

## THE CHARACTERISTICS OF A VARIANT STRAIN OF *BRUCELLA MELITENSIS* REV 1

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### ABSTRACT

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Circumstantial evidence is presented for the occurrence of a variant of a vaccine strain of *B. melitensis* Rev 1, designated "FSA" (foreign South African). FSA resembles Rev 1 in its reactions to penicillin and streptomycin but reacts closer to a field strain of *B. melitensis* as regards dye (thionine and basic fuchsin) sensitivity and colony size.

Although colonies of Rev 1 were consistently smaller than other *B. melitensis* strains, their size was 0,75 mm as opposed to the 1-2 mm reported in the literature, while *B. melitensis* 16M colonies were 1,25-1,5 mm as opposed to the 3-4 mm previously reported.

Rev 1 was found to be urease positive, unless a test of low sensitivity was applied.

### INTRODUCTION

During the period 1979-1984 no field strains of *Brucella melitensis* were isolated or typed by the Veterinary Research Institute, Onderstepoort, from anywhere within the borders of the Republic of South Africa (Herr, unpublished annual reports, 1982-1986). This is in agreement with previous reports that *B. melitensis* is rare in South Africa (Elberg, 1981). A few isolations of *B. melitensis* Rev 1 vaccine strain (Rev 1) were made from aborted sheep fetuses in cases where the owner, contrary to directions, had inoculated pregnant ewes with the full dose ( $1-2 \times 10^9$  viable organisms) (Herr, unpublished data 1982-1986). This dose may cause abortion in pregnant sheep (Alton & Elberg, 1967).

A search of the literature revealed only a single case where the Rev 1 strain was implicated in the pathology of the genital tract of rams or billy goats (Verger & Plommet, 1985). Equally rare were reports of human infections with Rev 1 with a single case following on direct inoculation (Alton & Elberg, 1967). This was despite the fact that as few as  $10^4$  organisms were reported to cause mild symptoms and that  $2,8 \times 10^4$  organisms caused frank clinical disease in 4/6 human volunteers injected with this latter dose (Alton & Elberg, 1967).

Subsequent to 1984 a strain of *B. melitensis* with atypical characteristics was isolated from aborted sheep fetuses and Angora goat rams<sup>1</sup> with orchitis and/or epididymitis. The same strain was typed from 2 human isolates<sup>2</sup>. When the locally produced vaccine strain (originally obtained from a foreign source) was examined, it showed the same atypical characteristics. The vaccine was immediately withdrawn and replaced by a Rev 1 strain<sup>3</sup>, recommended by the World Health Organization (WHO Expert Committee on Brucellosis, 1986). The strain that typed atypically was named "FSA" (foreign South African) to avoid confusion with the Rev 1 strain. The atypical strains from the various sources were then subjected to typing in parallel with reference strains of *Brucella* in order to accurately record their characteristics.

### MATERIALS AND METHODS

#### Typing procedures

All strains were multiplied and typed according to the methods described by Corbel & Hendry (1983) with the following modifications:

1. The basic medium used was serum dextrose agar (SDA) as described but using 10 % inactivated equine serum and made up to a total volume of 1  $\ell$ .

2. A standard dilution of  $10^9$  viable *Brucella* organisms  $\text{m}\ell^{-1}$  was used on dye plates. A graduated platinum loop (0,01  $\text{m}\ell$ ) was used to streak out 5 lines without recharging on a quarter of the standard dye plate. Only if growth was seen on the 3rd line of the streak or beyond after 48 h incubation at 37 °C was it recorded as positive. All dye plates were incubated in air and in air plus 10 %  $\text{CO}_2$ .
3. Both the thionine and basic fuchsin dyes were manufactured by British Drug House<sup>4</sup> or affiliates.
4. The urease test was done on Christensen's urea agar slopes using:
  - (a) the method of Corbel & Hendry (1983) of inoculating a single loopful of  $10^9$  organisms  $\text{m}\ell^{-1}$ , as well as
  - (b) the method of Alton, Jones & Pietz (1975), where a loopful of colonies was picked up from an SDA plate and directly inoculated onto the slope.

In both cases the urea agar slopes were incubated at 37 °C in air and checked for a positive reaction at 15 min and then at hourly intervals up to 6 h and finally at 18 h post-inoculation. Any change in colour to pink or red was taken as a positive reaction. The quantitative method, using a liquid medium and variations of pH (Van Drimmelen, 1964), was not applied.

5. Phage sensitivity testing followed Corbel & Hendry's (1983) method, but only Tbilisi (Tb), Weybridge (Wb) and Firenze (Fi) phages were used at routine test dilution (RTD). The Berkeley (Bk<sub>2</sub>) phage available to us was giving unreliable results and was therefore not included.
6. Colony size was measured after 96 h incubation at 37 °C in air plus 10 %  $\text{CO}_2$  on SDA plates. The colonies were measured with a slide micrometer.
7. The antibiotic tolerance tests were done on SDA plates on which a lawn of *Brucella* organisms was created by spreading, 0,1  $\text{m}\ell$  of a suspension containing  $10^9$  organisms  $\text{m}\ell^{-1}$  with the use of a sterile glass rod. "Mastring"<sup>5</sup> discs were used.

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THE CHARACTERISTICS OF A VARIANT STRAIN OF *BRUCELLA MELITENSIS* REV 1.

TABLE 1 The typing reactions of *B. melitensis* 16M, FSA and Rev 1

Test	Typing procedure		<i>B. melitensis</i>		
	Method		16M	FSA	Rev 1
CO <sub>2</sub> requirement			—	—	—
Urease activity:	Loopful of suspension of 10 <sup>9</sup> organisms ml <sup>-1</sup> applied to slope		—	—	—
	Loopful of colonies applied to slope		+	+	+
Growth on dyes: 1. Thionine acetate in 10 % CO <sub>2</sub> :	1/23 000		+	+	—
	1/62 000		+	+	+
	1/100 000		+	+	+
2. Thionine acetate in air:	1/23 000		—	—	—
	1/62 000		+	+	—
	1/100 000		+	+	+
3. Basic fuchsin in 10 % CO <sub>2</sub> :	1/50 000		+	+	+
	1/100 000		+	+	+
4. Basic fuchsin in air:	1/50 000		+	+	—
	1/100 000		+	+	+
Agglutination in monospecific sera	<i>B. abortus</i>		—	—	—
	<i>B. melitensis</i>		+	+	+
	<i>B. ovis</i>		—	—	—
Lysis by phage at RTD	Tbilisi		NL	NL	NL
	Weybridge		NL	NL	NL
	Ferenzi		NL	NL	NL
Colonial morphology	No agglutination with acriflavine (0,1 % m/v)		S	S	S
Colony size	Growth in 10 % CO <sub>2</sub> at 96 h on SDA (mm)		1,25–1,5	1,25–1,5	0,75
Growth in presence of antibiotic discs: Penicillin	5 i.u.		+	—	—
	10 i.u.		+	—	—
Streptomycin	5 µg		—	+	+

RTD = Routine test dilution      S = Smooth colonies  
NL = No lysis                      SDA = Serum dextrose agar

8. Various reference strains were typed in parallel to check the correctness of the various typing procedures. The strains used were:

- B. abortus* biovar 1 (544) NCTC 10093
- B. melitensis* biovar 1 (16M) NCTC 10094
- B. ovis* (63/290) NCTC 10512
- B. suis* biovar 1 (1330) NCTC 10316
- B. melitensis* biovar 1 (Rev 1) from The National Institute of Agronomical Research (INRA), Nouzilly, France.

9. FSA isolates from the following sources were typed:

Source	No.	Disease condition	Remarks
Man	2	Brucellosis	Denied having inoculated themselves
Angora rams	12	Orchitis/epididymitis	Inoculated as young rams
Sheep foetuses	4	Abortion	Owners denied inoculating pregnant ewes
Vaccine	2	—	—

10. The FSA cultures from the 2 vaccines and from 2 of the Angora rams were sent to the Central Veterinary Laboratory, Weybridge<sup>6</sup>, for confirmation of the typing.

RESULTS

Except for the urease test, all the reference strains typed as previously described (Alton & Elberg, 1967; Alton *et al.*, 1975; Corbel & Hendry, 1983; WHO Expert Committee on Brucellosis, 1986). In the urease test, Rev 1 strains were found to be urease positive if a loopful of colonies was inoculated onto the slope but urease negative if a loopful of a suspension of 10<sup>9</sup> organisms ml<sup>-1</sup> was used for inoculating the slope. Both *B. abortus* 544 and *B. ovis*, which are reportedly urease negative (Alton *et al.*, 1975), remained negative no matter what method was used. *B. suis*, which should show a positive urease reaction almost immediately (Alton *et al.*, 1975),

<sup>6</sup>Central Veterinary Laboratory, New Haw, Weybridge, Surrey KT 15 3NB, England

remained negative beyond 6 h when the loopful of suspension was applied to the slope, but became positive immediately with a loopful of colonies.

The FSA isolates typed as *B. melitensis* biovar 1. They differed from Rev 1 in their sensitivity to basic fuchsin and thionin when grown in air, as well as in the size of colonies compared to the reference strain of Rev 1. In their sensitivity to penicillin and streptomycin, the FSA isolates reacted the same as the Rev 1 reference strain (Table 1).

The Central Veterinary Laboratory, Weybridge, England, found that the 2 vaccine and 2 field isolate strains sent to them typed essentially the same as was found here (Corbel, personal communications, 1986). The colonies of FSA, like those of *B. melitensis*, were consistently twice the size of the Rev 1 reference strains. The actual sizes were 1.25–1.5 mm for FSA and 16M but only 0.75 for Rev 1 (Table 1).

#### DISCUSSION

The finding of a *B. melitensis* strain (FSA) simultaneously in the field and in the vaccine presents strong circumstantial evidence that this strain originated as a variant from a Rev 1 vaccine strain. Alton & Elberg (1967) cite Vershilova as reporting *in vitro* variation in virulence and dissociation in the Rev 1 strain but failed to experience any variations in the characteristics of Rev 1 themselves. Alton & Elberg (1967) also cite Ulasevich (1965) as failing to elicit change in the characteristics of Rev 1 by serial passage in sheep. If we are correct in surmising that a variant strain had occurred, it would be important for other countries using the Rev 1 vaccine to be on the lookout for a similar occurrence.

The work reported on here is insufficient to draw any conclusions as to the pathogenicity of the FSA strain. Although only a single case of epididymitis, caused by Rev 1, was reported on in the literature (Verger & Plommet, 1985), Rev 1's pathogenicity when used in Angora goat rams has not been investigated. When the numbers of reported cases of FSA infections are compared to the amounts of the vaccine used, it becomes clear that the FSA strain is far from being as fully pathogenic as field isolates of *B. melitensis*. Although the cases of sheep abortions and brucellosis in humans has no history of inoculation, this is mere hearsay evidence. It still remains possible, at least in 2 of the sheep abortion cases, that residues in syringes used for the inoculation of weaner sheep could have been the source of the FSA infection when used to inoculate pregnant adults with some other vaccine. In the 2 cases of brucellosis in humans the very low numbers of organisms needed to cause clinical disease in man (Alton & Elberg, 1967) means that a mere scratch of the skin by a vaccine contaminated needle could lead to brucellosis infection.

Whether FSA will survive for any great length of time in nature is also a mute point. It is well known that goats clear themselves of infection with Rev 1 within 14 weeks of inoculation (Alton & Elberg, 1967). It is to be hoped that the FSA strain will have a similar short life span *in vivo*.

Although *B. melitensis* 16M and FSA colonies were consistently twice the size of Rev 1 colonies, the actual size of 1.25–1.5 mm for 16M and 0.75 for Rev 1 compares poorly with the reported (Corbel & Hendry, 1983) sizes of 3–4 mm and 1–2 mm respectively. Our findings are, however, in agreement with the statement (Alton & Elberg, 1967) that Rev 1 strains are always smaller than other strains of *B. melitensis* and that Rev 1 colonies have a maximum size of 1–2 mm. The reasons for this consistently smaller size of colonies in our work is without explanation.

In the urease test, the Rev 1 strain gave different results, depending on the test method used. With the other reference strains used, the correct results were obtained with the placing of a loopful of colonies on the Christensen's urea slopes. With this method, the Rev 1 strain proves to be urease positive which is contrary to the findings of Alton & Elberg (1967), but they do not describe the method used. Van Drimmelen (1962) reported a low urease reaction with Rev 1 using his quantitative method and this is more in keeping with the findings in our work.

Our experience with the methods employed in the typing of these strains, especially with respect to the urease test and colony size, leads us to support the plea (Verger & Plommet, 1985) for greater standardization of methods of typing between laboratories.

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