International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Leptospira*

Minutes of the Meeting, 6 to 10 August 1982, Boston, Massachusetts

Session 1. Closed meeting

Minute 1. Call to order. The closed session of the meeting was called to order by the Chairman, S. Faine, at 19:00 on 6 August; this was followed by an open session at 20:00. Further open sessions were held at 14:00 on 8 August and at 14:00 on 10 August.

Minute 2. Record of attendance. Members present were: S. Faine (Chairman), N. Stallman (Secretary), A. Alexander, C. Cox, H. Dikken, R. Johnson, and R. Yanagawa. In addition to members of the Subcommittee, the following attended one or more of the open sessions: V. Bezjak, B. Cacciapuoti, C. Everard, K. Hovind-Hougen, M. Mailloux, R. Marshall, A. Schonberg, O. Stalheim, W. Terpstra, W. Van Eseltine, and S. Waitkins.

Minute 3. Minutes of the previous meeting. The minutes of the meeting held in Munich, Federal Republic of Germany, on 3 to 6 September 1978 and circulated in February 1979 (Int. J. Syst. Bacteriol. 32:245-247, 1982) were accepted as a true record of the meeting.

Minute 4. Changes in membership and officers. The Subcommittee recorded with deepest regret the death of Masami Kitaoka, who was an adviser and former member. As a mark of respect, a minute of silence was observed. A. Alexander was elected Chairman, and R. Yanagawa was elected Vice-Chairman. K. Hovind-Hougen, M. Mailloux, R. Marshall, and C. Sulzer were elected members. The lists of observers and collaborators were reviewed. B. Adler, V. Kiktenko, W. Terpstra, and S. Waitkins were elected observers. Y. Yanagihara was elected a collaborator.

Minute 5. Report on the activities of the Subcommittee. A report of Subcommittee activities for the period from 1979 to 1982 was read and accepted.

Minute 6. Appreciation expressed. The Chairman expressed his appreciation and that of the Subcommittee for the extensive time and effort expended by the committee which prepared a draft of the catalog of published serovars.

Minute 7. Preparation of the catalog of published serovars. A discussion was held on whether serovars that have not been adequately published should be given provisional status in the catalog. The matter was not resolved, and an ad hoc committee consisting of A. Alexander, H. Dikken, R. Johnson, R. Marshall, N. Stallman, W. Terpstra, and S. Waitkins was formed to investigate the matter further. This committee was also requested to prepare a revised draft of the catalog.

Minute 8. Method of listing serovars in the catalog. It was agreed that serovars should be listed alphabetically in their serogroups and that the serogroups should also be listed alphabetically in the catalog.

Minute 9. Revision of serogroups. It was agreed that the Autumnalis serogroup should be divided into three serogroups (Autumnalis, Djasiman, and Louisiana) and that the Hebdomadis serogroup also should be divided into three serogroups (Hebdomadis, Sejroe, and Mini).

Minute 10. List of strains of *Leptospira biflexa*. It was agreed that the lists of serovars of *Leptospira biflexa* submitted to the Subcommittee by M. Cinco should be accepted.

Minute 11. Record of culture collections. The Secretary was requested to write to laboratories to obtain lists of serovars and strains held in culture collections for the purpose of compiling a directory.

Minute 12. Alternative taxonomic criteria. Short papers were presented on alternative taxonomic criteria, as follows.

R. Marshall described an alternative method for the identification of leptospires based on restriction endonuclease analysis of the deoxyribonucleic acids. In studying the Pomona serogroup, he compared the patterns of reference strains and concluded that *pomona*, *monjakov*, *kennewicki*, and *cornelli* were the same. When *mozdok* and *dania* were compared, their patterns were shown to be the same as each other but different from that of *pomona*. Both *proechimys* and *tropica* gave unique patterns.

The Subcommittee agreed that the method described by Marshall was an extremely useful technique for the identification of leptospires, and it was recommended that other serogroups be investigated. It was also recommended that R. Marshall examine the possibility of differentiating *Leptospira interrogans* from *L. biflexa* by this method.

R. Yanagawa presented a brief review of leptospiral antigens. He suggested that to assist in solving some of the problems, chemically defined serovar-specific haptenic compounds are required. If these were available, the classification of leptospires could be done by a variety of serological methods.

Minute 13. Antigenic variation. A discussion was held on whether there is any loss of agglutinability in serovars when they are cultured in EMJH medium. The Subcommittee agreed that no loss of activity has been detected.

Minute 14. Family Leptospiraceae. The Subcommittee concurred with the *Spirochaetales* Subcommittee regarding the formation of the new family *Leptospiraceae* within the order *Spirochaetales*, which includes the genus *Leptospira*.

Minute 15. Recognition of new serovars. The Subcommittee discussed the previously recorded guidelines for the acceptance of new serovars. It was agreed that strains published as representing new serovars should be recognized if they are typed by the classical absorption method in at least one reference laboratory.

Minute 16. Recognition of reference laboratories. It was agreed that laboratories should be recognized as reference laboratories only if they are designated by the Subcommittee. The list of laboratories should be reviewed every 4 years. The laboratories of the following individuals were approved as reference laboratories: N. D. Stallman, Brisbane, Queensland, Australia; E. Kmety, Bratislava, Czechoslovakia; M. Mailloux, Paris, France; S. Waitkins, Hereford, England; W. Terpstra, Amsterdam, The Netherlands; M. Torton, Ness Ziona, Israel; B. Cacciapuoti, Rome, Italy; M. Cinco, Trieste, Italy; C. Sulzer, Atlanta, Ga.; and Y. G. Chernukha, Moscow, Union of Soviet Socialist Republics.

Minute 17. Differentiation of species. It was agreed that there is a need to define criteria which can be used to distinguish the species *L. interrogans* and *L. biflexa*. A. Alexander and R. Johnson were delegated to prepare a

statement specifying differential characteristics.

Minute 18. *Species identification of isolates.* It was recommended that the species identification of isolates be checked before proceeding to serological classification.

Minute 19. *Standardization of methods for the identification of serovars.* The guidelines described below were recommended for the identification of serovars.

(i) *Preparation of rabbit hyperimmune serum.* Healthy rabbits weighing at least 2 kg but preferably 3 to 4 kg are selected. These are pretested for leptospiral antibodies, and only those in which no antibodies are detected are used.

Each rabbit is injected intravenously in the marginal vein of an ear with a well-growing live of formalin-treated cloned culture with a density of approximately 2×10^8 leptospores per ml. The culture should be grown in rabbit serum-peptone medium or another appropriate medium. Five injections of 1, 2, 4, 6, and 6 ml are given at 7-day intervals. One week after the final injection, a sample of blood is withdrawn from the marginal ear vein of the rabbit, and the homologous titer is determined. If the titer is at least 1:12,800, the rabbit is bled by cardiac puncture 7 days later (i.e., 14 days after the final injection).

If the titer is less than 1:12,800, a further injection of 6 ml of culture can be given; 7 days after this injection, the homologous titer is again determined. Unless the titer is at least 1:12,800, the procedure should be repeated with a fresh rabbit.

Two rabbits are used to prepare antisera. If the titers are satisfactory in both, the sera may be pooled.

To preserve potency, it is preferable to freeze-dry the antiserum in 2-ml amounts and store it at 4°C. Alternatively, it can be stored in 2-ml amounts at -20°C.

(ii) *Microscopic agglutination test.* Well-growing live cloned cultures with densities of approximately 2×10^8 leptospores per ml are used as antigens.

Serial two-fold dilutions of serum in phosphate-buffered saline (pH 7.2) starting at 1:25 are prepared. Equal amounts of antigen and diluted serum are mixed and incubated 1.5 to 4 h at 30°C. The serum-antigen mixtures are then examined by dark-field microscopy for agglutination. The endpoint is defined as that dilution of serum which shows 50% agglutination, leaving 50% free cells compared with a control culture diluted 1:2 in phosphate-buffered saline.

(iii) *Agglutinin absorption test.* Rabbit antiserum with a titer ranging between 12,800 and 51,200 is used for the agglutinin absorption test.

To prepare the absorbing antigen, 150 ml of a well-growing live cloned culture with a density of approximately 2×10^8 leptospores per ml is centrifuged at $12,000 \times g$ for 30 min. The supernatant is discarded, and the sediment is retained.

For absorption the sediment is suspended in 2.4 ml of phosphate-buffered saline. The suspension is added to 0.1 ml of serum in three equal amounts at intervals of 10 min. The

mixture is incubated for 1.5 to 4 h at 30°C, after which it is centrifuged at $12,000 \times g$ for 30 min. The supernatant, which constitutes the absorbed serum, is retained.

The absorbed serum should have a residual titer of 0.5 to 1% of the preabsorption titer against the absorbing strain to ensure that neither over- nor underabsorption has taken place. If the residual titer exceeds 1%, the absorption test should be repeated.

(iv) *Definition of serovar.* Two strains are considered to belong to different serovars if, after cross-absorption with adequate amounts of heterologous antigen, 10% or more of the homologous titer regularly remains in at least one of the two antisera in repeated tests.

Minute 20. *Publication of new serovars.* The Subcommittee recognized that publication of papers in which new serovars are described is not always favorably considered by editors of journals because of the large amount of supporting tabulated data. As an alternative to overcome this problem, it was recommended that when a new serovar is proposed, details be submitted to the Secretary, who will circulate the information to members of the Subcommittee for approval. If the proposal is accepted, the Secretary will submit the details to the *International Journal of Systematic Bacteriology* for publication.

Minute 21. *Funding for data collection.* It was recommended that the Secretary, on behalf of the Subcommittee, approach the International Committee on Systematic Bacteriology for funds to support collection of data on new serovars.

Minute 22. *Report of ad hoc committee.* The ad hoc committee referred to above (see Minute 7) met and submitted a revised draft of the catalog of published serovars to the Subcommittee.

Minute 23. *Expression of appreciation.* The Subcommittee expressed its thanks to the retiring Chairman, S. Faine, for his work during the past 9 years.

Minute 24. *Current membership.* The current membership of the Subcommittee is as follows: A. D. Alexander (Chairman), Chicago, Ill.; R. Yanagawa (Vice-Chairman), Sapporo, Japan; N. D. Stallman (Secretary), Brisbane, Queensland, Australia; T. G. Chernukha, Moscow, Union of Soviet Socialist Republics; M. Cinco, Trieste, Italy; C. D. Cox, Amherst, Mass.; H. Dikken, Groningen, The Netherlands; S. Faine, Melbourne, Victoria, Australia; K. Hovind-Hougen, Copenhagen, Denmark; R. C. Johnson, Minneapolis, Minn.; E. Kmetz, Bratislava, Czechoslovakia; M. Mailloux, Paris, France; R. Marshall, Palmerston North, New Zealand; C. Sulzer, Atlanta, Ga.; and M. Torten, Ness-Ziona, Israel.

The final session was closed by the Chairman at 18:00 on 10 August 1982.

N. D. STALLMAN, *Secretary*