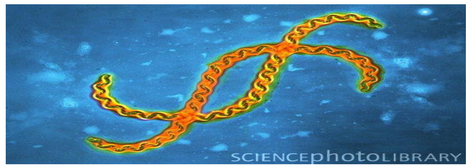

Diagnostic Accuracy of MAT and ELISA Assays in the Detection of *Leptospira* in Two, Low-prevalence, Populations

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Summary

- Leptospirosis is a bacterial zoonotic pathogen that exists at endemic levels throughout the world. Little is known about the accuracy of the traditional diagnostic assay, the microagglutination test (MAT), and more recent assays, the IgG ELISAs, in detecting prior infection for epidemiologic studies.
- This study evaluated three serologic tests in two low-risk populations. Population A (tested with MAT only) consisted of 393 women in urban Chile, and Population B (tested with MAT and two IgG ELISAs) consisted of 611 children from Wisconsin.
- Bayesian techniques estimated that the MAT assay had a specificity of 94-96% , and the specificity of the SERION ELISA was 74% in Population B. Sensitivity estimates were not as reliable due to few positive samples. Estimates of prevalence were 2.1% (Population A) and 1% (Population B).
- In order to determine the endemic levels of Leptospirosis globally, detection of non-clinical cases is important, and careful evaluation of diagnostic assays will be critical in ensuring valid results.
- **Objective:** ELISA-type assays are becoming more popular diagnostic tools for their ease of implementation. However, their field level evaluation has been limited. The objective of this study was to evaluate the performance of three serologic tests for leptospirosis as applied to detection of prior infection in traditionally low-risk populations.

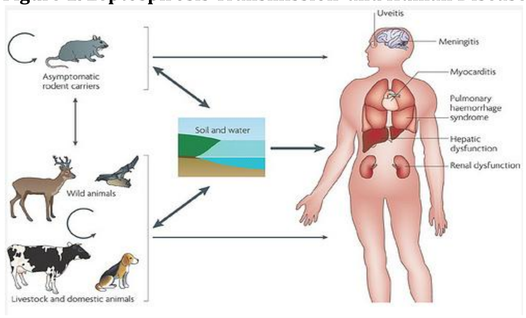
Background

Leptospirosis is a worldwide public health problem caused by pathogenic bacteria of the genus *Leptospira*. This zoonotic pathogen is transmitted directly or indirectly from animals (wild and domestic) to humans.

Risk Factors for Infection:

- Humid tropical and Subtropical Climates
- Heavy rains and flooding
- Close contact with animals for agriculture
- Poor housing
- Inadequate waste disposal
- Changes in density of animal reservoirs

Figure 1. Leptospirosis Transmission and Human Disease [1]



Methods

Study Populations

- A: 494 urban women of the Los Rios Region, Chile
- B: 611 children of Marshfield, Wisconsin

Serologic Tests

- Microagglutination test (MAT), commonly used reference assay
- SERION ELISA classic Leptospira IgG
- IVD Leptospira IgG Microwell ELISA

Statistical Analyses

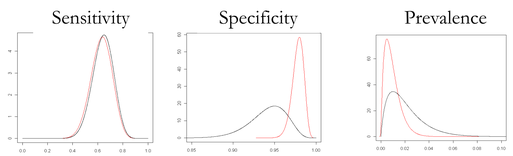
Bayesian statistical methods for evaluation of diagnostic accuracy in the absence of a gold standard test were used to estimate the diagnostic sensitivity (SE) and specificity (SP) of each test, as well as the resulting prevalence for each population. Priors were elicited from expert opinion and modeled using Beta distributions (Table 1).

Bayesian Algorithms Used for Estimation:

i) One test (MAT) and one population (population A)
ii) Two tests (MAT, SERION ELISA) and one population (population B)

Data from IVD ELISA in population B were not included because of poor assay performance. All models were run in WinBUGS[2] by adapting previously published codes[3] using 500,000 iterations after 50,000 burn-in period. Convergence was assessed with visual inspection of trace plots and using the Gelman and Rubin convergence statistic.[4]

MAT in Population A (urban women from Chile)



SERION ELISA and MAT in Population B

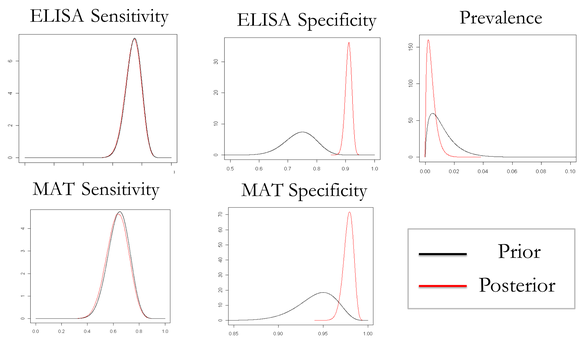


Figure 2. Plots of priors and posterior for MAT and ELISA tests

Results

Descriptive Comparisons

Population A: 27 of 393 (6.9%) samples tested positive by MAT.
Population B: 26 of 611 (4.3%) samples tested positive by MAT and 39 of 611 (6.4%) by SERION ELISA.

Proportion agreement between the MAT and SERION ELISA in population B was 90.7% (95% C.I.: 88.0%-92.8%), however the source of most of the agreement was from the negative results as 550 samples were negative by both tests. Only 4 samples were positive by both tests (Table 1).

Table 1: Comparison of agreement between microagglutination test (MAT) and SERION IgG ELISA for detection of *Leptospira* infection in children from Wisconsin, USA

SERION ELISA	MAT		Total
	Negative	Positive	
Negative	550	22	572
Positive	35	4	39
Total	585	26	611

Estimation of sensitivity, specificity, and prevalence

Sensitivity: SE for the MAT assay was similar for both populations; 64.1% (95% P.I.: 47.3%, 79.3%) and 63.9% (95% P.I.: 47.0%, 79.1%) for Populations A and B, respectively. SERION ELISA SE was higher than MAT SE in population B; 74.2% (95% P.I.: 63.1%-84.0%).

Specificity: SP for the MAT assay was similar for both populations; 94.3% (95% P.I.: 91.7%, 96.8%) and 96.0% (95% P.I.: 94.3%, 97.5%) for Populations A and B respectively. SERION ELISA SP was lower than MAT SP in population B; 77.3% (95% P.I.: 74.0%-80.5%).

Prevalence: The prevalence for urban women from Chile, population A, was 2.1% (95% P.I.: 0.3%, 5.4%) and from children from Wisconsin in the U.S., population B, was 1.0% (95% P.I: 0.1%, 2.7%).

Table 2: Prior and Posterior Estimates of SE, SP, and Prevalence in Populations A and B

	Parameter		Mean	2.5% tile	97.5% tile
Population A	MAT SE	Prior	64.3%	47.2%	79.3%
		Posterior	64.1%	47.3%	79.3%
	MAT SP	Prior	94.1%	89.0%	97.8%
		Posterior	94.3%	91.7%	96.8%
	Prevalence	Prior	2.1%	20.0%	5.9%
		Posterior	2.1%	30.0%	5.4%
Population B	MAT SE	Prior	64.3%	47.2%	79.3%
		Posterior	63.9%	47.0%	79.1%
	MAT SP	Prior	94.1%	89.0%	97.8%
		Posterior	96.0%	94.3%	97.5%
	SERION ELISA SE	Prior	74.2%	63.1%	84.0%
		Posterior	74.2%	63.0%	84.0%
	SERION ELISA SP	Prior	74.2%	63.1%	84.0%
		Posterior	77.3%	74.0%	80.5%
	Prevalence	Prior	1.2%	0.1%	3.6%
		Posterior	1.0%	0.1%	2.7%

Conclusions

- Percent agreement between the MAT and SERION ELISA assays in the Wisconsin population was high (91%), but driven mainly by the proportion of samples that were negative by both tests.
- SP of the MAT assay in both populations was 94-96% which is consistent with other studies.[5,6]
- SP of SERION ELISA was lower (74%) than SP of MAT; 35 children tested positive by SERION ELISA, but negative MAT.
- The Bayesian algorithm was less robust when estimating SE because of the low number of positive samples and low agreement between positive test results.
- The prevalence estimate was 1% for children in Wisconsin and 2% for urban women in southern Chile. Considering that not all infected individuals are detectable by ELISA or MAT assay at time of sampling, the true prevalence is expected to be higher.
- In the effort to estimate the global burden of Leptospirosis, sero-prevalence studies play an important role in quantifying infection. Properly evaluated assays will be critical in obtaining the best estimate of the burden of *Leptospira* infection in the varied populations and community settings worldwide.

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