REPORT TO THE INTERNATIONAL COMMITTEE ON
NOMENCLATURE OF BACTERIA BY THE
SUBCOMMITTEE ON TAXONOMY OF BRUCELLAE
MINUTES OF MEETING, JULY 1966

The Subcommittee on Taxonomy of Brucella met on July 22 and 23, 1966 at the Gamaleya Institute in Moscow prior to the IXth International Congress for Microbiology. Members present were Dr. A. W. Stableforth (Chairman) (Turkey), Dr. Lois M. Jones (Secretary) (USA), Dr. Margaret Meyer (USA), Dr. W. J. Brinley Morgan (England), Dr. N. B. McCullough (USA), Dr. R. Housewright (USA), Prof. P. A. Vershilova (USSR), and Prof. W. Wundt (Germany). Dr. M. Kaplan, Dr. M. M. Ivanov, Dr. E. I. Kajtmazova, Dr. N. N. Ostrovskaya and Dr. T. G. Todorov attended as observers at the invitation of the chairman.

Since the last meeting of the Subcommittee held in Montreal in 1962, there have been some changes in the membership of the Subcommittee. Prof. G. S. Wilson has retired. Dr. M. E. Meyer and Dr. W. J. B. Morgan were co-opted by the chairman and secretary as temporary members. At the Moscow Congress their names were submitted to the International Committee on Nomenclature of Bacteria, and they were accepted as ordinary members. Dr. H. D. Housewright said he was no longer actively engaged in Brucella work and, therefore, regretfully asked to be removed from the membership list. The Subcommittee elected Prof. W. Wundt as the new Chairman, and Dr. Jones was reelected Secretary.

The Subcommittee reviewed published work as well as unpublished written evidence sent to it on the working and on the merits of the taxonomic scheme proposed in 1962 (Intl. Bull. Bact. Nom. Taxon. 13:145-158, 1963). The general opinion was that the scheme had worked well and had been of particular value in the examination of strains whose taxonomic position had been in question and which were the common or predominant types in certain areas.

There was general agreement on the QO_2N values obtained in the oxidative metabolic tests in a number of different laboratories. A few exceptions were noted, e.g., the QO_2N values on l-lysine and l-glutamic acid were higher for some strains of B. suis than had previously been reported, and a few strains of B. abortus had lower rates on l-asparagine and l-glutamic acid. It was recommended that the full range of twelve substrates should be used. These are: d-alanine,

l-asparagine, l-glutamic acid, dl-citrulline, l-lysine, l-arginine, dl-ornithine, l-arabinose, d-galactose, d-ribose, d-xylose and l-alanine.

There was agreement on the results obtained with phage typing. The Subcommittee reaffirmed that the TB phage be the reference phage (type abortus strain 3 phage is the same as TB phage), using it at routine test dilution (RTD) and 10,000 x RTD and using either B. abortus strain 544 or 19 as propagating strain. It recommended that the results be recorded as: 4+ = complete lysis, 3+ = nearly complete lysis or confluent plaques, 2+ = lysis of about 50% of the culture population. Lesser degree of lysis should be considered as negative. It also recommended that the phage be propagated and used for typing as described in Annex 3 of the 4th Report of the FAO/WHO Expert Committee on Brucellosis.

The use of other tests such as inhibition of growth in presence of safranin O and determination of certain enzyme activities, such as oxidases, deaminases, transaminases, etc., were discussed. It was decided that further investigations must be made before their value can be assessed.

Some minor changes were recommended for the table of biotypes (see revised table). Carbon dioxide-requiring B. abortus biotype 3 cultures are more widely distributed than CO₂-independent cultures; therefore the table has been modified to read "usually positive, negative varieties occur." Many B. abortus biotype 6 cultures produce H₂S, and therefore H₂S production was made a variable character.

The Subcommittee recommended a change in the definition of the genus Brucella—the last sentence to read: "Facultative intracellular parasites which produce acute and chronic infection in animals and which may cause disease in man. The generalized infection is followed by localization which, in animals, is predominantly in the mammary gland and the reproductive tract."

At the last meeting of the Subcommittee the decision on the taxonomic position of Brucella ovis (Buddle, J. Hyg. 54: 351-364, 1956) as a member of the genus Brucella and the question of Brucella neotomae (Stoenner and Lackman, Am. J. Vet. Res. 18:947-951, 1957) as a separate species were deferred until further investigations had been made. Since then a new member of the genus Brucella, B. rangiferi tarandi (Davydov, Trudy vsesoyuz. Inst. eksp. Vet. 27:24-31, 1961), has been brought to the attention of the Subcommittee.

Additional studies have been made on the organism referred to as the ram epididymitis organism or Brucella ovis.

Table 1. Differential characters of Brucella species and biotypes.

Species	Type	CO ₂ re- quirement	H ₂ S pro- duction	Growth on dyes ¹			Aggl. mono- spec. sera	Phage Tb at RTD	Metabolic tests ²				Most common host reservoir
				thionin a b c	basic fuchsin b c				Gluc- acid	Orni- thine	Ri- bose	Ly- sine	
<u>B. melitensis</u>	1	-	-	- + +	+ + +		-	-	+	-	-	-	sheep, goats
	2	-	-	- + +	+ + +		+	-	+	-	-	-	"
	3	-	-	- + +	+ + +		+	-	+	-	-	-	"
<u>B. abortus</u>	1	+(-) ³	+	- - -	+ + +		+	+	+	-	+	-	cattle
	2	+	+	- - -	- - -		+	-	+	-	+	-	"
	3	+(-)	+	+ + +	+ + +		+	-	+	-	+	-	"
	4	+(-)	+	- - -	+ + +		-	+	+	-	+	-	"
	5	-	-	- + +	+ + +		-	+	+	-	+	-	"
	6	-	- or +	- + +	+ + +		+	-	+	-	+	-	"
<u>B. suis</u>	7	-	- or +	- + +	+ + +		+	+	+	-	+	-	"
	8	+	-	- + +	+ + +		-	+	+	-	+	-	"
	9	- or +	+	- + +	+ + +		-	+	+	-	+	-	"
<u>B. suis</u>	1	-	++	+ + +	- - -		+	-	-(+)	+	+	+	pigs
	2	-	-	- + +	- - -		+	-	+	+	+	-	pigs, hares
	3	-	-	+ + +	+ + +		+	-	+	+	+	+	pigs
	4	-	-	+ + +	+ + +		+	-	+	+	+	+	reindeer (<i>Rangifer tarandi</i>)
<u>B. neotomae</u>	1	-	+	- - +	- - -		+	-	+	-	-	+	(+ or -) + desert wood rat (<i>Neotomae lepida</i>)

(See footnotes next page)

Table 1. (cont.) (footnotes)

¹ Species differentiation is obtained on albimi or tryptose agar with the following graded concentrations of dyes from National Aniline Division, Allied Chemical and Dye Co., New York: 1:25,000 (a), 1:50,000 (b), 1:100,000 (c), thionin; and 1:50,000 (b), 1:100,000 (c), basic fuchsin. Other concentrations may be preferable with other growth media. Interpretation of results should be controlled with the reference strains of each species.

² Four substrates that provide for differentiation of the three species are given in the table. It is recommended, however, that all substrates be used for examining atypical strains.

³ Usually positive, negative varieties occur.

This organism has the oxidative metabolic pattern of B. melitensis on the substrates used for the definition of the species of Brucella, but it does not utilize glucose or erythritol, which nearly all recognized Brucella cultures do. This organism is not stained with fluorescent conjugated Brucella antibody. The soluble antigens of this organism are similar to those of rough and smooth B. melitensis as shown by indirect hemagglutination, Ouchterlony gel diffusion precipitation and immunoelectrophoresis precipitation tests. DNA homology studies with this organism and the recognized species of Brucella show similarity but not identity. As these results are somewhat conflicting, it was decided to defer a decision on the taxonomic position of this organism until the studies were completed and the interpretation of the data was clarified.

Cultures of Brucella neotomae have a metabolic pattern which is different from the other recognized species, and the Subcommittee unanimously agreed that they should constitute a new species, Brucella neotomae, which is defined as follows: "Aerobic, produces H₂S, does not grow on basic fuchsin, grows on thionin, has A antigen predominant, oxidizes l-alanine, d-alanine, l-asparagine, l-glutamic acid, l-lysine, l-arabinose, d-galactose and d-xylose; oxidation of d-ribose variable. Does not utilize l-arginine, dl-citrulline or dl-ornithine. Not lysed by phage at RTD but lysed at 10,000 x RTD. Occurs naturally in the desert wood rat (Neotomae lepida Thomas). Type strain 5K33.

The Subcommittee noted that a number of workers had examined cultures of Brucella rangiferi tarandi and found that they had a metabolic pattern similar to that given by B. suis biotype 3 but that they differed from the latter in their serology and in their natural host, the reindeer. It was therefore agreed that these cultures should be redesignated as B. suis biotype 4 (see Table 1).

The membership of the Subcommittee on Taxonomy of Brucella at the close of the Moscow meeting was:

- Wundt, Prof. W. (Chairman), Klinikum Mannheim Medizinische Fakultät der Universität Heidelberg, 68 Mannheim, West Germany
- Jones, Dr. L. M. (Secretary), Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706, USA
- Gargani, Dr. G., FAO/WHO Brucellosis Center, University of Florence, Florence, Italy
- McCullough, Dr. N. B., Laboratory of Bacterial Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014, USA
- Meyer, Dr. K. F., The George Williams Hooper Foundation, The Medical Center, University of California, San Francisco, California 94122, USA
- Meyer, Dr. M. E., School of Veterinary Medicine, Department of Veterinary Microbiology, University of California, Davis, California 95616, USA
- Morgan, Dr. W. J. B., Central Veterinary Laboratory, Weybridge, Surrey, England
- Parnas, Prof. J., Department of Microbiology, Academy of Medicine, Lubartowska 85, Lublin, Poland
- Renoux, Prof. G., Faculté de Médecine, Boulevard Tonnelé, 37, Tours, France
- Stableforth, Dr. A. W., United Nations Special Fund, Sheep Diseases Research Laboratories, Pendik, Istanbul, Turkey
- Van Drimmelen, Dr. G. C., Agricultural Counselor, South African Embassy, Washington, D. C. 20008, USA
- Vershilova, Prof. P. A., Brucellosis Department, Gamaleya Institute of Epidemiology and Microbiology, USSR Academy of Medical Science, Moscow D-98, USSR

- Lois M. Jones
Secretary