Immunodiagnostic Assays for Flea-Borne Typhus

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Abstract

Flea-borne typhus in humans is found worldwide and is etiologically related to both *Rickettsia typhi* and *Rickettsia felis.* Endemic foci are found in many regions, including Los Angeles and Orange Counties in Southern California. This poster describes some of our testing with both a 2-antigen micro-IFA and specific ELISA assays for these two agents. Although multiple assays for *R. typhi* have been commercially available for over 20 years, the *R. felis* ELISA has been developed more recently. Native rOmpB from octyl-glucoside-extracted *R. felis* was eluted in a pure form in the void volume of Sephadex G-100 gel chromatography. This assay is compared with values derived using the 2-antigen micro-IFA assay using normal healthy blood donor sera from the Texas Gulf Coast.

Materials and Methods

Purification of rOmpB from *R. felis:* *R. felis* was propagated in D.mel-2 cells adapted to serum-free medium at 28°C. Harvests were pooled and stored at -85°C°C. Rickettsia were harvested by differential centrifugation, purified via standard Renografin density gradient and stored at -85°C. An aliquot of *R. felis* in PBS was then brought to 1% octyl-β-D-glucopyranoside and extracted for 15 minutes at 25°C. The cell-free supernate was removed after centrifugation and layered over Sephadex G-100 equilibrated with PBS. Fractions were collected by gravity flow and analyzed by a BCA protein assay (Pierce) and SDS-PAGE using 10% Bis-Tris gels (Life Technologies). A single peak of 120 kDa protein was found in the void volume and these peak fractions were pooled and stored in aliquots at -85°C.

MIF assay for flea-borne typhus: *R. felis* was propagated as described (above) and *R. typhi* was propagated in Vero-6 cells at 32°C. Both were purified as described and inactivated for slide preparation in 0.25% methanol-free formaldehyde. Antigens were blended to a standard density in a background matrix, robotically applied to 12-well teflon-masked IFA slide stock, acetone-fixed and packaged under vacuum.

***R. felis* ELISA development:** Purified rOmpB isolated as described above was coated onto medium-binding ELISA wells (Costar) in a series of dilutions. Sera which were negative, weakly or strongly positive for *R. felis* by MIF assay (above) were used to optimize the titer of antigen and horseradish peroxidase (HRP) conjugated anti-human IgG. Test sera were diluted 1:100 in sample diluent and added to the coated wells. A serum incubation of 60 minutes and conjugate incubation of 30 minutes (both at ambient temperature) were separated by 4X washing with PBST. TMB substrate was added for a 10 minute incubation followed by a stop solution. Results were measured at 450 nm on an ELISA plate reader and compared with MIF titers, especially with sera giving weak reactions at 1:32 (typhi) or 1:64 (felis) using the MIF technique. These sera define the cut-off calibrator for the ELISA results, with absorbance values within 10% of this cutoff absorbance value defining an “equivocal” range. Titers within this equivocal range are expected to be evenly divided between high negative and low positive.

***R. typhi* ELISA formats:** Two formats were used from Inventory, with kit components including ELISA modules coated with either rOmpB antigen or LPS antigen.

Individual Serum Data

180 serum samples (n= 180) from the Gulf Coast Regional Blood Center were randomly selected from plasma units processed May 13-14, 2013 that had been cleared by required donor blood testing. Donor age ranged from 17-75 years of age and donor residence is expected to be within the region serviced by Gulf Coast Regional Blood Center in Houston, Texas.

Serum ID	*Rickettsia felis*		*Rickettsia typhi*		
	OmpB	MIF	OmpB	LPS	MIF
GC001	NEG	NEG	NEG	NEG	NEG
GC002	NEG	NEG	NEG	NEG	NEG
GC003	NEG	NEG	NEG	NEG	NEG
GC004	NEG	NEG	NEG	NEG	NEG
GC005	NEG	NEG	NEG	NEG	NEG
GC006	NEG	NEG	NEG	NEG	NEG
GC007	NEG	NEG	NEG	EQ	POS
GC008	NEG	NEG	NEG	NEG	NEG
GC009	NEG	NEG	NEG	NEG	NEG
GC010	NEG	NEG	NEG	NEG	NEG
GC011	NEG	NEG	NEG	NEG	NEG
GC012	NEG	NEG	NEG	NEG	NEG
GC013	NEG	NEG	NEG	NEG	NEG
GC014	NEG	NEG	NEG	NEG	NEG
GC015	NEG	NEG	NEG	NEG	NEG
GC016	NEG	NEG	NEG	NEG	NEG
GC017	EQ	POS	NEG	NEG	NEG
GC018	NEG	NEG	NEG	NEG	NEG
GC019	NEG	NEG	NEG	NEG	NEG
GC020	NEG	NEG	NEG	NEG	NEG
GC021	NEG	NEG	NEG	NEG	NEG
GC022	NEG	NEG	NEG	NEG	NEG
GC023	NEG	NEG	NEG	NEG	NEG
GC024	NEG	NEG	NEG	NEG	NEG
GC025	NEG	NEG	NEG	POS	POS
GC026	NEG	NEG	NEG	NEG	NEG
GC027	POS	POS	NEG	NEG	NEG
GC028	NEG	NEG	NEG	NEG	NEG
GC029	NEG	NEG	NEG	NEG	NEG
GC030	NEG	NEG	NEG	NEG	NEG
GC031	NEG	NEG	NEG	NEG	NEG
GC032	NEG	NEG	NEG	NEG	NEG
GC033	NEG	NEG	NEG	NEG	NEG
GC034	EQ	EQ = 1:64	NEG	NEG	NEG
GC035	NEG	NEG	NEG	NEG	NEG
GC036	NEG	NEG	NEG	NEG	NEG
GC037	NEG	NEG	NEG	EQ	NEG
GC038	NEG	NEG	NEG	NEG	NEG
GC039	NEG	NEG	NEG	NEG	NEG
GC040	NEG	NEG	NEG	NEG	NEG
GC041	EQ	EQ = 1:64	NEG	NEG	NEG
GC042	POS	POS	NEG	NEG	NEG
GC043	NEG	NEG	POS	POS	EQ = 1:64
GC044	NEG	NEG	NEG	NEG	NEG
GC045	NEG	NEG	NEG	NEG	NEG

Serum ID	*Rickettsia felis*		*Rickettsia typhi*		
	OmpB	MIF	OmpB	LPS	MIF
GC046	NEG	NEG	NEG	NEG	NEG
GC047	POS	POS	NEG	POS	POS
GC048	POS	POS	NEG	NEG	NEG
GC049	NEG	NEG	NEG	NEG	NEG
GC050	NEG	NEG	NEG	NEG	NEG
GC051	NEG	NEG	NEG	NEG	NEG
GC052	NEG	NEG	NEG	NEG	NEG
GC053	POS	POS	NEG	NEG	NEG
GC054	NEG	NEG	NEG	NEG	NEG
GC055	NEG	NEG	NEG	NEG	NEG
GC056	NEG	NEG	POS	POS	POS
GC057	NEG	NEG	NEG	NEG	NEG
GC058	NEG	NEG	NEG	NEG	NEG
GC059	NEG	NEG	NEG	NEG	NEG
GC060	NEG	NEG	NEG	NEG	NEG
GC061	NEG	NEG	NEG	NEG	NEG
GC062	NEG	NEG	NEG	NEG	NEG
GC063	POS	POS	NEG	NEG	NEG
GC064	POS	EQ = 1:64	NEG	NEG	NEG
GC065	NEG	NEG	NEG	NEG	NEG
GC066	NEG	NEG	NEG	NEG	NEG
GC067	NEG	NEG	NEG	NEG	NEG
GC068	NEG	NEG	NEG	NEG	NEG
GC069	NEG	NEG	NEG	NEG	NEG
GC070	NEG	NEG	NEG	NEG	NEG
GC071	NEG	NEG	NEG	NEG	NEG
GC072	NEG	NEG	NEG	NEG	NEG
GC073	EQ	POS	NEG	NEG	NEG
GC074	NEG	NEG	EQ	EQ	EQ = 1:32
GC075	NEG	NEG	NEG	NEG	NEG
GC076	NEG	NEG	NEG	NEG	NEG
GC077	NEG	NEG	NEG	NEG	NEG
GC078	NEG	NEG	NEG	NEG	NEG
GC079	NEG	NEG	NEG	NEG	NEG
GC080	NEG	NEG	NEG	NEG	NEG
GC081	NEG	EQ = 1:64	POS	POS	NEG
GC082	EQ	POS	NEG	POS	POS
GC083	POS	POS	NEG	EQ	POS
GC084	NEG	NEG	NEG	NEG	NEG
GC085	NEG	NEG	POS	POS	EQ = 1:32
GC086	EQ	NEG	NEG	NEG	NEG
GC087	NEG	NEG	NEG	NEG	NEG
GC088	POS	POS	NEG	NEG	NEG
GC089	NEG	NEG	NEG	NEG	NEG
GC090	POS	EQ = 1:64	NEG	NEG	NEG

Individual Serum Data

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Serum ID	*Rickettsia felis*		*Rickettsia typhi*		
	OmpB	MIF	OmpB	LPS	MIF
GC091	NEG	NEG	NEG	NEG	NEG
GC092	NEG	NEG	NEG	NEG	NEG
GC093	NEG	NEG	NEG	NEG	NEG
GC094	NEG	NEG	NEG	NEG	NEG
GC095	NEG	NEG	NEG	NEG	NEG
GC096	NEG	NEG	NEG	NEG	NEG
GC097	NEG	NEG	NEG	NEG	NEG
GC098	NEG	NEG	NEG	NEG	NEG
GC099	POS	POS	EQ	EQ	NEG
GC100	NEG	NEG	NEG	NEG	NEG
GC101	NEG	NEG	NEG	NEG	NEG
GC102	NEG	NEG	EQ	POS	NEG
GC103	NEG	NEG	POS	NEG	NEG
GC104	NEG	NEG	NEG	NEG	NEG
GC105	NEG	NEG	NEG	NEG	NEG
GC106	NEG	NEG	NEG	NEG	NEG
GC107	NEG	NEG	NEG	NEG	NEG
GC108	NEG	NEG	NEG	NEG	NEG
GC109	NEG	NEG	NEG	NEG	NEG
GC110	NEG	NEG	EQ	EQ	NEG
GC111	NEG	NEG	NEG	NEG	NEG
GC112	NEG	EQ = 1:64	NEG	NEG	NEG
GC113	NEG	NEG	EQ	NEG	NEG
GC114	NEG	NEG	NEG	EQ	EQ = 1:32
GC115	NEG	NEG	NEG	NEG	NEG
GC116	NEG	NEG	NEG	NEG	NEG
GC117	EQ	EQ = 1:64	NEG	NEG	NEG
GC118	EQ	NEG	NEG	NEG	NEG
GC119	EQ	NEG	NEG	NEG	NEG
GC120	NEG	NEG	NEG	NEG	NEG
GC121	POS	POS	NEG	NEG	NEG
GC122	NEG	NEG	EQ	EQ	NEG
GC123	NEG	NEG	NEG	NEG	NEG
GC124	NEG	NEG	NEG	EQ	EQ = 1:32
GC125	NEG	NEG	NEG	NEG	NEG
GC126	NEG	NEG	NEG	POS	NEG
GC127	NEG	NEG	NEG	POS	NEG
GC128	NEG	NEG	NEG	NEG	NEG
GC129	NEG	NEG	NEG	NEG	NEG
GC130	NEG	NEG	NEG	NEG	NEG
GC131	NEG	NEG	NEG	NEG	NEG
GC132	NEG	NEG	NEG	NEG	NEG
GC133	NEG	NEG	NEG	EQ	EQ = 1:32
GC134	NEG	NEG	NEG	NEG	NEG
GC135	NEG	NEG	NEG	NEG	NEG

Serum ID	*Rickettsia felis*		*Rickettsia typhi*		
	OmpB	MIF	OmpB	LPS	MIF
GC136	NEG	NEG	NEG	NEG	NEG
GC137	NEG	NEG	POS	POS	POS
GC138	NEG	NEG	NEG	NEG	NEG
GC139	NEG	NEG	NEG	NEG	NEG
GC140	NEG	NEG	NEG	NEG	NEG
GC141	NEG	EQ = 1:64	POS	POS	POS
GC142	NEG	NEG	NEG	NEG	NEG
GC143	NEG	NEG	NEG	NEG	NEG
GC144	NEG	NEG	NEG	EQ	NEG
GC145	NEG	NEG	NEG	NEG	NEG
GC146	EQ	EQ = 1:64	NEG	NEG	EQ = 1:32
GC147	POS	POS	NEG	NEG	NEG
GC148	NEG	NEG	NEG	NEG	NEG
GC149	NEG	NEG	NEG	NEG	NEG
GC150	NEG	NEG	NEG	NEG	NEG
GC151	NEG	NEG	NEG	NEG	NEG
GC152	NEG	NEG	NEG	NEG	NEG
GC153	NEG	NEG	NEG	NEG	NEG
GC154	EQ	NEG	NEG	NEG	NEG
GC155	POS	NEG	NEG	NEG	NEG
GC156	POS	NEG	NEG	NEG	NEG
GC157	NEG	NEG	NEG	EQ	NEG
GC158	EQ	NEG	NEG	NEG	NEG
GC159	NEG	NEG	NEG	NEG	NEG
GC160	NEG	NEG	NEG	NEG	NEG
GC161	NEG	NEG	NEG	NEG	NEG
GC162	NEG	NEG	NEG	NEG	NEG
GC163	NEG	EQ = 1:64	NEG	NEG	NEG
GC164	NEG	NEG	NEG	NEG	NEG
GC165	NEG	NEG	NEG	EQ	EQ = 1:32
GC166	NEG	NEG	NEG	NEG	NEG
GC167	NEG	NEG	POS	POS	POS
GC168	NEG	NEG	NEG	NEG	NEG
GC169	NEG	NEG	NEG	NEG	NEG
GC170	NEG	NEG	NEG	NEG	NEG
GC171	NEG	NEG	NEG	NEG	NEG
GC172	NEG	NEG	NEG	NEG	NEG
GC173	POS	NEG	NEG	NEG	NEG
GC174	NEG	NEG	NEG	NEG	NEG
GC175	NEG	NEG	NEG	NEG	NEG
GC176	NEG	NEG	NEG	NEG	NEG
GC177	NEG	NEG	NEG	NEG	NEG
GC178	NEG	NEG	NEG	NEG	NEG
GC179	NEG	NEG	NEG	NEG	NEG
GC180	NEG	NEG	NEG	NEG	NEG

TABLE 1A

	Texas Gulf Coast Normals			
Result	RF(n)	RF(%)	RT(n)	RT(%)
POS	16	9%	16	9%
EQ	12	7%	13	7%
NEG	152	84%	151	84%
Total	180	100%	180	100%

TABLE 1B

	R. felis ELISA			
	Result	POS	EQ	NEG	Total
MIF	POS	11	2	0	13
	EQ	3	4	3	10
	NEG	3	6	148	157
	Total	17	12	151	180

TABLE 1C

	R. typhi rOmpB ELISA			
	Result	POS	EQ	NEG	Total
MIF	POS	4	0	5	9
	EQ	2	1	5	8
	NEG	2	4	157	163
	Total	8	5	167	180

TABLE 1D

	R. typhi LPS ELISA			
	Result	POS	EQ	NEG	Total
MIF	POS	7	2	0	9
	EQ	2	6	1	9
	NEG	4	6	152	162
	Total	13	14	153	180

Table 1. Seroprevalence of *R. felis* and *R. typhi* in a population of normal healthy blood donors in an endemic region. The Equivocal range was initially defined as MIF titers between 1:32-1:64 for each ELISA assay, with the ELISA Cutoff Calibrator defined by these minimal positive sera. For total RT prevalence values positive reactivity with either rOmpB or LPS antigens was counted as a positive serum. The correlation of these assays demonstrates the higher sensitivity with the LPS antigen and the LPS-centric sensitivity of the MIF assay. For RF assays the lack of competing antigens allows for better correlation.

TABLE 2

	Rickettsia felis			
	Result	POS	EQ	NEG	Total
R. typhi	POS	3	2	11	16
	EQ	1	1	11	13
	NEG	13	10	128	151
	Total	17	13	150	180

Table 2. Comparison of *R. felis* and *R. typhi* results in normal healthy blood donors. Results were classified as Positive if at least 1 assay result was positive. The Equivocal designation is for sera with at least 1 equivocal titer and no positive results. Only 3 sera were positive for both agents (1.7%), with another 4 sera (2.2%) showing equivocal reactivity to both. The results with the 2 rOmpB ELISA assays show no dual reactivity (0%), further suggesting that dual-reactivity seen in endemic typhus is more probably a true dual reactivity based on multiple flea bites.

Conclusions

- **For serodiagnosis of *R. felis* infection the MIF and ELISA assays correlate closely, even with the low reactivity of healthy blood donor sera.**
- **For serodiagnosis of *R. typhi* infection both rOmpB and lipopolysaccharide (LPS) antigens are important, with 50% of positive sera reacting with only a single antigen.**
- **Our data suggests that the *R. typhi* MIF antigen we used is much more sensitive to anti-LPS reactivity.**
- **Seropositivity rates are similar for *R. felis* and *R. typhi.***
- **Our data suggests that these assays could all be screened at lower dilutions or lower cutoffs without losing specificity.**