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Authors: Lord, Veronica R., and Lord, Rexford D.

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Brucella suis Infections in Collared Peccaries in Venezuela

Veronica R. Lord[1] **and Rexford D. Lord,**[2] [1] Laboratory of Brucellosis, Institute of Veterinary Investigations, Box 70, Maracay 2102, Venezuela; [2] Biology Department, Indiana University of Pennsylvania, Indiana, Pennsylvania 15705, USA

ABSTRACT: A bacteriologic and serologic study was conducted on two ranches in the states of Apure and Guarico, Venezuela for brucellosis in collared peccaries (*Tayassu tajacu*). One hundred thirty-nine peccaries were necropsied and tissues were cultured. Forty-three isolations of *Brucella suis* biovar 1, were made from lymph nodes and spleens of 25 males and 18 females. Antibody to *Brucella* sp. was detected in sera from 122 animals by the rapid plate agglutination, standard tube agglutination, 2-mercaptoethanol, rivanol, complement fixation and card tests. Young animals had infection and reactor rates nearly as high as the older animals indicating most were infected at a relative early age. Results suggest that this species may transmit brucellosis when living with domestic animals. This is the first report of *B. suis* biovar 1 from collared peccaries in Venezuela.

Key words: Brucellosis, collared peccary, *Brucella suis*, prevalence, sero survey, *Tayassu tajacu*.

Brucellosis, caused by several species of *Brucella*, has been detected in numerous wild animals (Moore and Schnurrenberger, 1981; Witter, 1982; Garin-Bastuji et al., 1990). Some of these species might become reservoirs of infection for susceptible domestic animals (Corey et al., 1964; Hayes, 1977).

In several countries serum antibody has been detected and *Brucella* spp. isolated from wild animals but it remains uncertain whether they serve as reservoirs of the disease (Szyfres and Tome, 1966; Davis et al., 1979; De La Vega et al., 1979; Boer et al., 1980). Collared peccaries (*Tayassu tajacu*) are well distributed in Venezuela and are found on ranches with cattle which are of considerable economic importance to the country.

The principal objectives of this study were to sample tissues and sera from peccaries from the Llanos of Venezuela, for the purpose of isolating *Brucella* sp. and surveying for serum antibody against *Brucella* sp.

One hundred thirty-nine peccaries were collected by shooting with a rifle on two ranches, one in the state of Guarico (07°45′ to 07°55′N, 69°18′ to 69°23′E) and the other in the state of Apure (09°16′ to 09°21′N, 67°15′ to 67°22′E). Spleen, liver, mesenteric and retropharyngeal lymph nodes, sera and eyes were taken from all animals.

Spleens were washed in a solution of 0.85% NaCl, immersed in 95% alcohol, flamed, seared with a hot spatula and a 1 cm^3 piece removed for inoculation of solid culture media (Albimi *Brucella* sp. agar, trypiticase soy agar, and Kuzdas Morse) to which 5% fetal bovine serum was added (Alton et al., 1975). Lymph nodes were ground with mortar and pestal with a solution of 0.85% NaCl (pH 6.8) and inoculated on the same culture media.

Plates were incubated ≤10 days at 37 C in 10% CO_2, and examined daily after 48 hr. Colonies were observed with a steroscopic microscope according to the method of Henry (Alton et al., 1975). Possible *Brucella* sp. isolates were stained by the Gram and Koster technique (Alton et al., 1975). Several colonies from each sample, with typical characteristics of *Brucella* sp., were harvested and inoculated on agar slants (potato agar and trypticase soy agar) and on Petri plates. Isolates were incubated at 37 C to determine CO_2 dependency and growth on media containing 5% fetal bovine serum. They were examined further by the following tests: acriflavine (1:1,000), immersion in crystal violet (1:40), motility, urease activity, production of catalase, production of oxidase, production of H_2S, fermentation of lactose (MacConkey agar), production of hemolysis (blood agar), and reduction of nitrates

TABLE 1. Characteristics of the 43 isolates of *Brucella* sp. from collared peccaries in Venezuela.

Bacteriological morphology:		Cocabacilli
Motility:		37 C—negative, 22 C—negative
Requirement for serum:		none
Dependency on CO_2:		none
Sensitivity to thionin:		yes
Sensitivity to fuchsin:		no
Sensitivity to safranin:		no
Development in 1 mg/ml i-erythritol:		positive (100% growth)
Development in 5 IU[a]/ml penicillin:		positive (50% growth)
Production of H_2S (days):		positive for 5 days
Activity of urease (minutes):		positive (after 15 min)
Activity of catalase (seconds):		positive (immediate)
Fermentation of lactose (MacConkey agar):		negative
Reduction of nitrates:		positive
Utilization of citrate:		negative
Monospecific sera—A:		positive agglutination
—M:		negative agglutination
—R:		negative agglutination
Sensitivity to Tbilisi phage:	1 RTD	no lysis
	$\times 10^4 \times$ RTD	lysis

[a] International Units.

and citrate (Alton et al., 1975; Cowan and Steele, 1979; MacFaddin, 1980). The dye sensitivity of *Brucella* sp. isolates was determined by adding basic 0.1% fuchsin (1:25,000, 1:50,000, 1:100,000), 0.1% thionin (same dilutions), and 1% safranin (1:5,000) to Albimi *Brucella* sp. agar. Growth on media containing i-erythritol (1 mg/ml) and penicillin (5 International Units/ml) was also studied (Huddleson, 1931). The media were inoculated with bacterial suspensions prepared in 0.85% sterile NaCl solution at a similar density with reference strains (*B. abortus* 544, *B. melitensis* 16 M, *B. suis* 1330). Plates were divided into four quarters for inoculation with a calibrated platinum loop and incubated at 37 C for 72 hr. Monospecific antisera, anti-A, anti-M and anti-R, were used to determine which of the agglutinins predominated in the isolates. Two concentrations of the Tbilisi phage (Routine Test Dilution (RTD) and 10,000 × RTD) were used (Alton et al., 1975).

For the metabolic tests, the following substrates were used in sequential groups:

TABLE 2. Oxidative rates $QO_2(N)$ of 43 isolates of *Brucella* sp. from peccaries in Venezuela with four amino acids and four carbohydrate substrates.

	Group I		Group II		Group III			
Isolates or reference strain	L-alanine	L-glutamic acid	D,L-ornithine	L-lysine	L-arabinose	D-galactose	D-ribose	D-glucose
B. suis biovar 1 observed range . . .	60–68	61–69	160–166	141–149	120–127	280–287	304–308	372–378
544[a]	140	322	41	18	160	289	108	86
16M[b]	82	98	37	36	25	38	42	57
1,330[c]	65	68	159	148	124	283	304	375

[a] *B. abortus*, reference strain.
[b] *B. melitensis*, reference strain.
[c] *B. suis*, reference strain.

TABLE 3. Antibody levels to *Brucella* sp. in peccaries from Venezuela.

Number of animals[a]	RPA test[b]	STA test[c]	Card test	Rivanol	2-mercapto-ethanol test[d]	Complement fixation	Group
12	100 IU[e]	200 IU	positive	200 IU	200 IU	104 IU	1
39	200	100	positive	200	200	52	
18	100	100	positive	50	50	26	
11	50	100	positive	—	25	13	2
19	25	50	positive	—	25	13	
10	50	50	positive	—	50	13	
4	50	50	positive	—	25	13	
2	25	50	—	—	—	—	3
3	50	25	—	—	—	—	
4	25	25	—	—	—	—	

[a] 17 animals were negative for all serological tests.
[b] Rapid plate agglutination test.
[c] Standard tube agglutination test.
[d] 2-mercaptoethanol test.
[e] International Units.

Group I used L-alanine and L-glutamic acid; Group II used amino acids of the urea cycle, D,L-ornithine and L-lysine; and Group III used the carbohydrates, L-arabinose, D-galactose, D-ribose and D-glucose (Meyer and Morgan, 1962).

A 1% Sorensen solution buffered with phosphates to pH 7.0 was prepared for each of the substrates (Meyer and Cameron, 1959). Packed bacteria cells were resuspended in Sorensen solution and adjusted to a dilution of 1:40 similar to a normal suspension. The density was determined in a spectrophotometer at a wave length of 420 nm. The normal suspension contained approximately 0.8 mg of nitrogen per ml. Manometric determinations were made using the Warburg apparatus (Clark, 1969). A substrate was considered to have been oxidated when the value of $QO_2(N)$ (μl oxygen uptake/mg nitrogen/60 min) was equal to or more than 50 μl (Meyer and Cameron, 1961a, b; Lord and Flores, 1983).

TABLE 4. Relation between age of peccaries, isolation of *Brucella* sp. and presence of *Brucella* sp. antibodies.

Estimated age in years	Isolations/animals cultured (%)	Reactors/sera tested (%)
1	4/18 (22)	14/18 (78)
2	7/23 (30)	23/23 (100)
3	18/51 (35)	43/51 (84)
4	14/47 (30)	42/47 (89)
Total	43/139 (31)	122/139 (87.8)

Blood samples were obtained from the jugular vein. The samples were allowed to clot, and were centrifuged, separated, and stored in a refrigerator until transport to the laboratory on wet ice. Peccary sera were tested in the rapid plate agglutination (RPA) test, standard tube agglutination (STA) test, 2-mercaptoethanol, rivanol, card test and complement fixation (CF) test, utilizing an antigen of *B. abortus* (strain 1119-3) (Alton et al., 1975).

Ages of peccaries were determined by the lens technique (Lord, 1959; Lord and Lord, 1988). All work was conducted in the Laboratory of Brucellosis (Institute of Veterinary Investigations, Maracay, Venezuela).

One hundred thirty-nine peccaries (70 males and 69 females) were taken from two ranches, and 556 tissue samples were cultured. *Brucella suis* was isolated from 43 (31%) of the peccaries (Tables 1, 2). On the basis of oxidative rates, all 43 isolates were determined to be biovar 1.

Serologic tests were considered positive when agglutination was observed in the card test, when there were 13 IU in the

CF test, and/or when titers of 100 IU were seen in the other tests. Reactors were animals whose sera reacted in any of the tests. High titers (26 IU in CF tests, 100 IU in other tests, or greater) of antibody were found in 69 (50%) of the 139 sera tested; 44 (32%) had low titers of antibody in all tests except rivanol; 9 (7%) of 26 had low levels of antibody in the RPA and STA tests, and 17 (12%) sera were negative to all tests.

Table 4 shows the relation between estimated age and the isolations and serologic reactors. The youngest animals had the lowest rates, though they were nearly as high as those of older animals. There was a slight tendency for higher isolation rates in animals with higher titers (32% of Groups 1 and 2; 22% of Group 3 of Table 3). Isolations were made from 25 males and 18 females, but serologic reactors were similar for both sexes.

Neither of the two ranches have feral or tame domestic pigs (*Sus scrofa*) running free on the open range. Nevertheless, there are other ranches in these states where domestic pigs range freely and *B. suis* has been detected in capybaras (*Hydrochaeris hydrochaeris*) from the Llanos (Lord and Flores, 1983). The peccaries could have been infected by transmission from capybaras or by a chain of transmission through peccaries, leading back to domestic pigs elsewhere. The high infection rate (31%) and a high rate of sero positively indicates an important segment of the peccary population was infected with *B. suis* biovar 1.

Although 1-yr-old animals had both the lowest isolation and reactor rates, these rates were nearly as high as those of the older animals, indicating that most became infected with *B. suis* at a relatively early age. This is the first report of *B. suis* biovar 1 in collared peccaries in Venezuela.

Peccaries are widely distributed in Venezuela, and likewise throughout many Latin American countries where bucellosis is a problem. These animals live on ranches with domestic animals such as swine, equines, bovines, and numerous wild animals, many of which may be adversely affected by *B. suis*. The epidemiologic significance of *B. suis* in peccaries is not known, but merits further investigation.

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