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Research article

### Lyme Disease in Clinically Sick Dogs in Southern US: Assessing Geographical and Seasonal distribution during year 2012 – 2011 in Texas

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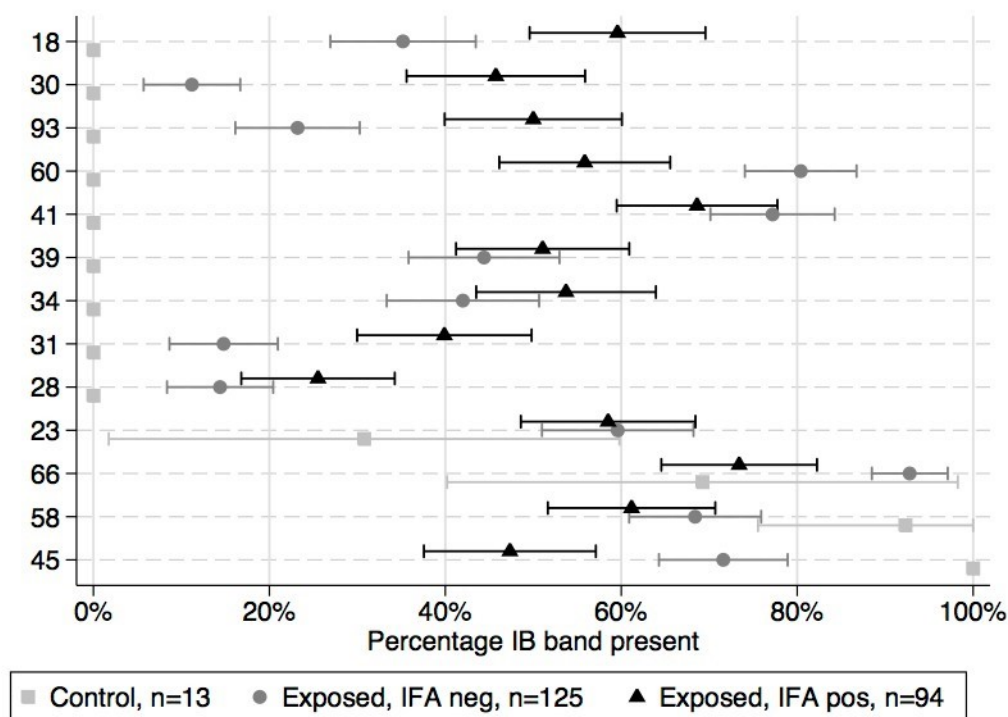
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## Supplemental Information



**Figure S1.** Percentages of samples with IB antigen band present, with exact binomial 95% confidence intervals, for control (n=13), non-control and IFA negative (n=125) and non-control and IFA positive (n=94) groups (see Table S1).

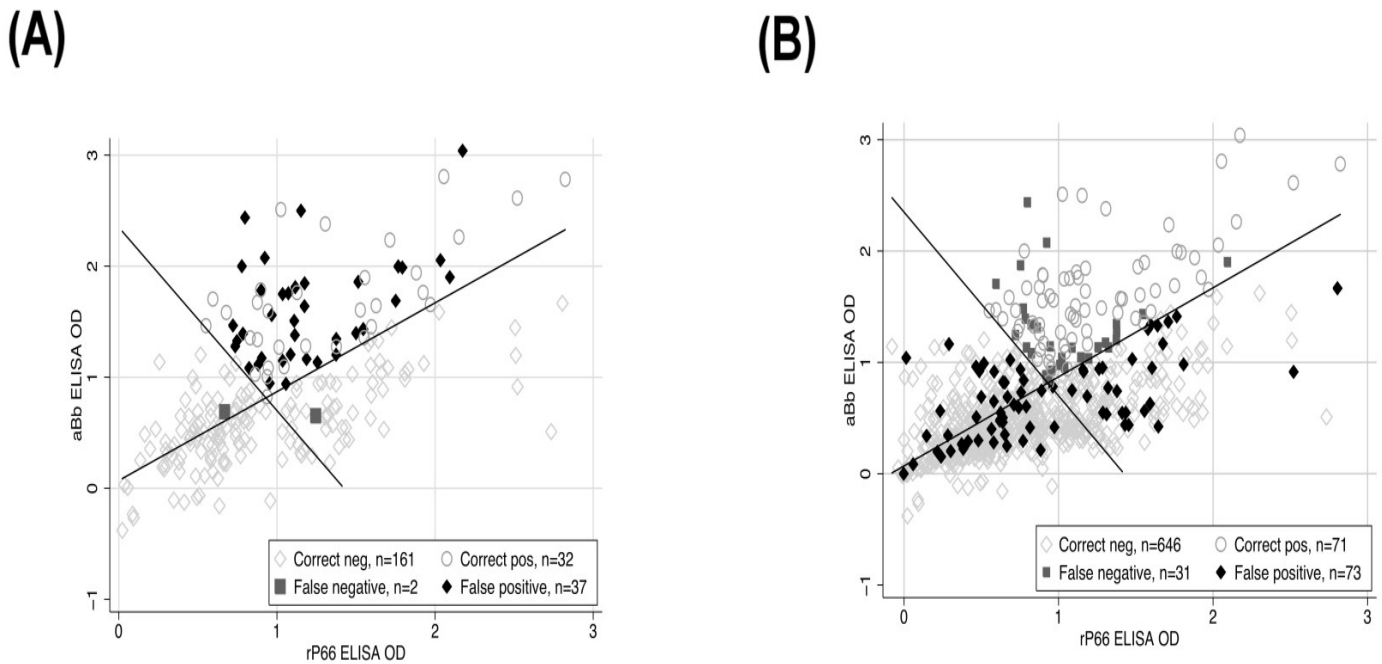
### Additional Information

Since IB provides more information regarding the antigens that each dog sample can react to, and based on the gold standard in human diagnosis, we used IB as gold standard in our studies. To do so, we evaluated the bands that strongly correlated with high ELISA values. For each subset of IB bands: 18, 23, 30 and 93kDa, and a variety of slopes on the two ELISA cut off-lines, sensitivity and specificity of ELISA diagnosis compared to an IB diagnosis was calculated. The highest average of sensitivity and specificity was obtained with the IB bands 18, 30 and 93 (all present) and ELISA cut-lines  $[(aBb OD_{450nm} + 1.65 * rP66 OD_{450nm}) > 2.35]$  and  $[(aBb OD_{450nm} - 0.8 * rP66 OD_{450nm}) > 0.07]$ : sensitivity 94.1% and specificity 81.3%, in which there were only two false negatives (Figure S2A). We note that both false negative samples had all six IB bands present (18, 23, 30, 34, 41 and 93). The false positive sample with an aBb ELISA OD greater than 3, had five IB bands present (18, 23, 30, 34, 41 but not 93).

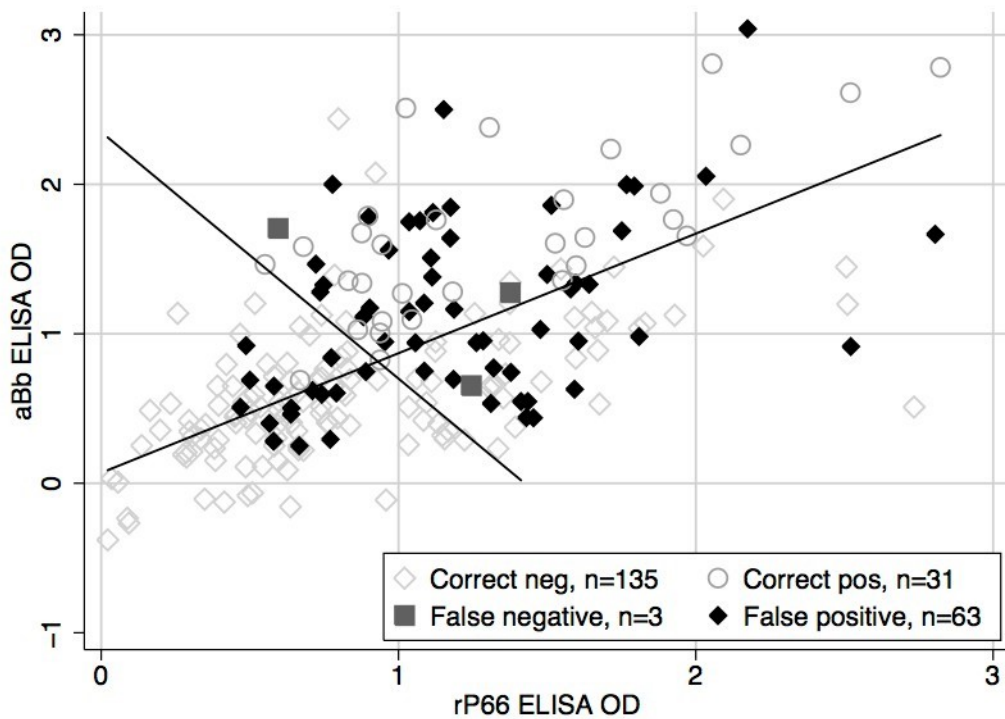
### Comparison of IB and ELISA diagnosis to IFA diagnosis

On the same 232 samples, the IFA test performance compared to IB (used as the gold standard) was not as good as the ELISA: 135 true negatives, 31 true positives, 3 false negatives and 63 false positives, resulting in sensitivity 91.2% and specificity 68.2% (see supplemental material, Figure S2). All three false negatives had the following IB Bands present: 18, 23, 30, 34, 41 and 93kDa.

For the 821 ELISA tested samples, the comparison of the IFA diagnosis to the ELISA test (being the ELISA the gold standard for this testing) resulted in 646 correct negatives, 71 correct positives, 31 false negatives and 73 false positive, giving a sensitivity of 69.6% and a specificity of 89.8% for the IFA assay (Figure S2B). Therefore, the ELISA test had an overall better performance than the IFA test.



**Figure S2.** Sensitivity and specificity of assays used in this study. (A) Sensitivity and specificity of ELISA LD test compared to IB LD as a gold standard (n=232). IB positive was defined as the presence of three IB bands 18, 30 and 93kDa. ELISA positive was defined as  $aBb > 2.35 - 1.65rP66$  and  $aBb > 0.07 + 0.8rP66$ . (B) Sensitivity and specificity of IFA LD diagnoses compared to ELISA LD as gold standard (n=821). ELISA positive was  $aBb > 2.35 - 1.65rP66$  and  $aBb > 0.07 + 0.8rP66$ .



**Figure S3.** Sensitivity and specificity of IFA LD diagnoses compared to IB LD diagnosis on sample size n=232. IB positive was the presence of the three IB bands 18, 30 and 93kDa.

IB antigen (kDa)	Control N=13		Non-control, IFA negative N=125		Non-control, IFA positive N=94		Total N=232		P-value
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	
18	0	0%	41	32.8%	55	58.5%	96	41.4%	<0.001
23	4	30.8%	73	58.4%	53	56.4%	130	56.0%	0.768
28	0	0%	16	12.8%	22	23.4%	38	16.4%	0.094
30	0	0%	13	10.4%	42	44.7%	55	23.7%	<0.001
31	0	0%	17	13.6%	36	38.3%	53	22.8%	<0.001
34	0	0%	51	40.8%	50	53.2%	101	43.5%	0.156
39	0	0%	52	41.6%	44	46.8%	96	41.4%	0.443
41	0	0%	92	73.6%	61	64.9%	153	65.9%	0.321
45	13	100%	81	64.8%	40	42.6%	134	57.8%	<0.001
58	12	92.3%	76	60.8%	53	56.4%	141	60.8%	0.179
60	0	0%	93	74.4%	48	51.1%	141	60.8%	<0.001
66	9	69.2%	114	91.2%	67	71.3%	190	81.9%	<0.001
93	0	0%	24	19.2%	45	47.9%	69	29.7%	<0.001

**Table S1:** Descriptive statistics (number and percentage) of IB antigen bands present in 232 canine serum samples, with p-values from Fischer's exact test comparing control, non-control, IFA negative and non-control, IFA positive groups.

County	N	EL	% pos	County	N	EL	% pos	County	N	EL	% pos
Anderson	1	0	0%	Gonzales	2	0	0%	Nacogdoches	13	0	0%
Angelina	3	0	0%	Grayson	1	0	0%	Navarro	3	0	0%
Aransas	2	2	100%	Gregg	4	0	0%	Nolan	0	0	0%
Archer	23	1	4.3%	Grimes	5	0	0%	Nueces	16	1	6.3%
Atascosa	11	0	0%	Guadalupe	1	0	0%	Panola	1	0	0%
Austin	27	4	14.8%	Hardin	2	0	0%	Pecos	1	0	0%
Bastrop	28	1	3.6%	Harris	20	1	5.0%	Polk	1	0	0%
Bee	7	2	28.6%	Harrison	2	0	0%	Robertson	1	0	0%
Bell	21	4	19.0%	Hays	3	0	0%	Rockwall	2	0	0%
Bexar	6	0	0%	Henderson	10	2	20.0%	Rusk	11	0	0%
Bowie	9	1	11.1%	Hidalgo	3	0	0%	Sabine	1	0	0%
Brazoria	3	0	0%	Hill	1	0	0%	San Jacinto	1	0	0%
Brazos	82	6	7.3%	Houston	4	1	100%	San Patricio	2	0	0%
Brewster	6	1	16.7%	Hunt	7	0	25.0%	Smith	28	5	17.9%
Burleson	3	0	0%	Jack	1	0	0%	Somervell	3	1	33.3%
Burnet	3	0	0%	Jackson	3	0	0%	Starr	1	0	0%
Caldwell	1	0	0%	Jasper	1	0	0%	Tarrant	13	2	15.4%
Calhoun	1	0	0%	Jefferson	9	0	0%	Taylor	3	1	33.3%
Callahan	1	0	0%	Jim Wells	3	1	0%	Travis	18	7	38.9%
Cameron	1	0	0%	Karnes	7	3	33.3%	Trinity	1	0	0%
Camp	1	0	0%	Kaufman	3	1	42.9%	Tyler	1	0	0%
Cass	5	1	20.0%	Kerr	2	0	33.3%	Upshur	3	0	0%
Collin	2	0	0%	Kleberg	1	0	0%	Van Zandt	6	0	0%
Colorado	1	0	0%	Lamar	2	0	0%	Victoria	3	0	0%
Comal	3	1	33.3%	Lampasas	1	0	0%	Walker	15	5	33.3%
Comanche	1	0	0%	Lee	1	0	0%	Waller	5	1	20.0%
Coryell	19	6	31.6%	Leon	2	0	0%	Washington	17	1	5.9%
Dallas	23	3	13.0%	Limestone	4	0	0%	Webb	23	2	8.7%
Denton	1	0	0%	Live Oak	13	0	0%	Wharton	1	0	0%
Eastland	2	1	50.0%	Llano	4	0	0%	Wichita	6	1	16.7%
Ellis	8	0	0%	Lubbock	5	0	0%	Williamson	5	1	20.0%
Erath	3	0	0%	Madison	3	0	0%	Wilson	1	0	0%
Fayette	0	0	0%	Mason	1	0	0%				
Fort Bend	11	2	18.2%	Matagorda	1	1	100%				
Franklin	1	0	0%	McLennan	23	1	4.34%				
Freestone	5	0	0%	Medina	3	2	66.7%				
Frio	4	0	0%	Menard	1	0	0%				
Gillespie	1	1	100%	Milam	1	0	0%				
Goliad	4	0	0%	Montgomery	6	0	0%				

**Table S2.** The number of non-control canine serum sample, by Texas county, in this study (n=831). (N=total number of samples, EL=ELISA tested, %pos=tested positive in ELISA testing).