. Vandamme further suggested that it would be difficult to address this issue in this way since many publishers were involved. It was thus decided to write to the International Union of Microbiological Societies (IUMS) in

order to 'strengthen' policy regarding peer review of papers published outside of the IJSEM that are subsequently sent to appear on its validation lists. One suggestion was that papers for validation be sent to members of the relevant subcommittee for review. P. Vandamme volunteered to prepare the letter to the IUMS, whilst S. L. W. On volunteered to send letters to key publishers expressing the subcommittees views on this issue.

(b) Taxonomic status of *H. nemestrinae*. The 16S rRNA gene of *H. nemestrinae* has been resequenced and reanalysed by F. E. Dewhirst, who commented on its high similarity to *H. pylori*. Similar observations on the biochemical (S. L. W. On) and protein (P. Vandamme) profiles of these taxa were also noted. It was agreed to re-examine the taxonomic position of *H. nemestrinae*. The G+C content would be redetermined by P. Vandamme. R. J. Owen agreed to analyse the fatty acid content of this species and collect additional taxonomic data from the other investigators.

(c) '*H. westmeadii*'. The taxonomic position of this organism as a potentially distinct species had been questioned in a polyphasic taxonomic study involving P. Vandamme and S. L. W. On but the details were at present confidential. A report in an international journal was however expected in due course.

(d) '*H. suncus*'. F. E. Dewhirst remarked on the quality of this description and suggested that it should be validated. However, none of the subcommittee members were involved with its initial description.

(e) Species diversity in HIV-positive children. A. J. Lastovica reported on a recent initiative at the Red Cross Hospital, South Africa, to investigate the prevalence of *Campylobacter* and related bacteria in diarrhoea samples of 600 HIV-positive children. Animals would also be examined to assess possible sources of infection. Dr Lastovica remarked that this would undoubtedly result in a rich source of strains for other scientists to collaborate on.

(f) Topics for the open meeting. An agenda for the open meeting of the subcommittee was agreed.

Minute 11. Next meeting. The next closed meeting of the subcommittee will be held in 2000. It was agreed to ballot all members to identify the preferred time and venue for holding the 2000 meetings.

Minute 12. Adjournment. The meeting was adjourned at 18:30 on 13 September 1999.

Session 2. Open meeting

Minute 1. Call to order. The Chairman, P. Vandamme, called the meeting to order at 8:30 on 16 September 1999 in the St George Room at the Renaissance Harbourplace Hotel in Baltimore, USA.

Minute 2. Record of attendance. The members present were P. Vandamme (Chairman), A. J. Lastovica, A. Lee, S. L. W. On (Secretary) and R. J. Owen. Apologies for absence were received from J.-P. Butzler, F. E. Dewhirst, J. G. Fox, F. Mégraud, T. J. Trust and I. V. Wesley.

Minute 3. Description of activities, and area of interest, of the subcommittee. The Chairman described the activities undertaken by the subcommittee to the delegates and outlined its area of interest.

Minute 4. Taxonomic status of '*Flexispira rappini*', '*H. heilmannii*' and '*Gastrospirillum*' spp. P. Vandamme related

to delegates the taxonomic and nomenclatural problems concerning these bacteria (as summarized above). It was further noted that two different nomenclatural proposals for what appeared to be the same species had been made during the host conference from different research groups, namely '*Candidatus H. suis*' and '*Candidatus H. heilmannii*'. The former name has been validly published. Both proposals referred to the so-called '*H. heilmannii*' type 2 group. A. Lee reported that his research group had obtained 20 16S rRNA gene sequences from various animal species (including the New Guinea wild dog and the red-fronted lemur) that showed a high similarity to those defined as '*H. heilmannii*' type 2. As a consequence, A. Lee felt that the name '*Candidatus H. suis*' may not appropriately reflect the wide host range of the candidate organism. It was agreed that this nomenclatural issue should be resolved and that only one name should be used to refer to these bacteria. The description of a '*Candidatus H. suis*'-specific assay by the authors would assist in resolving the prevalence of this organism in diverse animal species. The delegates agreed that the matter needed to be communicated to the wider scientific community. The importance of the matter was emphasized by A. Lee who reported a higher percentage of gastric lymphomas in infections with this bacterium.

Minute 5. Identification of *Campylobacter* and related bacteria. P. Vandamme presented a brief overview of the methods currently used to identify member taxa of the ϵ subclass of the *Proteobacteria*. Extensive phenotypic analysis, numerical comparison of whole-cell protein patterns, PCR-RFLP analyses and 16S rRNA gene sequence comparisons were all considered to be useful, but not always

infallible, tools for identification. R. J. Owen remarked on the potential of cellular fatty acid profiles for differentiating *Helicobacter* spp., a method which he considered had been somewhat overlooked for this purpose. Particular note was made of the use of 16S rRNA sequence comparisons, where the dangers in relying on a threshold similarity value of 97% to delineate species was discussed with special reference to *C. hyointestinalis* and *H. cinaedi* (discussed above). A. J. Lastovica mentioned his collection of unusual strains, many of which had not been identified to the species level, as an example of the problems in this area. The delegates agreed that collaboration in this area would be most beneficial and all members of the subcommittee present expressed their willingness to share their expertise to help to identify problem strains.

Minute 6. Questions from the delegates. A delegate asked if it was felt acceptable to allow the description of a new species based on the isolation of five strains from a single animal colony, or similar source material. The consensus opinion of the subcommittee was that such a description was not acceptable.

A delegate remarked on the problems of dealing with strains that demonstrated a swarming morphology and that did not appear to form colonies. Such cultures could contain more than one bacterial species and presented difficulties to workers aiming to accurately determine the prevalence of member taxa, and to those working in taxonomy.

Minute 7. Adjournment. The meeting was adjourned at 9:50 on 16 September 1999.

Stephen L. W. On, *Secretary*