REPORT OF THE SUBCOMMITTEE ON
TAXONOMY OF THE GENUS BRUCELLA

Speciation in the Genus Brucella¹

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The Joint FAO/WHO Expert Committee on Brucellosis in its third report recommended that the question of species designation in the genus Brucella be referred to the International Committee on Bacteriological Nomenclature. At the Seventh International Congress for Microbiology held in Stockholm in August, 1958, a Subcommittee on Taxonomy of Brucella was appointed to study the question. Research, some of which has been directly supported by WHO, has progressed meanwhile. The subcommittee did much of its business by correspondence but met in London in August, 1961 and again at the 8th International Congress for Microbiology, Montreal, August, 1962 where the ensuing report was prepared.

Taking into consideration the results of the conventional tests for species identification in the genus Brucella, as well as the results from newly-developed methods, such as

¹ This report was prepared on behalf of the Subcommittee on Taxonomy of Brucella of the International Committee on Bacteriological Nomenclature by Lois M. Jones and A. W. Stableforth following the meeting held in Montreal August 18, 1962. Members of the Subcommittee who attended were Jones, McCullough, Renoux, Stableforth, Wilson and Wundt. Dr. Margaret E. Meyer and Dr. M. Kaplan were present at the meeting as participating observers. The views of those members who could not attend were solicited in advance and considered in the discussion. The report was circulated after the meeting and general agreement was obtained from all members except Professor Parnas, who disagreed and is publishing his views elsewhere, and Professor Vershilova who has expressed agreement with the existing classification into three species—Brucella melitensis, B. abortus, and B. suis—but considers further study necessary on the question of biotypes.

² Chairman

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phage-typing and oxidative metabolic tests, the Subcommittee has concluded that brucella strains fall into three fairly well-defined groups which it considers worthy of specific rank. It would retain the present names Brucella melitensis, B. abortus and B. suis. It proposes a number of biotypes of each species.

INTRODUCTION

The conventional methods for species differentiation in the genus Brucella consist of:

- a) the need for added CO₂ for growth especially on primary isolation.
- b) the production of H₂S.
- c) differential growth on the dyes basic fuchsin and thionin.
- d) agglutination in monospecific sera.

Other tests, such as urease activity, use of other dyes (methyl violet and pyronin) and DEDTC tests are used by some workers.

It has long been recognized that the differences based on these tests are quantitative rather than qualitative but that, when methods are controlled and standardized and cultures in the S or SI phase are used, the majority of strains in most parts of the world can be identified as belonging to one or other of the three classical species as follows (Table 1):

Table 1

Species	CO ₂ require- ment	H ₂ S production	Growth on:		Agglutination in monospecific sera	
			thionin	basic fuchsin	abortus	melitensis
<u>B. melitensis</u>	-	-	+	+	-	+
<u>B. abortus</u>	+	+ 4 days	-	+	+	-
<u>B. suis</u>	-	++ 5 days	+	-	+	-

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In many areas, however, strains with different combinations of characters are found and in some areas these are the predominant or the only variety. A number of these have long been recognized:

a) A variety of B. abortus, which does not require CO₂ for primary isolation and which may be slightly more resistant to thionin than the classic abortus, was first isolated from cattle in Southern Rhodesia (Bevan, 1930) and is referred to as Rhodesian abortus by many workers. Another somewhat thionin resistant variety of B. abortus which requires CO₂ for primary isolation was isolated from cattle in Sumatra by van der Schaaf and Rosa (1940).

b) A variety of B. abortus which does not grow on thionin or basic fuchsin, occurs in many countries and seems particularly common in cattle in Japan. It is referred to as type 2 (Wilson, 1933).

c) A variety of B. abortus which is agglutinated by mono-specific melitensis rather than abortus serum was first isolated from a cow in Palestine by Gilbert (1930). Strains with these characteristics have also been isolated in France and were examined by Wilson (1933) and have been recovered from cattle in the United States (Veazie and Meyer, 1936).

d) A number of strains isolated from man, cattle, sheep and goats in France by Lisbonne and Taylor were examined by Wilson (1933). A considerable proportion of the strains were found to be B. melitensis according to H₂S production and growth on dyes but to be serologically like B. abortus. Renoux (1952) designated this variety B. intermedia.

e) A few strains in the Lisbonne-Taylor group examined by Wilson (1933) were like B. melitensis on all tests but were agglutinated by both melitensis and abortus mono-specific sera.

f) CO₂ requiring B. melitensis and H₂S producing B. melitensis have occasionally been isolated. (Taylor et al. 1932; van Drimmelen 1953.)

g) A Danish variety of B. suis which does not produce H_2S (isolated by Thomsen 1929, characterized by Kristensen 1931) has been isolated from pigs and hares and appears to be of very low pathogenicity for man.

h) A variety of B. suis isolated from pigs in the U.S.A. which grows in the presence of basic fuchsin and does not produce H_2S has been designated B. suis type 3 by Huddleston (1957).

i) Other less common varieties have been described (see Renoux et al. 1955; Stableforth 1959).

OXIDATIVE METABOLIC TESTS

Meyer and Cameron (1961a,b) and Meyer (1961a) determined the oxidative uptake rate on a series of amino acids and carbohydrates for 75 strains identified by conventional tests as typical B. melitensis, B. abortus or B. suis. They found that each species displayed a characteristic and definitive metabolic pattern. Strains which were atypical by conventional tests gave the metabolic pattern of one or other of the three species.

In these studies, and in an extension of this work on additional strains (Meyer 1962; Meyer and Morgan 1962), it was shown that cultures with the biochemical and serological properties of B. melitensis isolated from cattle in Great Britain and also from Germany and Uganda had the oxidative metabolic pattern characteristic of B. abortus. Cultures which had the cultural-biochemical properties of B. melitensis and the serological properties of B. abortus fell into two groups. Some had the metabolic pattern of B. abortus and some the pattern of B. melitensis. Furthermore, some cultures which had the cultural and biochemical properties of B. melitensis but were agglutinated by both abortus and melitensis monospecific sera had the oxidative metabolic pattern of B. melitensis.

Wundt (1962b) using the methods described by Meyer also found that each of the species of Brucella had a characteristic metabolic pattern. Many B. melitensis cultures isolated

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from cattle had the metabolic pattern of B. abortus. Cultures which had the cultural-biochemical properties of B. melitensis and the serological properties of B. abortus all had the metabolic pattern of B. melitensis.

BRUCELLAPHAGE

Stable brucellaphage was first isolated in the Soviet Union (Popkhadze and Abashidze 1955) and was reported to be lytic for B. abortus cultures but not for B. melitensis or B. suis. Additional phages have been obtained from manure, foetuses, human blood and stock cultures of *Brucella* in Russia and Poland (Drozhevskina 1957; Ostrovskaya 1961; Parnas *et al.* 1958; Parnas 1961a,b; and others). These workers and Dimitriu *et al.* (1959) reported that phage was able to lyse nearly all B. abortus cultures and only some B. melitensis cultures.

Phage Tbilisi (Tb) has been obtained from the Soviet Union and studied by van Drimmelen (1959, 1960), Morgan, Kay and Bradley (1960) and Gargani (1961). Phage from a different source in the Soviet Union was used by Stinebring and Braun (1959), Jones (1960) and Meyer (1961b). Several phages from Parnas were examined by Wundt (1961, 1962a), Gargani (1961), Jones (1961) and Morgan (1961). The results of these workers agreed in showing that cultures of B. abortus of both the classical type and the dye-sensitive type were lysed; as were also cultures with the cultural-biochemical properties of B. abortus and the serological properties of B. melitensis. Cultures of B. suis were not lysed. Most of the cultures of B. melitensis were not lysed. The few cultures of B. melitensis that were lysed had been isolated from cattle and included cultures isolated in Britain where no *melitensis* infection exists in goats or sheep. Jones (1960) and Morgan (1962) showed that strains of B. melitensis freshly isolated from cattle in Malta (which were probably in contact with infected goats) were not lysed by phage.

Meyer (1961b) using oxidative metabolic studies and phage susceptibility, showed that only those cultures with the metabolic pattern of B. abortus, irrespective of their properties by conventional tests, were susceptible to lysis by phage.

Wundt (1962b) found that cultures from cattle which were identified as B. melitensis by conventional tests could be

divided into two groups on the basis of phage tests. Those that were lysed by phage had the metabolic pattern of B. abortus, whereas those not lysed had the metabolic pattern of B. melitensis.

Morgan (1962) examined approximately 4,000 Brucella cultures for susceptibility to Tb phage and four phages obtained from Parnas. Results were the same as those summarized above and were identical with all phages. The similarity of the five phages was further confirmed by neutralization studies using antiphage serum. Morgan used phage at routine test dilution (RTD) and 10,000 x RTD. All phage susceptible cultures were lysed by both dilutions. None of the 22 B. suis cultures tested were lysed by the RTD but with 10,000 x RTD many of them showed changes suggestive of lysis. No effect was seen on B. melitensis with phage at either dilution.

Meyer and Jones reported to the Subcommittee that they also had observed that undiluted phage or phage at 10,000 x RTD produced a reaction on many B. suis cultures which could be confused with lysis. Growth in the area of the phage drop was partly inhibited, it was sometimes translucent and sometimes of a different colour and texture from that of the surrounding lawn. The appearance resembled the phenotypic alteration in the colonial morphology of an abortus culture described by Jones, McDuff and Wilson (1962). A similar effect has also been described in the phage typing of staphylococci (Blair and Williams, 1961) and enteric pathogens (Anderson and Williams, 1956) and is not regarded as true lysis. In support of this view is the fact that phage Tb does not replicate on B. suis (Morgan 1962). The nature, however, of this pseudolytic effect needs further investigation before its significance can be assessed.

COMMENT

Three points emerge from what has been said:

1. The differences displayed by the conventional tests are quantitative rather than qualitative and when clearly + or - often cut across one another in such a way that a strain can be variously named according to the value put on particular tests by different observers. No test is specific. Need for added CO₂ is usual for B. abortus but many

cultures (not derived from vaccine Strain 19) are aerobic on first isolation; B. abortus cultures usually do not grow on thionin but some do; most grow on basic fuchsin but many are dye sensitive. B. suis cultures seldom grow on basic fuchsin but occasionally do so. B. melitensis usually does not produce H_2S but absence of H_2S production can be a character of B. suis or B. abortus. CO_2 requirement, H_2S production and growth on dyes cannot always be correlated with serological characters and so on. The use of dye tests in different dye concentrations and of reciprocal absorption tests to extinction may help to clarify the position of otherwise doubtful strains.

2. Both the oxidative metabolic tests and the phage results agree in general* with the results of the conventional typing methods but are of particular value in characterizing unusual biotypes.
3. Provided cultures in the S or SI phase are used for the phage tests, the metabolic and phage results agree completely with one another.

The metabolic tests have been used to examine a large number of strains covering all the varieties described and of diverse origin and source, and have divided them into 3 species—B. melitensis, B. abortus and B. suis. It is also possible to distinguish between the 3 varieties of B. suis on the basis of oxidative rates on the substrate L-lysine, but there are no corresponding varieties in the other two species. It is immaterial whether the strains are smooth or completely rough. Phage-resistant mutants of B. abortus as well as streptomycin-resistant mutants of B. abortus all behave metabolically like the parent strain.

The metabolic method is of great help in the classification of irregular strains. It has, however, important drawbacks. The equipment is expensive to buy and the method is laborious and slow. At present it is a method for use by reference rather than routine laboratories.

The phage susceptibility test has the great advantage of simplicity and fits in easily with conventional tests. The

* "In general" refers mainly to the classical types which are the preponderant types in most areas.

phage preparation can be standardized, stored for long periods and transported without loss of titre, and the results are easy to read and interpret.

A limitation of the phage test is the absence of phages which are specific for B. suis and B. melitensis. Until these are available it is difficult to know exactly what weight can be placed on the use of phage susceptibility in the definition of species of Brucella.

Our present knowledge is not complete enough to decide which test or tests reveal the essential characters of a culture. It is not possible to prepare a dichotomous key of identification. Taking into account all the characters mentioned above we are of the opinion that brucella strains fall into three fairly well defined groups which we think are worthy of specific rank. We would retain the present names: Brucella melitensis, B. abortus, and B. suis.

Only 7 cultures of Brucella neotomae (Stoenner and Lackman 1957) have been isolated to date. Studies on these have shown that they fall into the genus Brucella but a decision on their position in the genus cannot be made until more strains have been isolated and studied (see Cameron and Meyer 1958; Beal et al. 1959).

The etiological agent of ram epididymitis has been described and named Brucella ovis (Buddle 1956). We are not satisfied that it is a member of the genus Brucella. Although it fits the description of the genus as defined below, it bears little antigenic relation to other members of the genus and its metabolic pattern differs from brucellae (Meyer and Cameron 1956, 1957). Comparisons of this organism with species of the genera Brucella, Pasteurella, Haemophilus and Bordetella are needed before a decision can be made on its position in the taxonomic scheme. (See also Kennedy et al. 1956.)

Speciation in the Genus Brucella

The following definitions of the genus Brucella and its species are presented by the Subcommittee on Taxonomy of Brucella. Table 2 summarizes the differential characters of the three species and their biotypes.

Genus Brucella

Small, nonmotile, nonsporing, Gram-negative cocco-

bacilli. Grow rather poorly on ordinary media or may require special media. Aerobic, no growth under strict anaerobic conditions. Growth often improved by CO₂. Little fermentative action on carbohydrates in usual media. Urea hydrolysed to a variable extent. Parasites occurring in animals and producing characteristic infections in animals and man.

Brucella melitensis

Aerobic. Produce no H₂S or no more than a trace on ordinary media. Usually grow in the presence of basic fuchsin and thionin. Usually have M antigen predominant. Oxidize L-alanine, D-alanine, L-asparagine and L-glutamic acid. Do not oxidize L-arabinose, D-galactose, D-ribose, D-xylose, L-arginine, DL-citrulline, DL-ornithine or L-lysine. Not lysed by brucella phage Tb at routine test dilution. Usually pathogenic for goats and sheep but can also affect other species including cattle and man.

Reference strain B. melitensis 16M.

Brucella abortus

Usually require added CO₂ for growth especially on primary isolation. Usually produce moderate amounts of H₂S but may be negative. Usually grow in presence of basic fuchsin but inhibited by thionin. Usually have A antigen predominant. Oxidize L-alanine, D-alanine, L-asparagine, L-glutamic acid, L-arabinose, D-galactose and D-ribose; do not oxidize D-xylose, L-arginine, DL-citrulline, DL-ornithine or L-lysine. Cultures in the smooth or smooth-intermediate phase are lysed by brucella phage Tb at routine test dilution. Usually pathogenic for cattle causing abortion but can also affect other species including man.

Reference strain B. abortus 544.

Brucella suis

Aerobic. Produce large amounts of H₂S or none at all. Grow in the presence of thionin but usually inhibited by basic fuchsin. Usually have A antigen predominant. Oxidize L-alanine, D-alanine, L-glutamic acid, L-arabinose, D-galactose, D-ribose, D-xylose, L-arginine, DL-citrulline, DL-ornithine and L-lysine. Do not oxidize L-asparagine. Not lysed by brucella phage Tb at routine test dilution. Usually pathogenic for pigs but can also affect hares and other species including man.

Reference strain B. suis 1330.

Table 2. Differential characters of the three species of the genus Brucella and their 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		
				basic		thionin	fuchsin			Glu- tamic acid	Orni- thine	Ri- bose	Ly- sine			
				a	b c		b								c	
				a	b	c										
<u>B. melitensis</u>	1	-	-	-	+	+	+	+	-	+	-	+	-	-	sheep, goats	
	2	-	-	-	+	+	+	+	-	-	+	-	-	-	" "	
	3	-	-	-	-	+	+	+	+	+	-	+	-	-	" "	
<u>B. abortus</u>	1	+(⁻) ³	+	-	-	-	+	+	+	-	+	+	-	+	cattle	
	2	+	+	-	-	-	-	-	+	-	+	-	+	-	"	
	3	-(⁺)	+	-	-	+	+	+	-	+	+	-	+	-	"	
	4	+(⁻)	+	-	-	-	+	+	-	+	+	-	+	-	"	
	5	-	-	-	+	+	+	+	+	-	+	+	-	+	-	"
	6	-	-	-	+	+	+	+	+	-	+	+	-	+	-	"
	7	-	- or +	-	+	+	+	+	+	+	+	+	-	+	-	"
	8	+	-	-	+	+	+	+	+	-	+	+	-	+	-	"
	9	- or +	+	-	+	+	+	+	+	-	+	+	-	+	-	"
<u>B. suis</u>	1	-	++	+	+	+	-	-	+	-	-	-	+	+	+	pigs
	2	-	-	-	+	+	-	-	+	-	-	+	+	+	-	pigs, hares
	3	-	-	-	+	+	+	+	+	-	-	+	+	+	+	pigs

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.

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