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Evaluation of Blood Assays for Detection of *Mycobacterium bovis* in White-Tailed Deer (*Odocoileus virginianus*) in Michigan

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EVALUATION OF BLOOD ASSAYS FOR DETECTION OF *MYCOBACTERIUM BOVIS* IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) IN MICHIGAN

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ABSTRACT: Surveillance and control activities related to bovine tuberculosis (TB) in free-ranging, Michigan white-tailed deer (*Odocoileus virginianus*) have been underway for over a decade, with significant progress. However, foci of higher TB prevalence on private lands and limited agency ability to eliminate them using broad control strategies have led to development and trial of new control strategies, such as live trapping, testing, and culling or release. Such strategies require a prompt, accurate live animal test, which has thus far been lacking. We report here the ability of seven candidate blood assays to determine the TB infection status of Michigan deer. Our aims were twofold: to characterize the accuracy of the tests using field-collected samples and to evaluate the feasibility of the tests for use in a test-and-cull strategy. Samples were collected from 760 deer obtained via five different surveys conducted between 2004 and 2007. Blood samples were subjected to one or more of the candidate blood assays and evaluated against the results of mycobacterial culture of the cranial lymph nodes. Sensitivities of the tests ranged from 46% to 68%, whereas specificities and negative predictive values were all >92%. Positive predictive values were highly variable. An exploratory analysis of associations among several host and sampling-related factors and the agreement between blood assay and culture results suggested these assays were minimally affected. This study demonstrated the capabilities and limitations of several available blood tests for *Mycobacterium bovis* on specimens obtained through a variety of field surveillance methods. Although these blood assays cannot replace mass culling, information on their performance may prove useful as wildlife disease managers develop innovative methods of detecting infected animals where mass culling is publicly unacceptable and cannot be used as a control strategy.

Key words: Bovine tuberculosis, diagnostic tests, *Mycobacterium bovis*, *Odocoileus virginianus*, white-tailed deer.

INTRODUCTION

Surveillance and control activities for bovine tuberculosis (TB; caused by *Mycobacterium bovis*) in free-ranging, Michigan white-tailed deer (*Odocoileus virginianus*; Schmitt et al., 1997) have now been underway for more than a decade (O'Brien et al., 2006). Significant progress has been made lowering the prevalence of TB in deer in the most affected area by approximately 70%. The primary factors likely responsible for this progress have been twofold: reduction of deer densities through hunting and restrictions on sup-

plemental feeding and baiting of deer by the public. These broad strategies, of the Michigan Department of Natural Resources (MDNR), implemented with the cooperation of Michigan deer hunters, have resulted in a halving of the deer population in the five-county TB area (Rudolph, 2006) and a substantial reduction in supplemental feeding (Matthews and Carlson, 2005). However, as hunters see fewer deer, their willingness to sustain aggressive harvests has waned (Van Deelen and Etter, 2003), and in general, public resentment of control measures for TB in wildlife has grown (O'Brien et al., 2006).

Although prevalence has been greatly reduced over the TB area as a whole, clusters of higher prevalence have long been known to exist on privately owned land and those rates persist. Epidemiologically, geographic area (O'Brien et al., 2002) and land ownership (Hickling, 2002) are the strongest predictors of TB occurrence. The current broad MDNR control strategies have limited ability to eliminate such clusters because private landowners can limit hunter access to their lands, effectively controlling harvest pressure and the size of the local deer population. Although large-scale agency culling of deer is theoretically an option, very little public or political support exists (Dorn and Mertig, 2005). Consequently, such culling is not a viable management option. Collectively, these factors can effectively preclude eradication of TB from the deer and from Michigan unless new, more publicly acceptable control strategies can be devised (O'Brien et al., 2006).

Beginning in January 2004, a new, cooperative, TB control strategy was instituted by MDNR with private landowners in the core area and with the US Department of Agriculture's Animal and Plant Health Inspection Service-Wildlife Services (APHIS-WS) branch (Schmitt et al., 2004). The program involved live trapping of deer in high-prevalence areas, testing them with a commercially available gamma-interferon blood test and culling of test-positive deer. Although logistically workable and well-supported by landowners and policymakers, performance of the gamma-interferon assay was a critical limitation (Schmitt et al., 2004). Others have also shown that assay to have limited usefulness with samples from white-tailed deer (Waters et al., 2007). Problems are not uncommon when using serologic tests to diagnose *M. bovis* in wildlife reservoirs (Cousins and Florisson, 2005). A TB test method with improved accuracy was clearly necessary for the test-and-cull program to be viable and cost effective as a disease-control strategy.

We report here on the ability of seven candidate blood assays (Waters et al., 2004) to determine the TB infection status of Michigan white-tailed deer. Our aims were twofold: to characterize the accuracy of the tests using field-collected samples and to evaluate the feasibility of performing the tests as part of the test-and-cull strategy.

MATERIALS AND METHODS

Study area and population

The study population inhabited parts of Deer Management Unit 452 (44°40'–45°00'N, 83°30'–84°05'W), considered to be the core of the TB outbreak area (O'Brien et al., 2002), in northeastern Lower Michigan. Deer samples were obtained from five sources: 1) hunter harvests in 2004 and 2005; 2) small-scale culls conducted by a hunt club in January 2006 and February 2007 as part of a herd-health program; 3) carcasses bearing gross lesions consistent with disseminated TB presented to the MDNR Wildlife Disease Laboratory (WDL) from 2005 to 2007; 4) nuisance deer shot on farms by APHIS-WS staff under MDNR-issued disease-control permits; and 5) depopulation of a captive cervid shooting facility confirmed to be positive for *M. bovis* in December 2006. All deer other than those from the captive cervid shooting facility were free ranging.

To obtain biologic samples from hunter-harvested deer, MDNR sought the cooperation of private land owners and hunt club managers in areas encompassing clusters of high TB prevalence (O'Brien et al., 2006) to maximize the likelihood of obtaining *M. bovis*-positive samples. Sampling kits, consisting of a uniquely numbered jaw tag, 2–10 cc Vacutainer (Becton-Dickinson, Cockeysville, Maryland, USA) blood collection tubes (one with serum separator, one with sodium edetic acid as an anticoagulant) prenumbered to match the tag, and instructions on how to collect blood from the chest cavity and tag the deer's head, were provided to club managers. They distributed the kits to members licensed to hunt deer during regular autumn hunting seasons.

At harvest, deer heads were disarticulated and tagged to document location (township, range, and section), date, and contact information for the hunter. Heads and blood samples from the same deer were bagged, sealed, and shipped to WDL. The sex (based

on presence or absence of antlers) and age (estimated by tooth eruption and wear; Severinghaus, 1949) of the deer were recorded. All data were archived (Microsoft Access 2002, Microsoft Corporation, Redmond, Washington, USA).

Blood samples were obtained from the chest cavity with a disposable pipette, immediately after the deer were shot (culled, depopulated, and nuisance deer) and at the time grossly lesioned carcasses were examined at WDL. Previous work (O'Brien et al., 2001) had shown deer with gross lesions were likely to yield positive cultures for *M. bovis*. Serum-separator tubes were centrifuged (Adams Compact II centrifuge, Becton-Dickinson) for 10 min at $1,163 \times G$, and the serum was decanted into 5-ml low-temperature freezer vials (VWR International, Inc., West Chester, Pennsylvania, USA). The subjective degree of hemolysis for all serum samples was assessed visually by a single technician.

The submandibular, parotid, and medial retropharyngeal lymph nodes and the palatine tonsils from hunter-harvested, culled, depopulated, and nuisance deer were dissected bilaterally and pooled for each deer in a sterile, 50-ml polypropylene, screw-top centrifuge tube (Corning Incorporated, Corning, New York, USA) for bacterial culture, as previously described (O'Brien et al., 2004). Tissue samples that were similarly collected from deer with gross lesions typically included parietal pleura, medial retropharyngeal lymph nodes, and/or lung.

Bacteriologic analyses

Tissue samples were tested at the Michigan Department of Community Health (MDCH) Tuberculosis Laboratory by methods described previously (O'Brien et al., 2004). Two smears were prepared from processed specimen sediments. One was heat-fixed, stained with fluorescent Auramine-O (Kent and Kubica, 1985) and examined microscopically for acid-fast bacteria. When positive or suspicious, the second smear was stained via Ziehl-Neelsen for confirmation. The remaining sediment was resuspended and inoculated to a Lowenstein Jensen medium slant (Becton-Dickinson), a Middlebrook 7H11S medium slant (Becton-Dickinson), and a Bactec 12B broth vial (Becton-Dickinson). Media were examined at least weekly for 8 wk for bacterial growth. Acid-fast growth, as determined by a Ziehl-Neelsen-stained smear, was tested by genetic probe (Accuprobe, Gen-Probe, San Diego, California, USA) to differentiate members of the *Mycobacterium tuberculosis* com-

plex (Risner et al., 1994). Biochemical testing and high-performance liquid chromatography distinguished *M. bovis* (Butler et al., 1991, Metchock et al., 1995).

Immunologic analyses

Workers performing all blood tests were blinded to the pathology and mycobacterial culture status of all samples.

Rapid test: A lateral-flow assay (CervidTB STAT-PAK[®]) was developed by Chembio Diagnostic Systems, Inc. (Medford, New York, USA) to detect antibodies in various host species (Lyashchenko et al., 2006). The test employs a unique cocktail of *M. bovis* antigens and blue, latex, bead-based signal-detection system. Thirty microliters of serum or whole blood and three drops of sample diluent were added sequentially to the sample pad. Results were read at 20 min. Any visible band in the test area was considered a positive result.

Multiantigen print immunoassay (MAPIA[™]): Eight recombinant proteins of *M. bovis* were used (Rv numbers per Cole et al. [1998] in brackets): ESAT-6 [Rv3875] and CFP-10 [Rv3874] (Statens Serum Institut, Copenhagen, Denmark); MPB59 [Rv1886c], MPB64 [Rv1980c], MPB70 [Rv2875], and MPB83 [Rv2873] (Veterinary Sciences Division, Stormont, Belfast, UK); AcrI [Rv3391] and PstS1 [Rv0934] (Standard Diagnostics, Seoul, South Korea). Two polyprotein fusions (CFP-10/ESAT-6 and AcrI/MPB83; Statens Serum Institut, Copenhagen, Denmark) and one native antigen (*M. bovis* culture filtrate T/91/1378; Veterinary Sciences Division, Stormont, Belfast, UK) were also employed.

The multiantigen print immunoassay was performed as described previously (Lyashchenko et al., 2000). Antigens were immobilized on nitrocellulose (Schleicher & Schuell, Keene, New Hampshire, USA) using a semi-automated airbrush-printing device (Linomat IV, Camag Scientific Inc., Wilmington, Delaware, USA). The membrane was cut into 4-mm-wide strips, blocked for 1 hr with 1% nonfat skim milk in phosphate-buffered saline with 0.05% Tween 20 (PBST; Sigma-Aldrich, St. Louis, Missouri, USA), and incubated for 1 hr with serum samples diluted to 1:40. After washing, strips were incubated overnight with peroxidase-conjugated Protein G (Sigma-Aldrich) diluted to 1:1,000. Deer immunoglobulin G (IgG) antibodies, bound to printed antigens, were visualized with 3,3',5,5'-tetramethyl benzidine (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Maryland, USA).

Immunoblot assay: Electrophoresis and immunoblot assays were performed using described procedures (Bannantine and Stabel, 2000) with the following modifications. Antigens for immunoblot were either a whole-cell sonicate of *M. bovis* strain 95-1315 or recombinant MPB83. The sonicate was prepared as described (Waters et al., 2005). Antigen was electrophoresed through preparative 10% (w/v) polyacrylamide gels and transferred to nitrocellulose. These membranes were placed in blocking solution consisting of PBST and 2% (w/v) bovine serum albumin (PBST-BSA), then into the slot-blotting device (Bio-Rad Laboratories, Richmond, California, USA) with individual sera, diluted to 1:200 in PBST-BSA, added to independent slots. After 2 hr incubation with gentle rocking, blots were washed five times with PBST and incubated with horseradish peroxidase-conjugated rabbit anti-deer (Kirkegaard and Perry) diluted to 1:20,000 in PBST-BSA for 1.5 hr. Blots were again washed four times with PBST and were developed for chemiluminescence in Super-Signal detection reagent (Pierce Chemical Company, Rockford, Illinois, USA).

Enzyme-linked immunosorbent assay (ELISA): Lipopolysaccharide-enriched antigen was prepared from *M. bovis* strain 95-1315 as described (Waters et al., 2002). Immulon II 96-well microtiter plates (Dynatech; Chantilly, Virginia, USA) were coated with 100 μ l/well (4 μ g) antigen diluted in carbonate/bicarbonate-coating buffer (pH 9.6). Antigen-coated plates, including control wells, were incubated for 15 hr at 4 C, washed three times with 200 μ l/well PBST, and blocked with 200 μ l/well of milk diluent/blocking solution (Kirkegaard and Perry). After incubation for 1 hr at 37 C, wells were washed nine times with 200 μ l/well PBST, and test sera added (100 μ l/well) in duplicate. Test and control sera were diluted 1:100 in PBS containing 0.1% gelatin. After incubation for 20 hr at 4 C, wells were washed nine times with 200 μ l/well PBST and incubated 1 hr at 37 C with 100 μ l/well of horseradish peroxidase-conjugated anti-cervine IgG heavy and light chains (Kirkegaard and Perry) diluted 1:1,000 in PBS plus 0.1% gelatin. Wells were again washed nine times with PBST and incubated 4 min at 20 C with 100 μ l/well of SureBlue™ TMB peroxidase substrate (Kirkegaard and Perry). The reaction was stopped by addition of 100 μ l/well of 0.18M sulfuric acid, and the absorbance (450 nm) of individual wells was measured using an automated ELISA plate reader (Molecular Devices, Menlo Park, California, USA). Data were evaluated as changes in

optical density readings (i.e., Δ OD) calculated by subtracting the mean optical density readings for wells receiving coating buffer alone (two replicates) from the mean optical density readings for antigen-coated wells (two replicates) receiving the same serum sample. Two cut-off values, Δ OD \geq 0.25 and Δ OD \geq 0.3, were evaluated separately as decision rules to determine whether a given sample was considered positive for *M. bovis*.

Statistical analyses

Seven blood assays were evaluated: rapid test on whole blood; rapid test on serum; multi-antigen print immunoassay on serum; immunoblot for *M. bovis* whole-cell sonicate on serum; immunoblot for MPB83 on serum; and ELISA with cut-off points of Δ OD \geq 0.25 and Δ OD \geq 0.3 on serum. The result of each test was binary, either TB positive or TB negative. All assays were evaluated against the gold standard of mycobacterial culture by calculating sensitivity, specificity, and the predictive values of positive and negative tests (Thrusfield, 1995) using freely available software (Lowry, 2007), with 95% percent confidence limits calculated by the continuity-corrected efficiency-score method (Newcombe, 1998).

Misclassification of true disease status may occur when factors affect the performance of diagnostic tests (Thrusfield, 1995). Consequently, exploratory analyses were carried out to assess the influence of two host factors (sex and age) and three sampling-related factors (source of sample, days elapsed between sampling and processing, and for tests on serum, degree of hemolysis) on the agreement between the blood assays and culture. Days elapsed, or lag days, was the number of days between the date the deer was killed and the date the rapid test on whole blood was run and the blood was spun down to separate serum. Factors were coded as follows: 1) sex (binary, reference level: female); 2) age (ordinal, by quartiles, reference level: \leq 1.5 yr); 3) source of sample (nominal, five levels, reference level: hunter harvested); 4) lag days (ordinal, three levels, reference level: 0 days); 5) hemolysis (ordinal, five levels, reference level: clear). For each assay on each deer, a binary variable named *agreement* took the value *yes*, if blood test and culture results agreed, and *no* otherwise. A two-tailed Fisher's exact test (Proc Freq, SAS version 9, Cary, North Carolina, USA) assessed whether significant variation in agreement (considered attained at $P\leq$ 0.1) occurred across levels of each host and sampling factor. Those found significant for a given assay were retained for multivariable modeling.

Retained factors were used as predictors in a series of multivariable logistic regression models (Kleinbaum et al., 1998) for each blood assay with agreement as the response, modeling the probability that *agreement*=yes. Candidate models were ranked, and the best-approximating models chosen by methods previously described (Burnham and Anderson, 1998), using Akaike's Information Criterion corrected for small sample bias (AIC_c ; Lebreton et al., 1992), the AIC_c difference (Δ_i) between each candidate model and the best approximating model, and normalized Akaike weights (w_i). Candidate models where $\Delta_i \leq 2$ were considered to have substantial support from the data, and those where $2 < \Delta_i \leq 4$ were considered reasonably plausible. Both groups were considered competing. Models where $\Delta_i > 4$ were considered a poor fit to the data. Associations between test agreement and the host/sampling factors in best-approximating models were assessed via odds ratios (Kleinbaum et al., 1998). Where more than one model was competing for best-approximating model, model averaging of parameter estimates was used to determine odds ratios. Associations were considered significant at $P \leq 0.05$.

RESULTS

Blood and tissue samples from 760 deer were obtained, and tissues were cultured for *M. bovis*. The volume and quality of blood samples was highly variable, particularly among hunter-harvested deer. In some cases, whole blood or serum was not submitted, or volumes were insufficient for all seven assays. The immunoblot on whole-cell sonicate and the two ELISA assays were not performed after February 2006. The sensitivity of the immunoblot was insufficient to justify its expense, whereas the ELISAs were discontinued following departure of the staff who had conducted them. Consequently, not all assays were run on all deer. Nevertheless, blood samples from culture-positive deer were distributed relatively evenly among the assay methods (Table 1). There was no significant variation in the proportion of samples from culture-positive deer that were tested by each blood assay ($\chi^2=3.45$, $df=6$, $P=0.75$). Results for the immunoblot on whole-cell sonicate and the ELISAs were obtained for 45% of the

TABLE 1. Diagnostic performance^a of *Mycobacterium bovis* blood tests, white-tailed deer, Michigan, 2004–2007.^b

Diagnostic culture, <i>n</i>	Rapid test			Immunoblot			ELISA		
	Whole blood	Serum	Multiantigen print immunoassay	<i>M. bovis</i> whole-cell sonicate	MPB83 antigen	AOD \geq 0.25	AOD \geq 0.3	AOD \geq 0.25	AOD \geq 0.3
Mycobacterial culture, <i>n</i>	25 531	22 724	22 727	13 333	20 671	12 329	12 329	12 329	12 329
Sensitivity	56.0 (35.3, 75.0)	54.5 (32.7, 74.9)	68.2 (45.1, 85.3)	46.2 (20.4, 73.9)	55.0 (32.0, 76.2)	66.7 (35.4, 88.7)	66.7 (35.4, 88.7)	58.3 (28.6, 83.5)	58.3 (28.6, 83.5)
Specificity	98.9 (97.4, 99.5)	98.1 (96.7, 98.9)	97.1 (95.5, 98.2)	92.5 (89.0, 95.0)	99.3 (98.2, 99.7)	95.1 (92.1, 97.1)	95.1 (92.1, 97.1)	97.3 (94.7, 98.7)	97.3 (94.7, 98.7)
Predictive value	+ ve test - ve test	70.0 (45.7, 87.2) 98.6 (97.4, 99.3)	41.7 (26.0, 59.1) 99.0 (97.9, 99.6)	19.4 (8.1, 38.1) 97.8 (95.3, 99.0)	68.8 (41.5, 87.9) 98.7 (97.4, 99.3)	33.3 (16.4, 55.3) 98.7 (96.6, 99.6)	33.3 (16.4, 55.3) 98.7 (96.6, 99.6)	43.8 (20.8, 69.4) 98.5 (96.2, 99.4)	43.8 (20.8, 69.4) 98.5 (96.2, 99.4)

^a Point estimates of sensitivity, specificity, and predictive values expressed as a percentage with 95% confidence limits calculated by the continuity-corrected efficient-score method (Newcombe, 1998).

^b ELISA = enzyme-linked immunosorbent assay; OD = optical density; + ve = positive; - ve = negative.

deer, whereas the rapid test on whole blood and the immunoblot on MPB83 antigen were performed on 72% and 90%, respectively. More than 97% of the samples were tested by both the rapid test on serum and the multiantigen print immunoassay (Table 2).

Point estimates of the sensitivity of the blood assays ranged from 46% to 68%, whereas specificities and negative predictive values were all >92% (Table 1). Positive predictive values were highly variable, ranging from 19% for the immunoblot on whole-cell sonicate to 70% for the rapid test on whole blood.

Best-approximating models of the influence of host and sampling factors on blood assay vs. culture agreement were dominated by sample source and the lag days between sample collection and processing (Table 3). There were no significant predictors of agreement in five of 13 (39%) of the competing models. Sample source was a significant predictor in the other eight models, with significant variation attributable to grossly lesioned WDL carcasses. Regardless of blood assay, results for those samples were less likely to agree with culture than hunter-harvested samples. Assay results for hunter-harvested samples were between eight and 125 times more likely to agree with culture than samples from lesioned carcasses. Hemolysis was a significant predictor of agreement only in the best-approximating model for the rapid test on serum, where results on more hemolyzed samples were more likely to agree with culture (odds ratios and 95% confidence limits compared with *clear* samples: light red, 6.3 [1.4, 29]; medium red, 14.0 [1.4, 136]; dark red, 10.6 [1.6, 72]).

DISCUSSION

The North American paradigm of public ownership of wildlife has far-reaching, sometimes unanticipated, implications. Even where they are accepted by wildlife managers as justified and scientifically sound, disease control strategies, such as

mass depopulation, that are standard practice for privately owned livestock may be publicly unacceptable for use on free-ranging wildlife (Heberlein, 2004), and so, may not be implemented on the scale necessary to be effective. With such tools made politically unusable, managers of wildlife diseases may increasingly be forced to develop more innovative methods of discriminating infected from non-infected animals as an antecedent to control.

The intent of this study was to evaluate a number of candidate blood assays simultaneously under realistic conditions and with specimens likely to be representative of those available from TB surveillance in Michigan. Although the sensitivity, specificity, and the predictive values for the various tests are reported collectively here, direct comparisons between tests should be approached with caution. Because of limitations in sample volume and test availability across years, no two blood tests evaluated exactly the same data set, although overlap was substantial (Table 2). There was no significant variation in the proportion of samples from culture-positive deer that were tested by each blood assay (Table 1), providing little evidence that differential testing of culture-positive animals by some assays, and not others, biased the results.

All of the blood assays displayed high specificities and negative predictive values. Public distaste for the killing of large numbers of "innocent" (i.e., TB-negative) deer in the pursuit of a few TB-positive animals is the fundamental basis for opposition to widespread culling as a disease-management tool. These blood tests were all quite unlikely to falsely identify a TB-negative deer as being positive, boding well for public acceptance. Granted, the public would have difficulty knowing when false-positive deer were culled. However, if close scrutiny of publicly accessible disease-control plans should reveal a lack of agency candor with respect to the accura-

TABLE 2. Frequency of white-tailed deer by *Mycobacterium bovis* test status and host/sampling factors, Michigan, 2004–2007.^a

Factor	Level	Mycobacterial culture				Rapid test				Immunoblot				ELISA			
		Whole blood		Serum		Multiantigen print immunoassay		<i>M. bovis</i> whole-cell sonicate		MPB83 antigen		$\Delta OD \geq 0.25$		$\Delta OD \geq 0.3$			
		+	-	+	-	+	-	+	-	+	-	+	-	+	-		
<i>n</i>		28	732	17	532	23	716	33	709	31	315	13	671	22	319	18	323
Sex	Female	11	425	7	332	12	413	14	411	18	168	7	378	13	173	8	178
	Male	17	307	10	200	11	303	19	298	13	147	6	293	11	144	8	147
Age ^b	≤ 1.5 yr	5	240	3	188	3	236	11	228	7	85	2	219	3	89	1	91
	2–2.5 yr	2	163	2	99	3	158	5	158	4	84	1	148	5	82	4	83
	3–3.5 yr	13	158	5	113	9	156	11	155	8	75	6	149	9	70	6	73
	≥ 4 yr	8	171	7	132	8	166	6	168	12	71	4	155	7	76	5	78
Source	Cull	4	128	4	128	7	123	4	126	7	45	3	127	2	50	1	51
	Depopulation	3	258	4	257	4	257	11	250	0	0	3	258	0	0	0	0
	Hunter harvest	6	306	2	99	8	293	14	290	22	264	6	279	19	262	12	269
	Lesioned carcasses	15	3	7	11	2	8	4	6	2	6	1	7	3	5	3	5
	Nuisance	0	37	0	37	2	35	0	37	0	0	0	0	0	0	0	0
Lag days	None	8	414	8	392	12	408	17	403	8	72	7	412	3	77	2	78
	<1 wk	12	246	7	91	7	237	14	232	16	179	4	191	19	175	12	182
	≥ 1 wk	6	29	2	38	2	29	2	30	2	26	1	27	1	23	1	23
	NR	0	0	0	11	2	42	0	44	5	38	1	41	1	42	1	42
Hemolysis	Clear	4	96			9	91	9	91	2	22	3	94	2	22	2	22
	Yellow	2	98			5	95	3	97	2	27	2	93	2	27	2	27
	Light red	6	238			4	240	7	237	7	88	4	220	9	85	3	91
	Medium red	0	136			1	132	6	130	4	78	0	120	3	77	2	78
	Dark red	8	148			3	153	8	148	16	99	4	139	8	105	7	106
	NR	8	16			1	5	0	6	0	1	0	5	0	1	0	1

^a ELISA = enzyme-linked immunosorbent assay; OD = optical density; NR = not recorded.^b By quartiles.

TABLE 3. Host- and sampling-related factors, competing models, numbers of estimated parameters (K), model selection results (AIC_c), difference from the best model (Δ_i), model weights (w_i), and significant predictors of agreement between results of *Mycobacterium bovis* blood assays and mycobacterial culture, white-tailed deer, Michigan, 2004–2007.^a

Blood test	Factors ^b	Competing models ^c	K	AIC_c	Δ_i	w_i	Significant ^d predictors
Rapid test, whole blood	Source	Source	5	100.5	0	1.0	Source
Rapid test, serum	Source, lag days, hemolysis	Source + lag days + hemolysis	13	166.0	0	0.84	Source, hemolysis
Multiantigen print immunoassay	Sex, source, lag days	Source + lag days	8	212.8	0	0.54	None
		Sex + source + lag days	10	214.1	1.3	0.29	None
		Sex + lag days	5	216.2	3.4	0.09	None
Immunoblot, <i>M. bovis</i> whole cell sonicate	Age, source, hemolysis	Source	5	204.50	0	0.29	Source
		Source + hemolysis	11	204.51	0.003	0.29	Source
		Source + age	7	205.1	0.6	0.22	Source
Immunoblot, MPB53 antigen	Source, lag days	Age + source + hemolysis	13	205.4	0.91	0.19	Source
ELISA, AOD ≥ 0.25	Source, lag days	Source + lag days	8	98.7	0	0.97	Source
		Lag days	3	134.2	0	0.85	None
		Source + lag days	8	137.8	3.5	0.15	None
ELISA, AOD ≥ 0.3	Source	Source	3	117.7	0	1.0	Source

^a ELISA = enzyme-linked immunosorbent assay; OD = optical density.

^b Factors showing significant variation in agreement ($P \leq 0.1$, Fisher's exact test) between the blood assay and culture.

^c Models with $\Delta_i \leq 4$ compared with the best approximating model.

^d $P \leq 0.05$.

cy of the diagnostic tests, the distrust created could critically undermine the credibility of wildlife managers for years. Without that credibility, the already precarious public support for disease control could further weaken (Heberlein, 2004), and progress toward eradication could slow or cease.

As would be expected when specificities are high, sensitivities and positive predictive values were, in general, considerably lower. This imposes formidable obstacles. First, approximately one third to half of the truly TB-positive deer tested will be identified as TB negative. Such animals will not be removed from the population by a test-and-cull strategy, such as ours, and will continue to pose an exposure risk. On that basis alone, it is understandable that test-and-cull programs using these blood assays could be rejected as TB-control strategies out of hand. Clearly, such strategies are not yet, and may never be, a substitute for mass culling, where depopulation is a tool actually available to wildlife managers. However, in situations like Michigan's where depopulation simply cannot be used because of public opposition, assays with even modest sensitivity would allow removal of some positive animals that would otherwise remain in the population. Whether test-and-cull strategies based on such tests could remove enough deer to have a meaningful effect on overall transmission and maintenance of TB remains to be determined. Second, and more important from a practical standpoint, false-negative deer adversely affect the cost effectiveness of test-and-cull programs, increasing the money and effort expended to remove a positive deer from the population. As the prevalence of TB in the population decreases, those costs may increase prohibitively. Incorporating vaccination of test-negative deer into a test-and-cull strategy should improve cost effectiveness. Early investigations of bacillus Calmette-Guérin vaccination of white-tailed deer are promising (Palmer et al., 2007; Nol et al., 2008).

The blood assays in this study were minimally affected by variations in the host and sampling factors. All the tests were robust to differences in sex, age, and sample quality that are likely to occur across the surveillance methods typically used to collect wildlife samples. The Office International des Épizooties has stipulated that estimates of diagnostic sensitivity should be made in populations that mimic those in which the diagnostic tests will be used (Cousins and Florisson, 2005). Thus, quantitative information on how host- or sampling-related factors affect the performance of the tests is critical, so that variations in accuracy can be properly attributed either to those factors or to the tests themselves. Some of the deer anticipated to show the strongest agreement between the blood assays and culture (grossly lesioned carcasses sampled at WDL), in fact, showed the poorest agreement. Fifteen (83%) of the 18 lesioned carcasses cultured positive for *M. bovis*. In all but one instance where the assay results disagreed with culture, a false-negative resulted. Thus, lowered sensitivity of the assays was the principal cause of this disagreement. The carcasses were often eviscerated and sometimes quite desiccated. The red fluid sampled from these deer and presumed to be blood may well have been a mixture of blood, water, rumen contents, and other fluids, which may not have contained sufficient antibodies to diagnose TB using the assays. In addition, because these carcasses comprised only about 2% of all blood samples tested, it is also possible that their effect on agreement would be moderated, or made insignificant, with a larger sample size.

Cousins and Florisson (2005) reviewed tests available to diagnose TB in a variety of wildlife species and the difficulties of test validation. They note that, in general, detection of humoral antibody is considered a poor indicator of infection, and that antibody-based tests, such as those we evaluated, are likely to have limited

application in TB-control programs. That said, they also point out that recent advances in availability of purified antigens, technologies for antibody detection, and the undeniable logistic advantages of antibody tests with respect to cost and ease of use, have sustained continued interest in data on their diagnostic performance. In particular, results from large samples of naturally exposed animals in low-TB-prevalence populations that have been simultaneously tested via the gold standard, mycobacterial culture, continue to be needed (Cousins and Florisson, 2005). Our study meets all of these criteria and has documented the performance of several available blood assays for *M. bovis* on specimens obtained through a variety of field surveillance methods, where use of the intradermal tuberculin test is not an option. The majority of the tests evaluated require a well-equipped, climate-controlled laboratory and skilled laboratory personnel to conduct. Where these needs can be satisfied, the tests may be valuable additions to the tools available for diagnosis of *M. bovis* in white-tailed deer. They may be of limited utility, however, in field operations where testing necessarily must be performed in adverse weather by staff unskilled in laboratory technique. In such situations, the logistic advantages of rapid, animal-side screening assays like the CervidTB STAT-PAK[®] may prove difficult to ignore.

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LITERATURE CITED

- BANNANTINE, J. P., AND J. R. STABEL. 2000. HspX is present within *Mycobacterium paratuberculosis*-infected macrophages and is recognized by sera from some infected cattle. *Veterinary Microbiology* 76: 343–358.
- BURNHAM, K. P., AND D. R. ANDERSON. 1998. Model selection and inference: a practical information-theoretic approach. 1st Edition. Springer-Verlag, New York, New York, 353 pp.
- BUTLER, W. R., K. C. JOST, AND J. O. KILBURN. 1991. Identification of mycobacteria by high performance liquid chromatography. *Journal of Clinical Microbiology* 29: 2468–2472.
- COLE, S. T., R. BROSCHE, J. PARKHILL, T. GARNIER, C. CHURCHER, D. HARRIS, S. V. GORDON, K. EIGLMEIER, S. GAS, C. E. BARRY, F. TEKAIA, K. BADCOCK, D. BASHAM, D. BROWN, T. CHILLINGWORTH, R. CONNOR, R. DAVIES, K. DEVLIN, T. FELTWELL, S. GENTLES, N. HAMLIN, S. HOLROYD, T. HORNSBY, K. JAGELS, A. KROGH, J. MCLEAN, S. MOULE, L. MURPHY, K. OLIVER, J. OSBORNE, M. A. QUAIL, M. A. RAJANDREAM, J. ROGERS, S. RUTTER, K. SEEGER, J. SKELTON, R. SQUARES, S. SQUARES, J. E. SULSTON, K. TAYLOR, S. WHITEHEAD, AND B. G. BARRELL. 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393: 537–544.
- COUSINS, D. V., AND N. FLORISSON. 2005. A review of tests available for use in the diagnosis of tuberculosis in non-bovine species. *Revue Scientifique et Technique Office International des Epizooties* 24: 1039–1059.
- DORN, M. L., AND A. G. MERTIG. 2005. Bovine tuberculosis in Michigan: Stakeholder attitudes and implications for eradication efforts. *Wildlife Society Bulletin* 33: 539–552.
- HEBERLEIN, T. A. 2004. “Fire in the Sistine chapel”: How Wisconsin responded to chronic wasting disease. *Human Dimensions of Wildlife* 9: 165–179.
- HICKLING, G. J. 2002. Dynamics of bovine tuberculosis in wild white-tailed deer in Michigan. Michigan Department of Natural Resources, Wildlife Division, Report 3363, Lansing, Michigan, 34 pp.
- KENT, P. T., AND G. P. KUBICA. 1985. Public health mycobacteriology: A guide for the level III

- laboratory. U.S. Department of Health and Human Services, Atlanta, Georgia, 207 pp.
- KLEINBAUM, D. G., L. L. KUPPER, K. E. MULLER, AND A. NIZAM. 1998. Logistic regression analysis. In *Applied regression analysis and other multivariable methods*. Duxbury Press, Pacific Grove, California, pp. 656–686.
- LEBRETON, J. D., K. P. BURNHAM, J. CLOBERT, AND D. R. ANDERSON. 1992. Modeling survival and testing biological hypotheses using marked animals—A unified approach with case-studies. *Ecological Monographs* 62: 67–118.
- LOWRY, R. 2007. VassarStats: Web site for statistical computation—Clinical calculator 1: From an observed sample. estimates of population prevalence, sensitivity, specificity, predictive values, and likelihood ratios. Vassar College, Poughkeepsie, New York, <http://faculty.vassar.edu/lowry/VassarStats.html>. Accessed August 2007.
- LYASHCHENKO, K. P., M. SINGH, R. COLANGELI, AND M. L. GENNARO. 2000. A multi-antigen print immunoassay for the development of serological diagnosis of infectious diseases. *Journal of Immunological Methods* 242: 91–100.
- , R. GREENWALD, J. ESFANDIARI, J. H. OLSEN, R. BALL, G. DUMONCEAUX, F. DUNKER, C. BUCKLEY, M. RICHARD, S. MURRAY, J. B. PAYEUR, P. ANDERSEN, J. M. POLLOCK, S. MIKOTA, M. MILLER, D. SOFRANKO, AND W. R. WATERS. 2006. Tuberculosis in elephants: Antibody responses to defined antigens of *Mycobacterium tuberculosis*, potential for early diagnosis, and monitoring of treatment. *Clinical and Vaccine Immunology* 13: 722–732.
- MATTHEWS, G., AND E. CARLSON. 2005. Winter deer feed surveillance in northeastern Michigan. In *Bovine tuberculosis in Michigan: 10th Annual Conference, Michigan Bovine TB Eradication Project, Lansing, Michigan, 7–8 June*. James B. Henry Center for Executive Development, Lansing, Michigan, p. 9.
- METCHOCK, B. G., F. S. NOLTE, AND J. W. WALLACE, JR. 1995. *Mycobacterium*. In *Manual of clinical microbiology*, B. Metchock, P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover and R. H. Tenover (eds.). American Society for Microbiology Press, Washington, D.C., pp. 400–437.
- NEWCOMBE, R. G. 1998. Two-sided confidence intervals for the single proportion: A comparison of seven methods. *Statistics in Medicine* 17: 857–872.
- NOL, P., M. V. PALMER, W. R. WATERS, F. E. ALDWELL, B. M. BUDDLE, J. M. TRIANTIS, L. M. LINKE, G. E. PHILLIPS, T. C. THACKER, J. C. RHYAN, M. R. DUNBAR, AND M. D. SALMAN. 2008. Efficacy of oral and parenteral routes of *Mycobacterium bovis* bacille Calmette-Guérin vaccination against experimental bovine tuberculosis in white-tailed deer (*Odocoileus virginianus*): A feasibility study. *Journal of Wildlife Diseases* 44: 247–259.
- O'BRIEN, D. J., S. D. FITZGERALD, T. J. LYON, K. L. BUTLER, J. S. FIERKE, K. R. CLARKE, S. M. SCHMITT, T. M. COOLEY, AND D. E. BERRY. 2001. Tuberculous lesions in free-ranging white-tailed deer in Michigan. *Journal of Wildlife Diseases* 37: 608–613.
- , S. M. SCHMITT, D. E. BERRY, S. D. FITZGERALD, J. R. VANNESTE, T. J. LYON, D. MAGSIC, J. S. FIERKE, T. M. COOLEY, L. S. ZWICK, AND B. V. THOMSEN. 2004. Estimating the true prevalence of *Mycobacterium bovis* in hunter-harvested white-tailed deer in Michigan. *Journal of Wildlife Diseases* 40: 42–52.
- , ———, J. S. FIERKE, S. A. HOGLE, S. R. WINTERSTEIN, T. M. COOLEY, W. E. MORITZ, K. L. DIEGEL, S. D. FITZGERALD, D. E. BERRY, AND J. B. KANEENE. 2002. Epidemiology of *Mycobacterium bovis* in free-ranging white-tailed deer, Michigan, USA, 1995–2000. *Preventive Veterinary Medicine* 54: 47–63.
- , ———, S. D. FITZGERALD, D. E. BERRY, AND G. J. HICKLING. 2006. Managing the wildlife reservoir of *Mycobacterium bovis*: The Michigan, USA, experience. *Veterinary Microbiology* 112: 313–323.
- PALMER, M. V., T. C. THACKER, AND W. R. WATERS. 2007. Vaccination of white-tailed deer (*Odocoileus virginianus*) with *Mycobacterium bovis* bacillus Calmette Guérin. *Vaccine* 25: 6589–6597.
- RISNER, B. S., A. M. GATSON, AND G. L. WOODS. 1994. Use of Gen-Probe Accuprobes to identify *Mycobacterium avium* complex, *Mycobacterium tuberculosis* complex, *Mycobacterium kansasii*, and *Mycobacterium goodii* directly from BACTEC TB broth cultures. *Journal of Clinical Microbiology* 32: 2995–2998.
- RUDOLPH, B. 2006. Deer population estimates, hunting and harvest trends, and attitudes toward deer management in northeast Michigan. In *Bovine Tuberculosis in Michigan: 11th Annual Meeting, Michigan Bovine TB Eradication Project, Henry Center for Executive Education, Lansing, Michigan, 7–8 June*. James B. Henry Center for Executive Development, Lansing, Michigan, pp. 15–16.
- SCHMITT, S., S. D. FITZGERALD, T. M. COOLEY, C. S. BRUNING-FANN, L. SULLIVAN, D. BERRY, T. CARLSON, R. B. MINNIS, J. B. PAYEUR, AND J. SIKARSKIE. 1997. Bovine tuberculosis in free-ranging white-tailed deer from Michigan. *Journal of Wildlife Diseases* 33: 749–758.
- , D. O'BRIEN, E. CARLSON, D. SMITH, Z. COOLEY, B. RUDOLPH, G. HICKLING, G. NUGENT, AND P. BUTCHKO. 2004. Evaluation of a new strategy for control of bovine tuberculosis in Michigan white-tailed deer—Progress report: Year 1. Wildlife Division, Michigan Department of Natural Resources, Lansing, Michigan, 14 pp.
- SEVERINGHAUS, C. W. 1949. Tooth development and

wear as criteria of age in white-tailed deer. *Journal of Wildlife Management* 13: 195–216.

THRUSFIELD, M. 1995. *Veterinary epidemiology*. 2nd Edition. Blackwell Science Ltd., Oxford, UK.

VAN DEELEN, T. R., AND D. R. ETTER. 2003. Effort and the functional response of deer hunters. *Human Dimensions of Wildlife* 8: 97–108.

WATERS, W. R., M. V. PALMER, J. P. BANNANTINE, R. GREENWALD, J. ESFANDIARI, P. ANDERSEN, J. MCNAIR, J. M. POLLOCK, AND K. P. LYASHCHENKO. 2005. Antibody responses in reindeer (*Rangifer tarandus*) experimentally infected with *Mycobacterium bovis*. *Clinical and Diagnostic Laboratory Immunology* 12: 727–735.

———, ———, ———, D. L. WHIPPLE, R. GREENWALD, J. ESFANDIARI, P. ANDERSEN, J. MCNAIR, J. M. POLLOCK, AND K. P. LYASHCHENKO. 2004. Antigen recognition by serum antibodies in white-tailed deer (*Odocoileus virginianus*) experimentally infected with *Mycobacterium bovis*. *Clinical and Diagnostic Laboratory Immunology* 11: 849–855.

———, ———, T. C. THACKER, K. ORLOSKI, P. NOL, N. P. HARRINGTON, S. C. OLSEN, AND B. J. NONNECKE. 2007. Blood culture and stimulation conditions for the diagnosis of tuberculosis in cervids by the Cervigam assay. *Veterinary Record* 162: 203–208.

———, ———, AND D. L. WHIPPLE. 2002. *Mycobacterium bovis*-infected white-tailed deer (*Odocoileus virginianus*): Detection of immunoglobulin specific to crude mycobacterial antigens by ELISA. *Journal of Veterinary Diagnostic Investigation* 14: 470–475.

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