

BIOTYPING OF SPANISH CATTLE AND SHEEP BRUCELLA STRAINS*

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The classification of the genus *Brucella* has been changing through the years, in accordance with our knowledge. HUDDLESON (1928) was able to divide them into three species, *B. abortus*, *B. melitensis* and *B. suis*, depending on their ability to grow in the presence of certain dyes (thionin and basic fuchsin) and H₂S production. WILSON and MILES (1932) by a quantitative agglutinin-absorption test were able to distinguish two different serological groups.

Later, different methods were tried with less significant results such as: the urease test; the sodium diethyldithiocarbamate test (RENOUX, 1952); antibiotic sensitivity (FARREL & ROBERTSON, 1967).

By means of the oxidative metabolic pattern, it was possible to differentiate the species and the biotypes (CAMERON & MEYER 1954; MEYER & CAMERON, 1957; MEYER & CAMERON, 1958; MEYER & CAMERON, 1961a, b; MEYER, 1961a, b; WUNDT 1963).

A new method of typing consisted in the use of phages. It seems that the first active phages against brucella were found in the U. S. S. R. by BOGDANOV (1938) (cit. DROZEVKINA, 1963), by subjecting the bacteriophage against the causative agent of paratyphoid abortion of mares (*Salmonella abortus-equi*) to multiple passages in culture of *Brucella*, he succeeded in adapting the phage to the lysis of *B. melitensis*. The reference phage used

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internationally is the so called Tb, isolated in the U. S. S. R. by POPHADZE & ABASIDZE (1957) from sewage in 1955.

The Subcommittee on Taxonomy of *Brucella* which met in 1958 in Stockholm, recommended a scheme (STABLEFORTH & JONES, 1963), that has been adopted internationally. At the meeting held in Moscow (1966), the scheme was reaffirmed (JONES, 1967) with minor changes. 4 species were admitted, *B. melitensis* with 3 biotypes, *B. abortus* with 9 biotypes, *B. suis* with 4 biotypes, and *B. neotomae* (STOENNER & LACKMAN, 1957). The acceptance of *B. ovis* (BUDDLE, 1956) as a member of the genus *Brucella* has been delayed until further studies have been made. On the basis of oxidative metabolism, phage and conventional typing methods (MEYER, 1966), *B. rangiferi tarandi* (DAVYDOV, 1961) should constitute a new biotype of *B. suis* (biotype 4).

Since the meeting in Moscow new biotypes and species have been described (Table I). KOROL & PARNAS (1967) and PARNAS et al. (1968) isolated a new type from wild rodents, called *B. murium*. Associated with abortion in dogs, CARMICHAEL & BRUNER (1968) in the U. S. A. described a new species of brucella (*B. canis*), recommended by MEYER (1969) to be included as biotype 5 of *B. suis*. Strains isolated from cattle and sheep studied by RENOUX & PHILIPPON (1969) have been considered as a new biotype (biotype 5) of *B. suis*.

In Spain we have only found a single paper by MARDONES SEVILLA (1968/69) in which three strains are typed, two from cattle (*B. abortus* type 1 and type 2) and one from a goat (*B. melitensis* type 1). Following the recommendations of different Committees (MORGAN, 1970) on the importance of surveys on the incidence of species and biotypes in planning initial control measures leading to eventual eradication, and in tracing the origin of infection in man, we have studied 24 strains from cattle and sheep.

MATERIALS AND METHODS

24 strains of brucella have been typed, 4 of cattle and 20 of sheep origin, obtained from the region of «Tierra de Campos» (León-Valladolid-Zamora).

The techniques used were the ones recommended by the Subcommittee on Taxonomy of *Brucellae* (STABLEFORTH & JONES, 1963; JONES, 1967): CO₂ requirement; H₂S production; growth on dyes (thionin and basic fuchsin, 1 : 50.000); agglutination in monospecific sera (A and M); lysis by phage (Tb) at RTD and 10⁴ RTD.

RESULTS AND DISCUSSION

The three biotypes of *B. melitensis* were found in the 20 sheep strains. Three strains were *B. melitensis* biotype 1, one *B. melitensis* biotype 2, and 16 strains *B. melitensis* biotype 3. All three strains of type 1, after 3 days, produce slight amounts of H₂S, a characteristic which has already been described (cit. STABLEFORTH & JONES, 1963).

The four cattle strains were typed as *B. abortus* biotype 3 (3 strains) and *B. melitensis* biotype 3 (1 strain). The incidence of *B. melitensis* biotype 3 in cattle is not surprising due to the high proportion of this type in sheep in this area. However, it is of great epidemiologic interest (Comité Mixto FAO/OMS de Expertos en Brucelosis, 1965) owing to the importance of the present vaccines in protecting cattle from *B. melitensis* infection.

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RESUMEN

Se hace primero una revisión del aspecto taxonómico del género *Brucella*. Se tipifican 20 cepas de origen ovino y 4 de origen bovino. Se encuentran en las ovejas *B. melitensis* tipo 1 (3 cepas), tipo 2 (1 cepa) y tipo 3 (16 cepas). Tres cepas bovinas correspondieron a *B. abortus* tipo 3 y una cepa bovina resultó ser *B. melitensis* tipo 3.

RESUME

On fait d'abord une revision de l'aspect taxonomique du genre *Brucella*. On typéfie 20 souches d'origine ovine et 4 d'origine bovine. Dans les brebis on trouve *B. melitensis* type 1 (3 souches), type 2 (1 souche) et type 3 (16 souches). Trois souches bovines correspondaient à *B. abortus* type 3 et une souche bovine était *B. melitensis* type 3.

SUMMARY

A review of the taxonomic aspect of the genus *Brucella* is made. 20 sheep strains were studied and typed as *B. melitensis* biotype 1 (3 strains), biotype 2 (1 strain) and biotype 3 (16 strains). 4 cattle strains were typed as *B. abortus* biotype 3 (3 strains) and *B. melitensis* biotype 3 (1 strain).

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TABLE I

POSSIBLE NEW SPECIES AND BIOTYPES

Species	Type	CO ₂ requirement	H ₂ S production	Growth on dyes		Aggl. mono-spec. sera A M	Phage T _h at		Metabolic tests				Most common host reservoir
				thionin a b c	basic fuchsin b c		RTD	10 ⁴ RTD	Glutamic acid	Omitine	Ribose	Lysine	
<i>B. murium</i>		—	+	+	—	— (+)	—	—	+	—	+	—	mouse (<i>Mus musculus</i>)
<i>B. ovis</i>		+	—	+	—	—	—	—	+	—	—	—	sheep
<i>B. canis</i> or <i>B. suis</i>	5	—	—	+	+	—	—	—	+	+	+	+	dog
<i>B. suis</i> ²	5	—	+	+	+	—	—	—	+	+	+	+	cattle sheep

1. The authors do not explicitly state this fact (Parnas et al. 1968).
2. Renoux & Philippon, 1969.