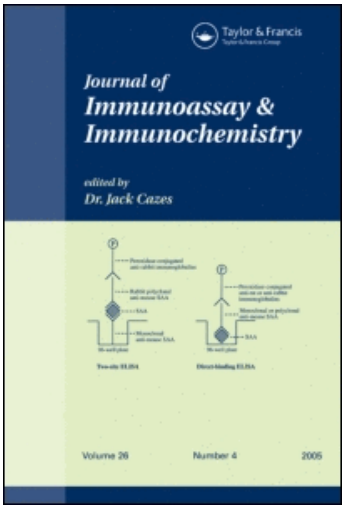

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Salmonella enterica Serotype Urbana Interference with Brucellosis Serology

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Abstract: Sheep were immunized with killed *Salmonella enterica* serotype Urbana cells and their sera were tested in various serological tests for antibody to *Brucella sp*., *Yersinia enterocolitica* O:9 and *Escherichia coli* O:157 H:7.

Of the eight sheep, all gave a positive agglutination reaction in the brucellosis buffered antigen plate agglutination test (BPAT), seven gave positive brucellosis standard tube agglutination test (TAT) and complement fixation test (CFT) results and four gave slightly positive reactions in a competitive enzyme immunoassay (CELISA). Seven sera were negative in an indirect enzyme immunoassay (IELISA-SLPS) using *B. abortus* smooth lipopolysaccharide (SLPS) antigen and all were negative in a fluorescence polarization assay (FPA-OPS) using *B. abortus* O-polysaccharide antigen. Two sheep gave a slight positive reaction in an IELISA using *Brucella* rough lipopolysaccharide antigen (IELISA-RLPS) and four sheep were slightly positive in an FPA using *Brucella* LPS core antigen (FPA-CORE). All sheep had high antibody responses to *S. enterica* serotype Urbana, *Y.* and *E. coli* O:157 and 7 were positive for antibody to *Y. enterocolitica* O:9 when tested by IELISA. The sheep were negative when tested in the FPA using OPS from *Y. enterocolitica* O:9 but all were strongly positive in the FPA using OPS from *E. coli* O:157 while seven sheep had titers to *S. enterica* serotype Urbana. The impact on diagnostic serology for brucellosis is discussed.

Keywords: Serology, Brucellosis, Cross reaction, Primary binding assays, Conventional tests

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INTRODUCTION

Serological cross reaction between *Brucella sp*. and *Salmonella sp*. was first noted by Gilman and Cameron[1] and subsequently, a number of other reports appeared. Interference with the serological diagnosis of brucellosis was not thought to be significant in spite of the antigenic relationship between *S. enterica* serotype Urbana and *Brucella sp*.[2–5] Naturally occurring *S enterica* serotype Urbana infection in cattle was also described[6] with the conclusion that there were no problems with serological diagnosis of brucellosis.

As the prevalence of brucellosis decreases in many areas of the world, false positive serological reactions due to antibody responses to cross reacting microorganisms will become more important. The various cross reactions have been well described by Corbel and coworkers[7,8] and a number of schemes have been developed to attempt to decrease reactivity of cross reacting antibody. These include the use of various antigens in precipitation tests and a caotropic indirect enzyme immunoassay,[9] rough lipopolysaccharide antigen in indirect enzyme immunoassay;[10] monoclonal antibody which detects mainly IgG2 subclass of antibody;[11] lipopolysaccharide specific monoclonal antibody;[12] and the use of multiple tests.[7] None of these tests were able to eliminate all serological cross reacting antibodies or alternately, if the specificity was high, the sensitivity was generally decreased.

Measurement of the cellular mediated immune response has also been used to distinguish brucellosis from other infections. In general, in vitro assays for gamma interferon have given variable results,[13–16] while the skin test has provided consistent specific discrimination.[13,17] Unfortunately, assessment of cellular immune reponse does not lend itself to diagnosis on a larger scale. Hence, a serological test that could be used to distinguish some cross reactions would be a useful tool for the diagnostician. In this communication, sheep exposed to *S. enterica* serotype Urbana were tested by a variety of serological tests to establish their relative sensitivity and specificity.

EXPERIMENTAL

Animals

Eight-year-old cross-bred sheep were prebled and immunized intramuscularly with 10^9 heat killed *S. enterica* serotype Urbana incorporated into 1 mL of Freund's complete adjuvant. Forty-two days later they were bled again and injected intramuscularly with an additional 10^9 killed bacteria in 1 mL of saline. Blood was taken 14 and 98 days later. Sera were stored at -20°C and tested simultaneously.

Serological tests: All sera were tested for antibody to *B. abortus* by the buffered antigen plate agglutination test (BPAT) as described by Angus and

Barton,[18] the complement fixation test (CFT),[19] tube agglutination test (TAT),[20] indirect and competitive enzyme immunoassay (IELISA and CELISA),[21] and fluorescence polarization assay (FPA).[21] In addition, IELISAs were performed using *E. coli* O157:H7, *Y. enterocolitica* O:9 and *S:enterica* serotype Urbana smooth lipopolysaccharide (SLPS) antigen and FPA using fluorescein isothiocyanate labelled O-polysaccharide (OPS) purified from the same bacteria.[10]

Data

The BPAT was expressed as positive or negative. Sera in the TAT at a dilution of 1:25 were considered suspicious if 50% agglutination occurred. Additional agglutination at 1:25 or higher serum dilution was considered as positive. The CFT was positive if 50% of the erythrocytes were lysed at a 1:5 serum dilution or higher. The IELISA results were expressed as % positivity using the following equation:

$$\%P = \text{optical density}_{\text{test sample}} \times 100/\text{average optical density}_{\text{strong positive serum control}}.$$

The CELISA results were expressed as % inhibition with a buffer control as 0% inhibition:

$$\%I = 100\text{-optical density}_{\text{test sample}} \times 100/\text{average optical density}_{\text{uninhibited control}}$$

The FPA was expressed as millipolarization (mP) units. Predetermined cutoff values for *Brucella* antigens of 20%P for the IELISAs, 26%P for the CELISA and 80 mP for the FPAs were used. Appropriate positive and negative control sera were included with all tests.

RESULTS

All serological results are tabulated in Table 1. Before immunization, the 8 sheep were negative when tested in IELISA using *B. abortus* SLPS, *E. coli* O157:H7 SLPS, Y. enterocolitica O:9 SLPS and one animal gave a weakly positive response to *S. enterica* serotype Urbana SLPS antigen. Two of the sheep gave slight responses when tested with combined *Brucella* S/RLPS antigens. All sheep were negative in the *Brucella* CELISA. Four sheep were positive in the FPA using OPS from *E. coli* O157:H7 and negative in FPA using *B. abortus* (OPS and Core antigens), *Y. enterocolitica* O:9 and *S. enterica* serotype Urbana OPS antigens. One sheep had a slight titer in the brucellosis TAT and was BPAT positive. Five sera activated complement in the absence of antigen.

Table 1. Serological test Results with IELISAs using *B. abortus* SLPS and S/RLPS antigens, *E coli* O157:H7 SLPS, *Y. enterocolitica* O:9 SLPS and *S. enterica* serotype Urbana SLPS; CELISA for anti-*Brucella* antibody and FPA using OPS from *B. abortus, E. coli O157:H7, Y. enterocolitica O:9* and *S. enterica* serotype Urbana as well as *B. abortus* CORE/OPS antigens

	IELISA						FPA							
#	SLPS	S/RLPS	YS	EC	SU	CE	OPS	O/RPS	YS	EC	SU	TAT	BPA	CFT
79	1	9	3	2	9	7	63	72	59	68	77	N	N	N
693	2	10	7	15	47	13	61	77	48	79	68	N	N	N
760	2	10	1	3	9	9	63	78	66	82	66	N	N	AC
761	13	24	7	6	13	13	62	54	67	153	64	N	N	AC
768	19	22	1	10	16	16	60	74	63	100	74	2−−	P	AC
772	7	10	11	12	13	13	70	73	55	70	60	N	N	AC
781	1	10	1	3	14	14	58	75	59	83	79	N	N	AC
785	2	12	9	9	8	8	59	72	58	90	77	N	N	N
42 days after primary immunization														
79	1	10	9	44	71	10	59	68	62	79	76	N	P	AC
693	6	11	40	90	87	20	60	81	64	142	122	42-	P	40
760	3	11	40	81	83	30	63	86	56	131	94	443	P	AC
761	18	23	39	82	93	23	61	80	61	131	98	42-	P	N
768	22	19	34	98	88	35	61	77	58	147	98	441	P	10
772	12	11	42	92	92	23	62	78	58	116	84	43-	P	AC
781	5	11	41	86	93	24	60	75	62	142	126	431	P	10
785	5	11	37	30	87	11	61	70	55	85	86	N	N	N
56 days after primary immunization, 14 days after reimmunization														
79	2	10	19	36	84	13	59	74	57	105	73	2−−	P	AC
693	7	10	40	101	90	18	59	75	51	154	157	42-	P	40
760	4	11	42	89	75	47	61	84	59	137	115	443	P	40/10
761	18	25	39	86	90	38	63	77	62	141	118	342	P	10
768	23	16	30	97	89	42	59	80	58	163	132	441	P	10
772	15	11	44	85	91	21	50	84	55	124	84	441	P	AC
781	6	10	37	87	87	70	60	83	61	160	191	444	P	20/5

785	7	10	35	33	92	10	55	66	55	134	62	N	P	AC
98 days after primary immunization, 56 days after reimmunization														
79	4	10	30	81	92	6	63	67	59	102	78	2– –	P	AC
693	6	11	41	109	97	19	63	86	60	155	159	42-	P	80
760	5	11	41	86	94	35	65	87	61	127	94	443	P	AC
761	15	23	44	102	94	37	57	78	58	153	125	441	P	AC
768	26	22	38	98	90	61	64	84	57	157	117	43-	P	20/5
772	9	11	45	99	97	23	60	79	56	149	120	441	P	10
781	9	10	48	104	93	61	61	78	59	161	192	443	P	40/10
785	8	11	45	85	96	19	55	84	57	145	88	1–	P	10

is the animal number.

SLPS = *B. abortus* S1119.3 smooth lipopolysaccharide.

S/RLPS = *B. abortus* S1119.3 SLPS and *B. abortus* RB51 rough lipopolysaccharide.

YS = Yersinia enterocolitica SLPS (IELISA) and OPS (FPA).

EC = E. coli O157:H7 SLPS (IELISA) and OPS (FPA).

SU = *Salmonella enterica* serotype Urbana SLPS (IELISA) and OPS(FPA).

CORE/OPS = *B. abortus* S1119.3 OPS and *B. abortus* RB51 Core antigen.

CE = competitive ELISA for antibody to B. abortus.

TAT = standard tube agglutination test.

BPAT = buffered antigen plate agglutination test.

CFT = complement fixation test.

IELISA results are presented as % positivity relative to a strong positive control serum.

CELISA results reflect % inhiition relative to an uninhibited control (buffer control).

FPA data are presented as millipolarization units.

TAT results are from three serum dilutions with 1 indicating 25% of the cells agglutinated, 2 is 50%, 3 is 75% and 4 is complete agglutination.

BPAT results are positive (P) or negative (N).

CFT data are reciprocal serum dilutions giving 50% or less hemolysis. AC indicates activation of complement in the absence of antigen. Where two numbers occur, the first number is the last serum dilution giving 50% or less hemolysis and the second indicates the level of AC activity of the serum.

After immunization with *S. enterica* serotype Urbana, one serum resulted in weak responses in the *Brucella* IELISAs and 2 gave low responses in the CELISA. All animals were positive in the other IELISAs except for one serum in the *Y. enterocolitica* assay. The FPA using *B. abortus* and *Y. enterocolitica* OPS were negative while three sera were slightly positive in the brucellosis FPA with the combined antigen. Seven sera were positive in the *E. coli* and *S. enterica* serotype Urbana FPAs and in the BPAT while 6 sera were TAT positive and 3 were CFT positive.

After re-immunization, 1 animal remained a low positive reactor in the IELISA using *B. abortus* SLPS and 2 were slightly positive using the brucellosis combined antigen in IELISA. Seven sera were positive in the IELISA using *Y. enterocolitica* and all were positive with the FPAs for *E. coli* and *S. enterica* serotype Urbana SLPS antigens. Two sera gave low reactions and 2 gave intermediate reactions in the CELISA. All sera were negative in the brucellosis and the *Y. enterocolitica* FPAs while all were positive in the *E. coli* FPA and the BPAT. Six were positive in the *S. enterica* serotype Urbana FPA and seven in the TAT. Five sera were CFT positive and the remaining 3 sera gave anticomplementary reactions.

DISCUSSION

Sheep for this study were selected based on negative brucellosis tests using IELISA, CELISA and FPA. Unfortunately, 2 of the sheep had low levels of antibody to *B. abortus* RLPS. This may be due to exposure to a crossreacting organism and in this case both animals had FPA reactivity to *E. coli* O157:H7. One of the two sheep also gave a positive reaction in the BPAT and a small titer in the TAT. Five animals activated complement in the CFT in the absence of antibody. Three other sheep had low levels of antibody to *E. coli* as well. Based on the pre-immunization data, it is apparent that serological cross reactions can occur with some of the conventional assays. In *S. enterica* serotype Urbana- immunized animals, the conventional tests were largely positive while the primary binding assays using *B. abortus* antigens (IELISA, CELISA and FPA) or *Y. enterocolitica* O:9 (FPA) were generally negative or low positive. Primary binding assays using *E. coli* and *S. enterica* serotype Urbana antigens (IELISA and FPA) were highly positive.

From the data, it appears that *S. enterica* serotype Urbana shares more antigenicity with *E. coli* O157:H7 than with *B. abortus* and *Y. enterocolitica* O:9 although the *Y. enterocolitica* O:9 IELISA gave low positive reactions with all sera. It is also evident that the *Y. enterocolitica* O:9 IELISA measures antibody differently than the FPA using OPS antigen as the FPA was negative throughout. Thus the serological tests of choice for the diagnosis of ovine brucellosis are the FPA and IELISA.

In Canada, brucellosis in domestic animals was eradicated in 1984, however, on a few occasions animals with low levels of antibody have been

detected during regular surveillance activities. These animals usually react in the BPAT and give persistent low levels of reactivity in the CELISA. The sera examined to date have been FPA and IELISA negative with variable CFT reactivity. Based on the data presented, it is clear that crossreacting microorganisms can cause this kind of diagnostic problem and it is equally clear that those problems can be avoided by using a different test such as the FPA or the IELISA, both of which are accepted by the OIE as prescribed tests for international trade.

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