International Committee on Nomenclature of Bacteria

Subcommittee on the Taxonomy of *Brucella*

Minutes of Meeting, 7 August 1970

Hotel Maria Isabel, Mexico City, Mexico

The Subcommittee on Taxonomy of *Brucella* met on 7 August 1970 at the Hotel Maria Isabel-Sheraton in Mexico City prior to the Xth International Congress for Microbiology. Members present were: W. Wundt (Chairman), Germany; Lois M. Jones (Secretary), USA; Margaret Meyer, USA; W. J. Brinley Morgan, Britain; G. Renoux, France; and G. C. van Drimmelen, South Africa.

Since the last meeting, A. W. Stableforth (Britain) had retired and J. Parnas (Poland) had resigned from the Subcommittee. Proposals for new members were made, and their names will be submitted to the ICNB. W. J. Brinley Morgan was elected as the new Secretary.

The development of additional procedures since the last meeting of the Subcommittee in 1966 was reviewed.

The ability of single-stranded DNA to hybridize with single-stranded DNA from a heterologous source provides a measure of genetic relatedness among organisms. Whereas the DNA's of *Brucella* species are almost completely homologous, the DNA's from other gram-negative organisms show no homology with DNA from *Brucella* (5).

The present membership of the Subcommittee is as follows: W. Wundt (Chairman), Mannheim, Germany; W. J. B. Morgan (Secretary), Weybridge, Surrey, England; G. Gargani, Florence, Italy; R. D. Housewright, Bethesda, Maryland, USA; Lois M. Jones, Madison, Wisconsin, USA; N. B. McCullough, East Lansing, Michigan, USA; K. F. Meyer, San Francisco, California, USA; M. E. Meyer, Davis, California, USA; G. Renoux, Tours, France; G. C. van Drimmelen, South African Embassy, Washington, D.C., and P. A. Vershilova, Moscow, USSR.

Immune diffusion studies have shown that soluble antigens are very similar in all *Brucella* species and clearly different from those of other genera. Agglutination tests have shown that *Brucella* surface agglutinogens are one of two types, either smooth or rough, with the former showing quantitative differences in the

M and A antigens as shown by agglutinin-absorption.

Recent studies (1) have shown that cultures of *Yersinia enterocolitica* subtype IX share a common surface antigen with *Brucella*; anti-smooth *Brucella* sera and anti-*Yersinia* sera both agglutinate the homologous and heterologous antigens to titer. Diaz and Medrano (4) extracted the smooth *Brucella* agglutinin (lipopolysaccharide) with phenol, and by immune diffusion showed reactions of partial identity with a similarly extracted antigen from *Yersinia enterocolitica* IX, whereas soluble antigens (protein) obtained by cellular disintegration did not show cross-reactions between genera.

Oxidative metabolic tests have been conducted on many additional cultures, including those of the proposed new species and biotypes. Additional substrates have been examined in order to define more precisely the metabolic pattern of organisms in the genus *Brucella* (7).

The Subcommittee recommended that D-alanine and D-xylose be omitted, and D-glucose and D-erythritol should be added. The overall pattern given by the substrates should be taken into consideration. In general, QO_2N values above 50 should be considered positive and values below 50 negative. The patterns for the species and biotypes using the recommended substrates are shown in Table 1. It should be noted that, whereas the overall metabolic patterns for all biotypes of *B. melitensis* and *B. abortus* are the same as for biotype 1 of each species, those for biotypes of *B. suis* are not identical with *B. suis* biotype 1.

The use of Tb phage at 10,000 X RTD, as well as RTD, has provided an additional means of identifying *B. suis* and *B. neotomae* cultures. The lytic pattern obtained on various species is consistent with research showing that *B. abortus* adsorbs phage and goes through a lytic cycle; *B. suis* adsorbs phage, is susceptible to lysis from without, but does not go through a lytic cycle; and *B. melitensis* does not adsorb

TABLE 1. Differential characters of the species and biotypes in the genus *Brucella*

Species	Bio-type	Lysis by phage		CO ₂ Required	H ₂ S Produced	Growth on dyes ^a			Agglutination by		Substrate utilization ^b										Most common host reservoir					
						Thionin			Basic fuchsin		Mono-specific sera		Anti-rough serum	Amino acids			Urea cycle substrates			Carbohydrates						
														L-Alanine	L-Asparagine	L-Glutamate	DL-Ornithine	DL-Citrulline	L-Arginine	L-Lysine		L-Arabinose	D-Galactose	D-Ribose	D-Glucose	<i>D</i> -Erythritol
		10 ⁴ × RTD	RTD			a	b	c	b	c	A	M														
<i>B. melitensis</i>	1	—	—	—	—	—	+	+	+	+	—	—	+	+	+	—	—	—	—	—	—	—	+	+	Sheep, goats	
	2	—	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—		
	3	—	—	—	—	—	—	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—		
<i>B. abortus</i>	1	+	+	+ (—) ^c	+	—	—	—	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Cattle
	2	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
	3	+	+	+ (—)	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
	4	+	+	+ (—)	+	—	—	—	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—		
	5	+	+	—	—	—	+	+	+	+	—	+	—	—	+	+	+	—	—	—	—	+	+	+	+	
	6	+	+	—	—, +	—	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—		
	7	+	+	—	—, +	—	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—		
	8	+	+	+	—	—	+	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—		
	9	+	+	—, +	+	—	+	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—		
<i>B. suis</i>	1	—	+	—	+	+	+	+	—	—	+	—	—	—	±	—	—	—	+	+	+	+	+	+	+	Pigs
	2	—	+	—	—	—	+	+	+	—	—	+	—	—	—	—	±	+	+	+	+	—	+	±	+	Pigs, hares
	3	—	+	—	—	—	+	+	+	—	—	+	—	—	±	—	±	+	+	+	+	—	—	+	+	Pigs
	4	—	+	—	—	—	+	+	+	—	—	+	—	—	±	—	±	+	+	+	+	—	—	+	+	Reindeer
	5	—	—	—	+	—	—	+	—	—	—	—	+	—	+	+	+	±	+	+	+	—	±	+	+	Cattle, sheep
<i>B. neotomae</i>		—	+	—	+	—	—	+	—	—	+	—	—	—	±	+	+	—	—	—	—	+	+	±	+	Wood rat
<i>B. ovis</i>		—	—	+	—	+	+	+	+	+	—	—	+	—	±	+	+	—	—	—	—	—	—	—	—	Sheep (rams)
<i>B. canis</i> ^d		—	—	—	—	+	+	+	—	—	—	—	+	—	±	—	+	+	+	+	+	±	±	+	+	Dogs

^a Dyes from National Aniline Division, Allied Chemical and Dye Co., New York, are recommended. On albimi or tryptose agar the results shown should be obtained with the following concentrations of dyes: a, 1:25,000; b, 1:50,000; c, 1:100,000. Other concentrations may be preferable with other growth media.

^b +, Oxidized (OQ₂N value greater than 50) by all strains by manometric methods; -, not oxidized by any strain; ±, oxidized by some strains.

^c Usually positive, but negative varieties occur.

^d Proposed new species (3).

phage. Recent reports (8) of a specific *B. suis* phage must be confirmed in several laboratories before the usefulness of the phage can be assessed.

Prior to the meeting a questionnaire was circulated asking members (a) if they recommended any changes in the taxonomic scheme proposed in 1962 and amended in 1966, and (b) their opinion on proposed *Brucella* species which had not been decided upon by the Subcommittee. The replies of members unable to attend were taken into consideration in the formation of this report.

Minor changes were made in the definition of the genus *Brucella*, which now is as follows:

Genus *Brucella*

Small, nonmotile, nonsporing, gram-negative coccobacilli which grow rather poorly on ordinary media or may require special media. Aerobic, no growth occurring under strictly anaerobic conditions. Growth often improved by CO₂. Little fermentative action on carbohydrates in usual media. Urea hydrolyzed to a variable extent. Facultative intracellular parasites which produce acute and chronic infection in animals and which may cause disease in man. The G + C content of the DNA ranges from 56 to 58 moles per cent. Members of the genus comprise a closely knit and sharply demarcated genetic group as defined by DNA hybridization studies.

The Subcommittee discussed criteria for speciation. According to the taxonomic scheme proposed in 1962, all characters must be considered in establishing the species. The general opinion was that the scheme had worked well and no major changes in the scheme were proposed. It was recognized that for practical purposes of identification, the conventional and phage tests were the most widely used and were adequate for the examination of most strains. The oxidative metabolic tests were useful for examining cultures which did not fit easily in the existing biotypes or which were isolated from unusual hosts.

The Subcommittee considered the taxonomic position of the organisms described as *B. ovis* (2), *B. murium* (6), *B. canis* (3), and *B. suis* biotype 5 (9).

It was recommended that *B. ovis* (2) be

accepted as a new species and that *B. suis* biotype 5 (9) be accepted as a biotype.

Examination of cultures from rodents, including those described as *B. murium* (6), has shown that they belong in the genus *Brucella*, possibly in *B. suis*, but the cultures are unstable and their properties are variable. Further study was recommended.

The organism described as *B. canis* (3) has some characters in common with those of *B. suis*. It differs from *B. suis* in that cultures of *B. canis* are in the nonsmooth phase even on primary isolation, thus being similar to *B. ovis*. Its acceptance as a new species, rather than as a biotype of *B. suis*, is therefore provisional.

Lois M. Jones, Secretary
W. Wundt, Chairman

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