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Serological and Bacteriological Study of Swine Brucellosis

VERONICA R. LORD,* JOHN W. CHERWONOGRODZKY, MABEL J. MARCANO, AND GERARDO MELENDEZ

Instituto de Investigaciones Veterinarias, Centro Nacional de Investigaciones Agropecuarias, Laboratorio de Brucelosis, Maracay 2102, Venezuela, and Defence Research Establishment, Suffield, Medicine Hat, Alberta, Canada T1A 8K6

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A serological and bacteriological study was performed with sera taken from 2,228 swine from six states in Venezuela. None of the animals were vaccinated against brucellosis, and the prevalence of the disease varied from 5 to 89% on farms located in these states. Our studies indicated that the animals could be categorized into four groups depending on the degree of reactivity in serological tests. *Brucella suis* biovar 1 was isolated from the lymph nodes, spleens, and semen samples of seropositive animals and identified by oxidative metabolic techniques. *B. suis* could not be isolated from tissues of seronegative swine even from farms with cases of the disease (detected by serology). Results suggest that, although the immunodiffusion assay using *Brucella melitensis* B115 polysaccharide B or *B. abortus* 1119-3 O-polysaccharide could be useful in the detection of active infections, it is perhaps not as sensitive as some of the other standard serological tests used in this study for the detection of swine brucellosis.

Brucella suis is a zoonotic disease that causes orchitis in boars, abortion in sows, and undulant fever in humans (16).

Although sporadic cases occur throughout the world, the disease is enzootic in Latin America and this is considered to be the region where swine brucellosis is the most prevalent (10).

Previous serological surveys made by us within Venezuela suggest that the incidence of disease in swine is extremely variable among farms, ranging from 5 to 89%. As it is not economically feasible to eradicate entire herds that have one or a few seropositive reactors, and since it is likely that some animals may have only been exposed to rather than infected by *B. suis*, there is an urgent need to develop an assay that will identify swine that are carriers of brucellosis.

Diaz et al. (7) described polysaccharide B (Poly-B), isolated from *Brucella melitensis* B115, which was precipitated by the sera of *Brucella abortus*-infected cattle only and not by sera from cattle vaccinated with *B. abortus* S-19. Cherwonogrodzky and Nielsen (4) determined that O-polysaccharide (O-chain), a polymer of 4,6-dideoxy-4-formamido-∂-D-mannose, was the active component of Poly-B. To our knowledge these antigens have been used only in the differentiation of the sera of vaccinated and unvaccinated cattle. The present work shows that these reagents can be used to detect active *B. suis* infections in swine. Comparisons between standard serological tests and those using Poly-B or O-chain polysaccharide antigens are also made.

The methods used in this study are described below.

Sera. A total of 2,228 swine from 6 (Aragua, Carabobo, Guarico, Miranda, Portuguesa, and Zulia) of the 22 states of Venezuela were studied.

Blood was collected aseptically from either the jugular or ear veins of swine kept in large piggeries. The sera were separated by centrifugation in a field laboratory, transported cold to the principal laboratory, and kept frozen until testing.

Tissues. All sampling was done aseptically with individually wrapped forceps, scissors, and tweezers that were autoclaved at 121°C at 15 lb/in^2 for 30 min. The spleens and lymph nodes (as well as semen samples from boars) were removed, placed in sterile plastic bags, and then continually chilled on ice until they could be homogenized for bacterial examination. Samples of tissues were plated onto Albimi agar, Kudzas Morse medium, and Trypticase soy agar with 5% fetal bovine serum (1). Animals were categorized into four groups. Group 1 (from a single farm with a seroprevalence of 89% as determined by previous serological tests) had 285 animals; tissue samples were collected from all animals, and there were 45 semen samples. Group 2 (from farms with a seroprevalence of 20 to 35%) had 472 animals; tissue samples were collected from 236 swine, and semen was collected from 36 animals. Group 3 (from farms with a seroprevalence of 5 to 10%) had 839 swine, and tissue and semen samples were collected from 418 and 39 animals, respectively. Group 4 had 632 serologically negative swine, of which 126 were examined for infected tissues and semen.

Serological studies. The rapid plate agglutination test (RPAT), standard tube agglutination test (STAT), 2-mercaptoethanol agglutination test (2-ME), complement fixation test (CFT), rivanol test (RIV), and card test (CT) were performed with a whole-cell antigen of *B. abortus* 1119-3 prepared by the Instituto de Investigaciones Veterinarias by methods described by Alton et al. (1). Any serum that gave a titer greater than 50 IU by the RPAT, STAT, 2-ME, or RIV, gave a titer greater than 13 IU by the CFT, or caused noticeable agglutination on the CT was considered positive.

Agar-gel immunodiffusion assay (AGID). Assays were prepared with 0.8% agarose–10% NaCl–0.1 M Tris (pH 8.3), and the antigens used were *B. melitensis* B115 Poly-B (crude) (prepared by the Instituto de Investigaciones Veterinarias) and *B. abortus* 1119-3 (O-chain) (purified) (prepared by the Defence Research Establishment, Suffield, Medicine Hat, Alberta, Canada). All antigens were used at 1 mg/ml of buffer (8).

The AGIDs were incubated at approximately 22°C in a moist chamber and were observed from 8 to 48 h.

The appearance of any precipitin between antigen and antiserum wells was considered a positive result.

Bacteriological studies. Specimens studied were the spleen and mesenteric and submaxillary lymph nodes. They were processed as follows. The spleens were washed in a solution of

* Corresponding author. Mailing address: Instituto de Investigaciones Veterinarias, Centro Nacional de Investigaciones Agropecuarias, Laboratorio de Brucelosis, Apdo. 70, Maracay 2702, Venezuela.

TABLE 1. Bacteriological and serological examination of swine from farms with brucellosis-infected animals

Swine category[a]	Bacteriological isolation (%)[b] from:			Positive sera (%) by:							
	Lymph nodes	Spleen	Semen	RPAT	STAT	2-ME	CFT	RIV	CT	AGID with:	
										Poly-B	O-chain
Group 1 (n = 285; 89%)	86	77	49	100	100	100	100	100	100	100	100
Group 2 (n = 472; 20 to 35%)	59	50	43	89	100	60	40	55	40	40	40
Group 3 (n = 839; 5 to 10%)	49	35	31	22	100	40	30	39	20	30	30
Group 4 (n = 632; 0%)	0	0	0	0	0	0	0	0	0	0	0

[a] The total number of animals examined and the previous degree of porcine brucellosis in these farms are as noted in parentheses. Groups 2 and 3 consist of swine that were seropositive by at least one test. Group 4 is a collection of all seronegative swine from groups 2 and 3.
[b] Percentage of bacteria from lymph nodes, spleen, or semen samples that were identified as *B. suis* biovar 1.

0.85% NaCl, immersed in 95% alcohol, flamed, and seared with a hot spatula, and a 1-cm^3 piece, to which 5% fetal bovine serum was added, removed for inoculation of solid culture media (Albimi *Brucella* agar, Trypticase soy agar, and Kudzas Morse). The samples of semen were inoculated in the same media. The lymph nodes were homogenized in a Ten Broeck 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 h of inoculation. Colonies were observed with a stereoscopic microscope according to the method of Henry (1). Possible *Brucella* isolates were stained by the Koster technique (1).

Several colonies from each sample showing characteristics typical of *Brucella* were harvested and inoculated on agar (potato agar and Trypticase soy agar) slants 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 and oxidase, production of H_2S, and reduction of nitrates and citrate (1, 6, 9). 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% Thionine (same dilutions), 0.1% methyl violet (1:100,000), and 1% safranine (1:5,000) to Trypticase soy medium. Growth on media containing L-erythritol (1 mg/ml) and penicillin (5 IU/ml) was also studied. The media were inoculated with bacterial suspensions prepared in sterile 0.85% NaCl solution with reference strains (*B. abortus* 544-2, *B. melitensis* 16M, and *B. suis* 1330; provided by the Pan American Zoonoses Center) at similar densities. The plates were divided into four quarters for inoculation with a calibrated platinum loop and incubated at 37°C for 72 h. Monospecific antisera, anti-*B. abortus* (anti-A) and/or anti-*B. melitensis* (anti-M), were used to determine which of the agglutinins predominated in the isolates. Two concentrations of Tbilisi phage (the routine test dilution [RTD] and 10,000× RTD) were used.

For the metabolic tests, the substrates indicated were used for the groups as follows: group I, L-alanine and L-glutamic acid; group II, amino acids of the urea cycle, DL-ornithine and L-lysine; and group III, carbohydrates, L-arabinose, D-galactose, D-ribose, and D-glucose (3, 14).

A 1% Sorensen solution buffered with phosphates to pH 7.0 was prepared for each of the substrates (2, 11). Packed bacterium 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 wavelength of 420 nm. The normal suspension contained approximately 0.8 mg of nitrogen per ml. Manometric determinations were made with a Warburg apparatus (5). A substrate was considered to have been oxidated when the oxygen uptake (in microliters per milligram of nitrogen during 60 min) was equal to or greater than 50 (12, 13).

Several species of *Brucella* are zoonotic diseases, and there is a direct relationship between the level of brucellosis in livestock and the incidence of human infections on farms (15). At one farm located within Venezuela, a previous serological study found that the incidence of brucellosis in the piggery was 89% (data not shown). Of the 10 workers, 9 were afflicted with brucellosis (the 10th was a new employee), and all four dogs on the premise were highly seropositive for this disease.

Because of the high prevalence of brucellosis on this farm, bacteriological and serological examinations were done on all 285 swine (group 1). *B. suis* biovar 1 was isolated from the lymph nodes (86%), spleens (77%), and semen samples (49%) at rates comparable to those previously observed for serological tests (shown in Table 1). Isolates were identified as *B. suis* biovar 1 by the following culture characteristics: 100% growth in three dilutions (1:25,000, 1:50,000, 1:100,000) of Thionine, no growth in the same dilutions of fuchsin, and growth in methyl violet (1:100), I-erythritol (1 mg/ml), penicillin (5 IU/ml), and Albimi culture medium. Biochemical characteristics that confirmed isolates to be *B. suis* biovar 1 were the following: production of H_2S from day 1 to 5, urease activity from 15 to 120 min, observation of agglutination with monospecific antiserum anti-A without agglutination with anti-M, and sensitivity to the Tbilisi phage (no sensitivity with the RTD and sensitivity with 10,000× RTD).

All serological tests were positive, and considering the extent of brucellosis on this farm, it is likely that all the swine were infected. Both Poly-B and O-chain were precipitated in the AGID by the sera of these swine. It appears these antigens can be used to rapidly identify active infections of brucellosis in swine as well as in cattle (8).

Piggeries that previously had far lower rates of serological positivity (groups 2 and 3) were more difficult to assess. For these swine, all were positive by the STAT and 20 to 55% were positive by the CFT, CT, or AGID (using Poly-B or O-chain as the antigen) while the isolation of *B. suis* from the lymph nodes, spleens, and semen samples varied from 59% for group 2 to 49% for group 3. It could not be determined if the presence of this bacterium meant an active infection or a transient exposure that would lead to a clearing of disease and subsequent protection.

It was noticed that swine that had the highest STAT and 2-ME titers (i.e., 100 IU or more) usually had the bacterium in their lymph nodes and spleens. Therefore, although the assays appear to vary in their sensitivities, we recommend that swine

with high values for any of the serological test results should be either eliminated or removed from the rest of the farm.

We wished to determine if swine that were seronegative on all tests described in this study but were taken from farms that had *B. suis*-infected animals (group 4) had this bacterium. Of 632 such animals, none were culture positive.

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