COMPARISON OF TWO SEROLOGICAL TESTS FOR DETECTION OF ANTIBODIES TO *TOXOPLASMA GONDII* IN FERAL SWINE

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ABSTRACT

Disease risks associated with an estimated 5 million feral swine (*Sus scrofa*) occupying 38 states need to be investigated and validated to thoroughly address human health issues. However, approved tests for the serological detection of *Toxoplasma gondii* in feral swine are currently not available, and testing choices for the detection of *T. gondii* in feral swine are limited. *Toxoplasma gondii*, an obligate intracellular protozoan that utilizes felids as a definitive host, may be harbored as tissue cysts in many mammals, including feral swine. Transmission to humans can occur through ingestion of undercooked meat, with congenital infections being of particular concern. Therefore, the Modified Agglutination Test and Enzyme-Linked Immunosorbent Assay are compared for agreement on 250 serum samples collected from feral swine across the U.S. Although the diagnostic tests use different methodologies, the two tests are expected to agree when serum is screened as recommended by the manufacturers. While both tests have good specificity and sensitivity, labor intensiveness and testing time are important considerations when screening feral swine. Study findings may potentially guide testing choices for *T. gondii* in feral swine and help evaluate risks to human health.

INTRODUCTION

- *Toxoplasma gondii* is an obligate intracellular protozoan
- Felids are the only definitive host
- Mammals, including feral swine, can harbor infectious tissue cysts (Fig. 1)
- Estimated 5 million feral swine in the United States
- Concerns:
  - Devastating congenital human infections
  - Maintenance of infectious disease in feral swine population
- Evaluation of human health risk must be investigated
- No approved diagnostic test available for detection of *T. gondii* in feral swine

Fig. 1 Young feral swine

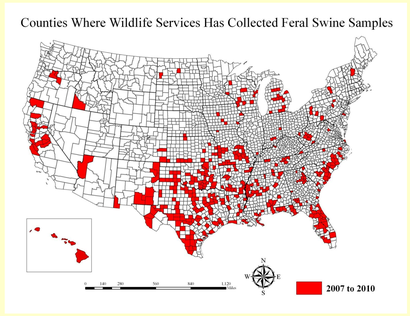

Fig. 2 Locations of sample collection

Fig. 3 A biologist collects blood samples

MATERIALS AND METHODS

256 serum samples collected in the U.S. by Wildlife Services for disease surveillance (Figs. 2 & 3). Frozen and thawed samples were tested with both Modified Agglutination Test (MAT) and Enzyme-Linked Immunosorbent Assay (ELISA)

MAT: Toxo-Screen DA, Biomerieux

- Agglutination of formalin-treated tachyzoites in the presence of anti-*T. gondii* IgG serum antibodies
- Non-specific IgM agglutination suppressed
- Serum samples tested at 1:40 dilution

- Positive reaction
  - IgG antibodies to *T. gondii* are present
  - Agglutination in a mat covering half or more of the well +/- ruffled edges
- Negative reaction
  - No *T. gondii*-specific IgG antibodies present
  - Sedimentation of tachyzoites in button or ring

ELISA: SafePath Antibody Test Kit

- *T. gondii* antigen coated test wells bind to anti-*T. gondii* IgG serum antibodies
- Color change to yellow if complex is present
- Quantified by Optical Density, 450nm
- Serum samples tested at 1:50 dilution

- Positive reaction
  - Color change to yellow
  - Optical Density ≥ 0.3
- Negative reaction
  - Contents of well are clear
  - Optical Density < 0.3

Statistical analysis

- Bayesian statistics used to estimate specificity and sensitivity in absence of "gold standard" test
- Specificity and sensitivity provided by test kit manufacturers used to assume prior distribution
- Uninformative prior distribution of disease prevalence assumed since samples were not randomly selected and number of positives unknown
- Prior distributions were characterized by specifying the modes and lower bounds of the 95% confidence intervals (Table 1)

Table 1 Prior Distributions

		Mode	Lower Bound 95% CI
ELISA	Sensitivity	99%	90%
	Specificity	87%	75%
MAT	Sensitivity	96%	60%
	Specificity	98%	60%

RESULTS

- 185 of 256 samples included in data analysis
  - Positive control of 1 ELISA plate did not meet manufacturer's standards and these samples were excluded from statistical analysis
- 69 of 185 samples positive by the MAT test
- 34 of 185 samples positive by the ELISA test

Agreement between tests:

- Concordance agreement: Kappa index=0.37 (0.07 SE)
- More agreement among negatives than positives (Table 2)

- Bayesian estimates of specificity and sensitivity shown in Table 3

- Computations were performed using R statistical software (R Development Core Team 2008) and the lme4 add-on package MCMCpack.

Table 2 Number of positive and negative samples

		ELISA	
		-	+
MAT	-	109	7
	+	42	27

Table 3 Bayesian estimates of specificity and sensitivity

		Median	Credible Interval*
ELISA	Sensitivity	70%	60-78%
	Specificity	92%	87-96%
MAT	Sensitivity	98%	94-99%
	Specificity	90%	84-95%

*Bayesian version of 95% confidence interval

DISCUSSION

- Kappa value indicates only fair agreement between the two tests
  - Both test have been validated in domestic swine
- Other testing considerations:
  - MAT more time-consuming to perform, faster results with ELISA
  - ELISA kit's reagents require less preparation
  - Interpreting MAT results can be somewhat subjective if agglutination is borderline
  - Serum samples must be diluted for both tests (equal time to set up)
- Cat bioassay could confirm actual status of samples but is not feasible
- Determining antibody titers of samples would allow additional comparison of the tests
- Specificity and sensitivity of both tests should be further characterized for testing of feral swine serum

ACKNOWLEDGEMENTS

Funding for this project was provided by:

- Merial Scholars Program
- Dept. of Microbiology, Immunology & Pathology, CSU
- USDA/APHIS/WS National Wildlife Disease Program
- Thank you to Dr. Jens Eickhoff for providing statistical analysis
- Feral swine photos provided by John A. Baroch