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BRUCELLA SPP. FROM THE CAPYBARA (*HYDROCHAERIS HYDROCHAERIS*) IN VENEZUELA: SEROLOGIC STUDIES AND METABOLIC CHARACTERIZATION OF ISOLATES

Veronica R. Lord[1] and Ricardo Flores C.[2]

ABSTRACT: A bacteriological and serological study of 201 wild capybara from the llanos, State of Apure, Venezuela was made to isolate *Brucella* from spleen and lymph node tissues and determine the role of this rodent as a reservoir of this bacteria. Twenty-three isolations were made, eight were identified as *B. abortus* and 15 as *B. suis* by oxidative metabolic techniques. A Poly B antigen in immunodiffusion in gel test was compared with other serologic tests. There was good correlation and 58% of sera were positive. The age and sex distribution of animals from which isolations were made and serological reactors indicated that this species may be an important alternate host of *Brucella* spp. in Venezuela.

INTRODUCTION

Brucellosis occurs in many species of wild animals which can serve as possible reservoir hosts to domestic animals and man (Witter, 1982). Reports from Argentina describe isolations of *Brucella suis* in the European hare (*Lepus europeus*), pampas and Patagonian gray foxes (*Dusicyon gymnocercus*, *D. griseus*), black-eared opossum (*Didelphis marsupialis*), and of *Brucella abortus* in the black-bellied ferret (*Grison cuja*, =*Galactis furax*) (Szyfres and Tomé, 1966; Gamarra and Szyfres, 1968; De La Vega et al., 1979).

In 1973 Plata (1973) found antibodies for *Brucella* in the sera of capybara from Venezuela, which suggested the possibility of these animals serving as a reservoir of *Brucella* spp. In subsequent years, Bello et al. (1976, 1978, 1979) found *Brucella* antibodies in capybara sera and isolated *Brucella abortus*, biotypes 1 and 2 from their tissues.

Isolates of the genus *Brucella* are usually classified by the conventional biochemical methods of Huddleson (1931) and Meyer and ZoBell (1932), and serological tests using monospecific sera (Wilson and Miles, 1932). The serological identification and sensitivity to different concentrations of dyes in media sometimes yield contradictory results. For example, isolates of *Brucella abortus* identified biochemically appear to be *Brucella melitensis* serologically (Pickett et al., 1953). It has been known since the original report by Wilson and Miles (1932) that *Brucella abortus* and *Brucella melitensis* contain (qualitatively) similar antigens which vary in quantitative distribution. Oxidative metabolic studies show that the quantitative distribution of antigens varies not only from species to species, but also within the species and the distribution of these antigens frequently is not related to other species characteristics (Meyer and Morgan, 1962).

The oxygen metabolism of members of the genus *Brucella* and utilization of substrates such as amino acids of the urea cycle and other amino acids (Cameron and Meyer, 1953, 1955) and carbohydrates can be considered as reliable methods for species and biotype identification (McCullough and Beal, 1951; Meyer and Cameron, 1959, 1961a, b). Meyer and Cameron (1961a) demonstrated that it was possible to correctly identify strains of *Brucella* which gave conflictive results by traditional methods as well as variants of a species which showed abnormal characteristics through the use of manometric techniques (Meyer, 1962; Clark, 1969).

The objective of the present study was to determine the prevalence of *Brucella* spp. in the capybara with the purpose of elucidating their possible role as a reservoir host of these bacteria.

MATERIALS AND METHODS

A total of 201 capybara (101 males and 100 females) were sampled from three ranches in the State of Apure, Venezuela. The first ranch, Hato El Frio, had an area of 78,000 ha, a bovine population of 36,000 and an estimated 40,000 capybara. On this ranch the samples were taken in two sites sufficiently

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separated (10 km) to be considered individually, called El Frio North and El Frio South. The second ranch belonging to the Ministry of Agriculture (called MAC) had an area of 6,000 ha, a bovine population of 3,600 and an estimated 3,000 capybara. The third ranch (Turagua) had an area of 65,600 ha, a population of 22,000 bovids and approximately 15,000 capybara. This ranch was also sampled in two sites (7 km apart) called Turagua East and Turagua West.

Each year in March a regulated number of capybara were harvested for meat and hides. Typically the capybara were rounded-up by men on horses and driven to where a group of men on foot surrounded them and dispatched each with a club.

Bacteriology

Specimens studied were the spleen and mesenteric and submaxillary lymph nodes. They were processed as follows: the 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* agar, trypticase soy agar, and Kuzdas Morse) to which 5% fetal bovine serum was added. The lymph nodes were homogenized in a Ten Broek grinder with a solution of 0.85% NaCl (pH 6.8) and inoculated on the same culture media.

The plates were incubated up to 9 days at 37 C in an atmosphere of 10% CO_2. They were examined daily after 48 hr post-inoculation. Colonies were observed with a stereoscopic microscope according to the method of Henry (Alton et al., 1975). Possible *Brucella* isolates were stained by the Koster technique (Alton et al., 1975). Several colonies from each sample showing typical characteristics of *Brucella* 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, catalase, oxidase, production of H_2S, reduction of nitrates and citrate (Alton et al., 1975; Cowan and Steele, 1979; MacFaddin, 1980). The dye sensitivity of *Brucella* isolates was determined by adding basic 0.1% fuchsin (1:25,000, 1:50,000, 1:100,000), 0.5% thionin (same dilutions), 0.1% methyl violet (1:100,000), and 1% safranin (1:5,000) to trypticase soy medium. Growth on media containing erythritol (1 mg/ml) and penicillin (5 IU/ml) was also studied. The media were inoculated with bacterial suspensions prepared in 0.85% sterile NaCl solution at a similar density with reference strains (*Brucella abortus* 544-2, *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 and/or anti-M, 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 × ATD) were used.

For the metabolic tests, the following substrates were used: Group I; L-alanine, L-glutamic acid; Group II; amino acids of the urea cycle, D,L-ornithine and L-lysine; Group III; 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 (Cameron and Meyer, 1953, 1955; 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 $QO_2(N)$ (microliters of oxygen uptake per mg of nitrogen during 60 min) was equal to or more than 50 μl (Meyer and Cameron, 1961a, b).

Serology

Blood samples were obtained from the jugular vein. The samples were allowed to clot, then were centrifuged, separated, and stored in a refrigerator until transport to the laboratory on wet ice.

The sera were tested with a polysaccharide antigen prepared from a rough strain of *Brucella melitensis* (B-115) (Díaz et al., 1979). In order to determine the quantity of polysaccharide contained in each ml of the antigen solution, the technique described by Dubois et al. (1956) was used.

A gel was prepared for use in the radial immunodiffusion (Díaz et al., 1979; Jones et al., 1980), Ouchterlony (Ouchterlony and Nilsson, 1973), immunoelectrophoresis (Kachwa, 1976) and counterimmunoelectrophoresis (Carrol et al., 1980) tests, by dissolving 0.8% agarose in 0.1 M glycine buffer (pH 8.6) (Chase, 1968). The antigen was dissolved in the glycine buffer to which had been added 10% NaCl. Antigen solution and gel were mixed in equal volumes at 65 C before application to the slides. The concentration of the antigen was from 73 to 292 μg/ml of gel. Tests using antisera from vaccinated cattle were negative while those with antisera from infected cattle were positive. The capybara sera were also tested in the tube test, Mercaptoethanol, Rivanol and card test utilizing an antigen of *Brucella abortus* (strain 1119-3).

The ages of the capybara were determined by the lens technique (Lord, 1959) with reference to lens weights of known age as reported by Ojasti (1973).

The results were analyzed by the standard chi-square test.

RESULTS

Twenty-three isolates of *Brucella* were made from 13 (13%) of the male and 10 (10%) female capybara. The difference was not significant ($\chi^2 = 0.90$, 1 df). Isolations were made from animals captured in four of the five sites (Table 5). The negative site, MAC, also had the lowest (40%) prevalence (Table 6).

TABLE 1. Characteristics of the 23 strains of *Brucella* isolated from capybara.

Strain no	CO_2 requirement	Serum requirement	Development* in: Thionin 1:25	Thionin 1:50	Thionin 1:100	Fuchsin 1:25	Fuchsin 1:50	Fuchsin 1:100	1:5 safranin	1:100 methyl violet	1 mg/ml erythritol	5 IU/ml penicillin	Base medium
3	Yes	No	4	4	4	–	–	–	–	2	4	4	4
30	Yes	Yes		–	–	2	3	3	4	3	4	2	4
46	No	Yes	4	4	4	1	2	2	4	4	4	4	4
74	No	Yes	4	4	4	1	1	–	2	4	4	4	4
75	Yes	No	4	4	4	–	–	2	1		4	3	4
78	No	Yes	4	4	4	2	–	–	2	4	4	4	3
87	Yes	No	4	4	4	–	–	–	–	2	4		4
98	Yes	No	4	4	4		–	–	–	4	4	3	4
102	No	Yes	4	4	4		–	2	–	3	4	4	4
103	Yes	No	4	4	4	–	–	2	–	3	4	4	4
105	Yes	No	4	4	4		–	–	2	2	4	3	4
148	Yes	No	4	4	4				2	2	4	3	4
149	Yes	Yes			–	4	4	4	2	3	4	3	4
157	No	Yes			–	4	4	4	1	4	4	–	4
166	No	Yes	–	3	4	4	4	4	3	2	4	2	4
168	Yes	Yes		–		3	3	4	4	3	3	3	4
171	No	Yes	4	4	4				2	3	4	4	4
174	Yes	No	3	3	4	4	4	4	4	3	4	4	4
177	Yes	No	2	3	4	4	4	4		4	4	4	4
179	No	No	4	4	4	–		–	–	4	4	3	4
192	No	No	2	3	3	–		–	–	4	4	1	4
198	Yes	No		2	3	1	2	3	4	4	4	4	4
199	Yes	Yes		–		2	3	4	4	4	4	3	4
16 M[a]	No	No	4	4	3	4	4	4	4	4	4	4	4
1330[b]	No	No	4	4	4	–		–	–	4	4	1	4
544-2[c]	Yes	Yes		–		4	4	4	3	4	4	3	4

The characteristics of the isolates are given in Table 1. All isolates exhibited smooth type colonies. By the criteria used for identification seven isolates were identified as *Brucella abortus* (biotypes 2, 3, 4, 5) and 15 strains as *Brucella suis* (biotypes 2 and 3). One isolate (strain 166) was originally identified as *Brucella melitensis* but oxidative metabolic tests suggested it should be considered *Brucella abortus*, biotype 7 (Tables 2, 3). When the 23 isolates were examined only on the bases of biochemical and serological reactions, the identification of 10 was

TABLE 1. Continued.

Production of H_2S (in days)					Urease (in min)				Monospecific antisera		Sensitivity to Tblisi phage		Species and biotype
1	2	3	4	5	15	30	60	120	A[b]	M[c]	1 RTD	$10^4 \times$ RTD	
−	−	−	−	−	±	±	±	±	+	−	−		*B. suis* type 2
++	++	++	++	++	−	±	±	±	−	+	+	+	*B. abortus* type 4
−	−	±	±	−	−	−	±	±	+	+	+	+	*B. abortus* type 7
−	−	−	−	−	−	−	±	±	+	−		±	*B. suis* type 2
−	±	−	−	−	−	−	±	±	+	−	−	±	*B. suis* type 3
−	−	−	−	−	−	−	−	±	+	−	−	−	*B. suis* type 2
−	−	−	−	−	−	−	±	±	+	−	−	−	*B. suis* type 2
−	−	−	−	−	−	±	±	+	−	−	−	+	*B. suis* type 2
−	−	±	±	−	±	±	±	±	+	−	−	±	*B. suis* type 2
−	−	−	−	−	±	±	±	±	+	−	−	+	*B. suis* type 2
−	−	−	−	−	±	±	±	±	+	−		+	*B. suis* type 2
−	−	−	−	−	±	±	±	±	+	−	−	+	*B. suis* type 2
±	±	++	++	++	−	−	±	±	−	+	+	+	*B. abortus* type 4
±	±	++	++	++	+	±	+	+	+	−	−	+	*B. abortus* type 2
−	−	−	−	−	−	−	±	±	+	+	−	±	*B. melitensis* type 3
+	++	++	++	++	−	−	±	±	−	+	+	+	*B. abortus* type 4
−	−	±	−	−	−	−	±	±	+	−	−	−	*B. suis* type 2
−	−	−	−	−	±	±	±	±	−	+	+	+	*B. abortus* type 5
+++	+++	+++	+++	+++	+	+++	+++	+++	+	−	−	+	*B. suis* type 3
−	−	−	−	−		−	+	±	+			+	*B. suis* type 2
−	−	−	−	−	±	±	±	±	+	−	−	+	*B. suis* type 2
++++	++++	++++	++++	++++	+	++	+++	+++	+	−	+	+	*B. abortus* type 3
+	+	++	++	++	−	±	±	±	−	+	+	+	*B. abortus* type 4
−	−	−	−	−	±	±	++	++	−	+	−	−	*B. melitensis* type 1
+++	+++	+++	+++	+++	++	+++	+++	+++	+	−	−	+	*B. suis* type 1
±	±	±	±	±	−	−	−	−	+	−	+	+	*B. abortus* type 1

[a] Development in dye, penicillin and base medium (4 = 100°C; 3 = 75°C; 2 = 50°C; 1 = 25°C).
[b] A = A antiserum.
[c] M = M antiserum.
[d] Goat reference strain.
[e] Swine reference strain.
[f] Bovine reference strain.

TABLE 2. Metabolic characterization of strains of *Brucella* of conflictive identity compared with biochemical methods.

Strain no	Sensitivity to dyes		Oxidative metabolism	
	Species	Biotype	Species	Biotype
46	*B. abortus*	7	*B. abortus*	5
74	*B. suis*	2	*B. suis*	3
75	*B. suis*	3	*B. suis*	2
78	*B. suis*	2	*B. suis*	3
102	*B. suis*	2	*B. suis*	3
157	*B. abortus*	2	*B. suis*	2
166	*B. melitensis*	3	*B. abortus*	7
171	*B. suis*	2	*B. suis*	2
198	*B. abortus*	3	*B. abortus*	3
199	*B. abortus*	4	*B. abortus*	4

difficult. The oxidative metabolic tests clarified the identification (Table 2). Table 3 presents the values of the oxidative rates $QO_2(N)$. In Table 4 the isolates are compared to the age of the infected animals. No isolations were made from animals less than 1 yr old nor 5 yr or older. The greatest number of isolations were from capybara 4 yr of age.

Table 6 presents the results of the immunodiffusion in gel tests of sera from the 201 capybara taken in the five different sites. Of the 201 sera 116 were positive in the immunodiffusion in gel tests, while 117 were positive in the other tests. Sixty-nine of the 101 males (68%) were positive and 47 of the 100 (47%) females were reactors, a significant difference ($\chi^2 = 9.19$, 1 df).

TABLE 4. Age distribution of capybara in relation to isolations of *Brucella*.

Age	Sample size	No. isolations	Percent
6–11 mo	27	0	0
1 yr	72	9	13
2 yr	40	6	15
3 yr	40	5	13
4 yr	14	3	21
5 yr	5	0	0

Table 7 shows the age of the capybara in relation to the results of the immunodiffusion in gel tests in separate sites. Ages varied from 6 mo to 5 yr or more. A high percentage (48%) of reactors were found in animals less than 1 yr. Reactor prevalences for the five sites varied considerably with the highest prevalence found in Turagua East (81%) and the lowest in MAC (40%) (significant difference, $\chi^2 = 42.03$, 1 df).

DISCUSSION

An analysis of the results obtained from three ranches (and five sites) sampled in the llanos, State of Apure, Venezuela was made for the purpose of determining whether the capybara could be considered as a possible reservoir of *Brucella* spp. Serological results showed that these animals became infected with *Brucella* at a young age, and could have remained as reactors for years, indicating that the capybara is an important alternate host of *Brucella* spp., with probable epizootiological repercussions.

TABLE 3. Oxidative rates $QO_2(N)$ of 10 strains of *Brucella* isolated from capybara with four amino acids and four carbohydrates.

Strain no.	Substrates							
	Amino acids				Carbohydrates			
	L-alanine	L-glutamic acid	D,L-ornithine	L-lysine	L-arabinose	D-galactose	D-ribose	D-glucose
46	122	58	36	37	176	110	90	73
74	42	22	79	62	39	26	85	56
75	16	42	137	41	529	111	183	55
78	57	85	98	91	179	166	81	69
102	47	55	130	48	82	71	82	60
157	34	236	135	34	267	135	346	423
166	183	364	44	35	222	144	84	81
171	12	56	56	33	131	76	66	87
198	63	93	46	33	378	377	172	227
199	102	197	44	34	183	112	358	200
16 M[a]	80	97	35	36	24	39	41	56
1330[b]	63	69	160	147	122	285	306	378
544-2[c]	139	320	40	19	160	288	109	85

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

TABLE 5. Distribution by species of *Brucella* isolated from capybara.

Site	Total animals	Species of *Brucella*	No. of isolations	Percent
El Frio North	68	*B. abortus*	2	2.9
		B. suis	1	1.5
El Frio South	37	*B. suis*	8	21.6
MAC	38	none		
Turagua East	26	*B. abortus*	3	11.5
		B. suis	2	7.7
Turagua West	32	*B. abortus*	3	9.4
		B. suis	4	12.5
Total	201	*B. abortus*	8	4.0
		B. suis	15	7.5
		Total	23	11.4

TABLE 6. Serological reactors to *Brucella* in male and female capybara (immunodiffusion in gel test).

Site	Males	Females	Total
El Frio North	21/32 (68)*	16/36 (44)	37/68 (54)
El Frio South	9/11 (82)	13/26 (50)	22/37 (60)
MAC	11/21 (52)	4/17 (24)	15/38 (40)
Turagua East	14/18 (78)	7/8 (88)	21/26 (81)
Turagua West	14/19 (74)	7/13 (54)	21/32 (68)
Total	69/101 (68)	47/100 (47)	116/201 (58)

* No. reactors/no. tested (%).

The significance of having isolated *Brucella suis* from the group of capybara studied lies in the co-habitation of these animals in pastures with cattle, presumably infecting and/or being infected by the bovids. This may result in serious problems when bovine serological reactors are detected, which normally leads to the decision to eliminate these animals from the herd.

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TABLE 7. Age distribution of serological reactors to *Brucella* in capybara (immunodiffusion in gel test).

Site	6–11 mo	1 yr	2 yr	3 yr	4 yr	5 yr
El Frio North	5/7 (71)*	15/24 (63)	6/14 (43)	8/15 (53)	3/5 (60)	0/3 (0)
El Frio South	0/3 (0)	12/17 (71)	6/8 (75)	1/6 (17)	2/2 (100)	1/1 (100)
MAC	2/5 (40)	2/9 (22)	5/12 (42)	6/6 (100)	0/2 (0)	0/1 (0)
Turagua East	2/3 (67)	9/12 (75)	4/5 (80)	4/4 (100)	2/2 (100)	
Turagua West	4/9 (44)	8/10 (80)	1/1 (100)	6/9 (67)	2/3 (67)	
Total	13/27 (48)	46/72 (64)	22/40 (55)	25/40 (63)	9/14 (64)	1/5 (20)

* No. reactors/no. tested (%).

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