

Smooth Phage-Resistant *Brucella abortus* from Bovine Tissue

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Conventional typing, oxidative metabolic, and virulence tests were conducted on a phage-resistant *Brucella abortus* strain isolated from the supramammary lymph node of a cow.

The susceptibility of smooth *Brucella abortus* strains to lysis by brucella phage is one of the criteria used to differentiate this organism from other *Brucella* species (1, 6). *B. abortus* phage-resistant strains occur; however, the majority of these have undergone dissociation to non-smooth forms during manipulation in the laboratory.

The purpose of this paper is to report the isolation of a smooth, phage-resistant (SPR) strain of *B. abortus* from the tissues of a cow and to report its characteristics and virulence for guinea pigs.

A culture of *B. abortus* was submitted to this laboratory for typing. It had been isolated from the supramammary lymph nodes of a cow in a herd known to be infected with *B. abortus*, biotype 1. *B. abortus* strain 19 and CO₂-dependent *B. abortus*, biotype 1, strains that were susceptible to brucella phage have been isolated from cattle in this herd. The isolate was characterized using conventional typing procedures and manometric techniques as previously described (1, 3, 7). The oxidative metabolic pattern on selected amino acid and carbohydrate substrates was typical of *B. abortus*. Conventional typing procedures revealed that the culture was *B. abortus*, biotype 1 (A antigen dominant). Additional criteria used to determine smoothness of the culture included examination of colony morphology under a low-power dissecting microscope using a reflected light source (5) and staining of colonies with crystal violet (8). The isolate was resistant to lysis by

the routine test dilution and a 10⁴ routine test dilution of brucella phage (Tb).

Virulence of the SPR isolate for guinea pigs was compared with *B. abortus* strains 2308 and 19. Three groups of 12, 350-g adult female guinea pigs were inoculated subcutaneously with the different *Brucella* strains. The number of viable organisms given were 0.72 × 10⁹ (SPR isolate), 0.75 × 10⁹ (strain 2308), and 0.97 × 10⁹ (strain 19). At 40 days postinoculation, the guinea pigs were weighed and necropsied, and the spleens were removed and weighed. The spleen/body weight ratios were calculated. Each spleen was cultured on tryptose serum agar plates. After incubation at 37°C with 10% CO₂, the plates were examined for colonies with morphological characteristics of brucella. Isolates recovered from the spleens of each group of guinea pigs were characterized using conventional typing procedures and manometric techniques as previously mentioned.

The spleen/body weight ratio in guinea pigs is one of the criteria used to assess the virulence of *Brucella* strains. The results of guinea pig inoculations are presented in Table 1. The spleen/body weight ratio of guinea pigs infected with strain SPR was significantly higher than those infected with strains 19 and 2308. Uninoculated guinea pigs have spleen/body weight ratios comparable to strain 19-infected guinea pigs (2). A number of spleens from guinea pigs infected with strains 2308 and SPR were rough and nodular; however, there were no visible lesions in spleens of strain 19-infected guinea

TABLE 1. Results of virulence tests in guinea pigs for *B. abortus* strains 2308, 19, and SPR

<i>B. abortus</i> strain	No. of animals inoculated	No. of viable organisms ^a	Avg spleen wt (g)	Avg spleen wt/body wt ratio (%)	No. of spleens culture positive
19	12	0.97 × 10 ⁹	0.566	0.13	7
2308 ^b	12	0.75 × 10 ⁹	0.976	0.22	12
SPR	12	0.72 × 10 ⁹	1.771	0.41	12

^a Number of organisms inoculated into each animal.

^b U. S. Department of Agriculture challenge strain.

pigs. Brucellae were recovered from the spleens of all guinea pigs inoculated with strains 2308 and SPR. Seven of 12 guinea pigs inoculated with strain 19 were positive for brucella. The SPR isolate from guinea pigs remained phage resistant, whereas strains 2308 and 19 were phage susceptible. Although a fully virulent smooth, phage-resistant variant of *B. abortus* has been isolated through the manipulation of laboratory stock cultures (4), this is the first reported isolation of a smooth, phage-resistant *B. abortus*, biotype 1, from bovine tissue.

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