Correspondence Report

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International Committee on Systematic Bacteriology

Subcommittee on the taxonomy of *Brucella*

Correspondence Report (Interim Report), 1991–1993

Introduction. The *Brucella* Subcommittee has decided to publish this Correspondence Report for 1991–1993 in order to document the history of the Subcommittee on the Taxonomy of *Brucella*. This interim report, written by G. Gargani (Chairman) and A. López-Merino (Secretary) and presented to the Subcommittee meeting held in Prague in 1994, completes the inter-meeting history of the *Brucella* Subcommittee; it is therefore published as a supplement to the minutes from the 1994 meeting [Corbel & Moriyón, *Int J Syst Evol Microbiol* 56 (2006), 1169–1170].

The meeting of the Subcommittee in Manchester in 1986 was attended by just two members (M. J. Corbel, UK, and J. M. Verger, France), who prepared a report on the basis of correspondence received from others. Very few members also attended the meeting in Osaka (1990; no report prepared). However, a correspondence report is now prepared, summarizing data received after the Manchester meeting.

Recent activities. The members have been active by correspondence. Significant publications include chapters by M. J. Corbel [Microbiology of the genus *Brucella*. In *Brucellosis: Clinical and Laboratory Aspects* (1989), pp. 53–72. Edited by E. J. Young & M. J. Corbel. Boca Raton, FL: CRC Press; Microbiological aspects. In *Brucellosis* (1989), pp. 29–44. Edited by M. M. Madkour. London: Butterworths; DNA analysis of *Brucella* species: an update. In *Brucella and Brucellosis in Man and Animals* (1991), Turkish Microbiological Society publication no. 16, pp. 11–25. Edited by E. Tümbay, S. Hilmi & Ö. Ang. Reading, UK: FEMS], by E. A. Dranovskaya [New approach to brucellosis vaccination of people with high risk of infections. *ibid.* pp. 87–100] and by G. Gargani [Il genere *Brucella*. In *I problemi della Moderna Biologia: Ecologia Microbica, Analitica di Laboratorio, Biotecnologia* (1991). Edited by H. Grimme, E. Landi & S. Dumontet. Sorrento: Ordine Nazionale dei Biologi (in Italian)].

A meeting of FEMS, organized by the Turkish Microbiological Society, was held in Izmir in September 1991. WHO/FAO Expert meetings were also held in Rome (September 1988), Geneva (April 1990), Rome (October 1991) and Geneva (June 1992), concerned particularly with epidemiology and with comparison of the Chinese strain 2 (brucellosis vaccine) and *Brucella suis* biovar 1.

Nomenclature and classification. The Manchester report assumed the paper by Verger *et al.* [*Int J Syst Bacteriol* 35 (1985), 292–295] on DNA hybridization studies and the proposition that all *Brucella* are just one species, with biovars; it was necessary to reclassify *Brucella abortus* biovar 9 as *Brucella melitensis* biovar Abortus 7, following deletion of biovars 7 and 8. It is remarkable that, according to the Manchester nomenclature, all 'biovars' Melitensis 1–3, Abortus 1–7 and Suis 1–5 were assigned to the same level of differentiation, irrespective of previous nomenclatures; this is undoubtedly correct based on genome studies, but misleading for brucellosis epidemiology considering the relatedness of nomenclatures with host animals and geographical spreading.

Moreover, McGillivray *et al.* [*Res Vet Sci* 45 (1988), 251–252] found that the restriction endonuclease profiles produced by *Bam*HI from DNA of five *Brucella abortus* isolates and the reference strain *B. abortus* biovar 2 were very similar. These results reinforced the existence of significant genetic homogeneity of the genus *Brucella*.

The report also emphasizes the relatedness of genus *Brucella* with the genera *Agrobacterium*, *Phyllobacterium* and *Rhizobium*. De Ley *et al.* [*Int J Syst Bacteriol* 37 (1987), 35–42] identified *B. abortus* as a member the alpha-2 subgroup of the *Proteobacteria* on the basis of 16S rRNA gene sequence comparison and Moreno *et al.* [*J Bacteriol* 172 (1990), 3569–3576] suggested a close phylogenetic relationship with the same group as a result of studying the composition of *Brucella* lipid A; later, Corbel [DNA analysis of *Brucella* species: an update. In *Brucella and Brucellosis in Man and Animals* (1991), Turkish Microbiological Society publication no. 16, pp. 11–25. Edited by E. Tümbay, S. Hilmi & Ö. Ang. Reading, UK: FEMS] also published a dendrogram showing relatedness with *Mycoplana*.

A genus-specific probe obtained in the Gamaleya Institute cross-reacted with representative strains of the genus, but not with species considered as serologically related (*Yersinia enterocolitica* O:9, *Yersinia pseudotuberculosis*, *Salmonella typhimurium*, *Vibrio cholerae*, *Francisella tularensis*, *Escherichia coli* O:157, *Pseudomonas putida*, *Rickettsia prowazekii*) [Gorelov *et al.*, The new tool for *Brucella* identification: genus specific DNA probes. In *Advances in Brucellosis Research* (1990), p. 490. Edited by L. G. Adams. College

Station, TX: Texas A&M University Press; Kulakov *et al.*, *Mol Gen Mikrobiol Virusol* issue 7–8 (1992), 23–27 (in Russian)].

Chromosome organization. Attempts made by the Nouzilly group [Verger *et al.*, *Ann Inst Pasteur Microbiol* **138** (1987), 235–238; Grimont *et al.*, *Res Microbiol* **143** (1992), 55–65] at better definition through the study of gene restriction patterns met with a lot of difficulty because of the exceedingly large number of restriction fragments, which gave almost the same pattern in all tested strains quite independently of their assignment to any of the six previous nomenspecies and of the epidemiological source. However, the utilization of an enzyme with low cleavage frequency allowed Allardet-Servent *et al.* [*J Bacteriol* **170** (1988), 4603–4607] to identify a specific pattern for five of the six nomenspecies and several patterns within the biovars of *B. melitensis*, *B. abortus* and *B. suis* (too few strains were studied; therefore, the results are not sufficient to support the existence of several species).

Allardet-Servent *et al.* [*J Bacteriol* **173** (1991), 2219–2224] estimated from the *SpeI* restriction pattern of the *B. melitensis* 16 M DNA that the genome size was about 3130 kb, which corresponds to a molecular mass of 1.9×10^9 ; the genome size of *B. abortus* was estimated previously as 2600 kb (2.61×10^9).

An insertion sequence was identified at locus BCSP31 in *Brucella ovis* by Halling & Zehr [*J Bacteriol* **172** (1990), 6637–6640]; they reported that the DNA polymorphism is due to this insertion sequence.

The polymorphism of locus *omp2*, with two genes *omp2a* and *omp2b*, which encodes a large protein of the outer membrane, offered the opportunity to identify five groups within the six species: group 1, *B. abortus* 1, 2 and 4; group 2, *B. abortus* 3, 5, 6 and 9 (now 7); group 3, *B. melitensis*; group 4, *B. suis*, *B. canis* and *B. neotomae*; group 5, *B. ovis*. The thionin-resistant groups 2, 3, 4 and 5 have a sequence of 120 bp in the *omp2a* gene that is not present in the thionin-sensitive group 1 [Ficht *et al.*, *Mol Microbiol* **4** (1990), 1135–1142]. It is evident that these

results are important for *Brucella* nomenspecies and biovar identification.

Phenotypic identification. In the range of classical biovars, some special marker was identified. Thionin-sensitive *B. melitensis* strains, previously reported by Gargani & Tolari [*Eur J Epidemiol* **2** (1986), 67–79] and Banai *et al.* [*J Clin Microbiol* **28** (1990), 1057–1059], were confirmed by Corbel [*J Clin Microbiol* **29** (1991), 1066–1068] in strains from six different countries. This observation must be correlated with those on variability of *omp2* gene by Ficht *et al.* [*Mol Microbiol* **4** (1990), 1135–1142]. Huang Jian [personal communication; journal unknown] reported the identity of 200 *B. canis* strains from China with the reference strains, also calling attention to the frequency of this nomenspecies in that country.

The report of the WHO working group meeting on oral/conjunctival brucellosis strain 2 vaccine (Rome, 2–4 October 1991; http://whqlibdoc.who.int/hq/1992/WHO_CDS_VPH_92.101.pdf) differentiated between *B. suis* strain 2 (Chinese vaccine strain) and *B. suis* biovar 1 strains by substrate-mediated tetrazolium reduction.

Rigby *et al.* [*Can J Vet Res* **53** (1989), 319–325] demonstrated that *Brucella* phages Tb, Fi, Wb, Iz and Np belonged to the same species, even though they come from different geographical areas. As the *Brucella* phages provide a simple and rapid means for identification of nomenspecies, more work is clearly needed on the phage genetics as well as their relations with virulence mechanisms of *Brucella*.

Current membership of the subcommittee – 1993.

The current membership of the Subcommittee is G. Gargani (Chairman) (Italy), A. López-Merino (Secretary) (México), M. Banai (Israel), E. S. Broughton (UK), M. J. Corbel (UK), F. Crespo León (Spain), R. Díaz (Spain), E. A. Dranovskaya (Russia), T. A. Ficht (USA), C. García Carrillo (Argentina), Huang Jian (China), Y. Isayama (Japan), Lu Shi-Liang (China), I. Moriyón (Spain), J. Payeur (USA), M. Ramuz (France) and J. M. Verger (France). Corresponding members are W. J. Brinley Morgan (UK), C. Rigby (Canada) and B. Stemshorn (Canada).