Minutes

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International Committee on Systematic Bacteriology

Subcommittee on the taxonomy of *Brucella*

Minutes of the meeting, 5 and 7 July 1994, Prague, Czech Republic

Minute 1. Call to order and record of attendance. The Subcommittee met in open session on 5 July 1994 and in closed session on 7 July 1994. The Chairman called the meeting to order. The following members were present: G. Gargani (Chairman), A. López-Merino (Secretary), M. Banai, M. J. Corbel, T. A. Ficht and I. Moriyón. Those present were considered to represent a quorum and able to make proposals on behalf of the Subcommittee taking into account proposals from the other members.

Minute 2. Report of previous meeting. Because of the poor attendance, no meeting was held at the Sixth IUMS Congress in Osaka, Japan, in 1990. On the basis of information received from the membership, the Chairman and Secretary had prepared an Interim Report. This was accepted with minor amendments. The report is published as a supplement to these minutes [Gargani & López-Merino, *Int J Syst Evol Microbiol* 56 (2006), 1167–1168].

Minute 3. Past activities. Since the last meeting, the Subcommittee has been active by correspondence. Relevant publications produced by the membership are listed in the Interim Report [Gargani & López-Merino, *Int J Syst Evol Microbiol* 56 (2006), 1167–1168]. In addition, several major conferences dealing with *Brucella* were held during this interval: a symposium entitled *Brucella and Brucellosis in Man and Animals* held in Izmir, Turkey, 24–26 September 1991; the 91st, 92nd, 93rd and 94th General Meetings of the American Society for Microbiology held in 1991, 1992, 1993 and 1994 in various venues in the USA; and an International Symposium on Advances on Brucellosis held in Mexico City, 10–11 March 1994.

Minute 4. Molecular genetic studies on *Brucella*. Very substantial progress has been made in this area in the past 4 years. The phylogenetic relationship of the genus to other groups of bacteria has begun to emerge. It is now evident from rRNA sequencing and other studies that *Brucella* is a member of the α -subclass of the *Proteobacteria*. It is related to the genera *Agrobacterium*, *Rhizobium* and *Phyllobacterium*, but the closest known relationship is to *Ochrobactrum anthropi*. There is also an affiliation to *Afipia*, *Bartonella* and *Rochalimaea* on the basis of rRNA sequence data. Evidence is emerging that the genetic relationship may be reflected in antigenic cross-reactions over a wider range than previously described. The

Subcommittee recommended that the position of *Brucella* in relation to the other members of the α -subclass should be defined more precisely.

The chromosome structure of *Brucella melitensis* has been determined to be similar to that of other members of the *Rhizobium*–*Agrobacterium* group. *B. melitensis* has two chromosomes [Michaux *et al.*, *J Bacteriol* 175 (1993), 701–705]. Both contain housekeeping genes and hence are essential for survival and replication. There is a need to extend these observations to other nomenclatures (unpublished studies have confirmed a similar genetic structure for *Brucella abortus*). However, this report opens the way for detailed genetic mapping which should clarify the relationships within the genus.

The Subcommittee noted the absence of a clear definition for the concept of species and biovars within the genus. Furthermore, the emerging data on phylogenetic relationships support the suggestion that the definition of the genus may itself require re-examination.

In the light of evidence from DNA hybridization and other studies, it is accepted that *Brucella* comprises a single genomic species. However, there is disagreement over the nomenclature to be adopted at the subgenus level. The nomenclature proposed by the Manchester meeting has not found widespread support. Members suggested that the current nomenclature for nomenclatures, which may be equivalent to ecospecies, should be retained until further genetic evidence emerges.

In relation to biovars, the present position was considered unsatisfactory. The biovars were defined on the basis of empirical observations using phenotypic characteristics which have proved of epidemiological value in the past. More recent studies using ribotyping, PCR, RAPD analysis and pulsed-field gel electrophoresis have provided additional information. At present, no clear picture has emerged; different groups have employed different sequences and the results are not directly comparable. There is a need to compile and evaluate this information. For particular sequences or probes to be acceptable for classification purposes, they must be readily available. The technology will also be applicable in routine typing laboratories.

Minute 5. Standardization of typing reagents and procedures. Although details of typing procedures using

conventional methods have been published on numerous occasions and are generally accepted, there is difficulty in obtaining standard reagents. Monospecific sera to A and M epitopes are difficult to obtain and there is concern over consistency. It was considered that sources of monoclonal antibodies to A and M epitopes should be identified. Ideally, International Reference Preparations of A- and M-specific antibodies should be established. Problems with supply and consistency of dyes (thionin and basic fuchsin) were also noted. It seems improbable that standard preparations of these will become available and the need to develop and validate alternative methods for biovar differentiation was emphasized. Changes in the behaviour of some phages have been quoted. They need to be subjected to careful evaluation of their lytic and host properties. It was recommended to establish banks of controlled phages in some laboratories. A. López-Merino and M. Banai agreed to set up some reference phage seed stocks.

Minute 6. *B. abortus* biovars 3 and 6. The position with regard to these was unsatisfactory. Because many strains do not correspond exactly to the original definitions of these biovars, emphasis has been placed on thionin sensitivity. This has blurred the differentiation of these biovars. Recent studies using ribotyping and probes specific for OMP genes have suggested that differentiation is achievable. The Subcommittee mentioned that these studies should be extended and that the definition of *B. abortus* biovar 3 and 6 should be re-examined.

Minute 7. Atypical *B. melitensis* strains. There was an increasing problem of atypical strains of *B. melitensis*; infections in cattle are now much more frequent than with *B. abortus* in some countries. There were also indications that vaccine-derived virulent strains may be occurring in some areas [Banai *et al.*, *J Clin Microbiol* **28** (1990), 1057–1059]. The current methodology was inadequate for characterization of these isolates. Molecular studies were being conducted on these and should be extended.

Minute 8. Strains from marine mammals. Several strains from marine mammals: dolphin, seals and porpoise cetaceans were isolated from around the coast of

Scotland. They did not fit into the current classification. On the basis of host animals, they were placed in three groups, with reference to papers in draft and published after the meeting [Ross *et al.*, *Vet Rec* **134** (1994), 359; Jahans *et al.*, *Vet Microbiol* **57** (1997), 373–382].

Minute 9. *Brucella canis* in China. In the last few years, an increasing number of *B. canis* isolates have been recorded in China. Around 23 % of them grew on thionin and fuchsin, as rough *B. melitensis* did (Huang Jian, personal communication). Based on these observations, it seems necessary to improve the identification methods in order to differentiate future isolates from China and in other countries.

Minute 10. Other matters. Finally, the members of the Subcommittee expressed recommendations to be taken into account for future research: homology of porin genes of *Rhizobium* and *Brucella*, ribosomal protein homology, use of standard nomenclature for gene classification, status of *B. abortus* biovars 3 and 6 in relation to genetic data, use of phagotypes as epidemiological markers and a better characterization of rough strains.

Minute 11. Election of officers and current membership. The Subcommittee thanked G. Gargani and A. López-Merino for their efforts in promoting its activities during their time of office. A. López-Merino was proposed as Chairman and M. Banai as Secretary for the forthcoming term. The current membership of the Subcommittee is: A. López-Merino (Chairman) (Mexico), M. Banai (Secretary) (Israel), E. S. Broughton (UK), M. J. Corbel (UK), F. Crespo León (Spain), E. A. Dranovskaya (Russia), T. A. Ficht (USA), Huang Jian (China), Y. Isayama (Japan), I. Moriyón (Spain), M. Ramuz (France) and J. M. Verger (France). Corresponding members: W. J. Brinley-Morgan (UK) and C. Rigby (Canada). New members proposed included E. Moreno (Costa Rica), N. Lucero (Argentina), S. El Gibally (Egypt) and X. Wang (China).

Minute 12. Next meeting. The membership recommended that more frequent meetings should be held. The annual Chicago meeting on brucellosis was suggested as an alternative. Other possibilities will be considered.