EVALUATION OF A NEW STRATEGY FOR CONTROL OF BOVINE TUBERCULOSIS IN MICHIGAN WHITE-TAILED DEER: YEAR 1 PROGRESS REPORT

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Abstract: The State of Michigan is striving to eliminate bovine tuberculosis (Tb) infection among free-ranging white-tailed deer in the northeastern Lower Peninsula of the state. Aggressive reduction in the overall deer population abundance may help to further reduce TB prevalence, but this course of action is unacceptable to many hunters and landowners. Targeted culling of sick deer would likely be far more acceptable to these stakeholders, so in the winter of 2003 the Michigan Department of Natural Resources pilot-trialed a new strategy based on live-trapping and Tb-testing of wild deer. The field study was conducted in a township with relatively high TB prevalence within Deer Management Unit 452 in the northeastern Lower Peninsula. Over a 2-month trapping period, 119 individual deer were live-trapped, blood sampled, fitted with a radio-collar, and released. A total of 31 of these deer were subsequently classified as Tb-suspect by at least one of five blood tests employed (however there was a low level of agreement among tests). A delay in testing meant that only six of these suspect deer were culled by sharpshooters before pre-programmed release of their radio-collars, after which they could no longer be located. *Mycobacterium bovis* was cultured from one of these six suspect deer; the other five were negative on culture. All target deer were located to within shooting range with 1 – 2 days of effort, and all the radio-collars on the apparently-healthy deer dropped off after the intended 90-day interval, and were thereafter recovered for re-use. There was considerable support for this pilot project among hunters, farmers, state and federal agriculture agencies, the media and the general public, and so we recommend that further field trials be undertaken using this technique. The initial focus of these trials should be on improving the efficacy and reliability of the blood testing procedure.

Key words: bovine tuberculosis, control strategm, deer, Michigan

INTRODUCTION

For the past decade, the State of Michigan has been striving to eliminate bovine tuberculosis (Tb) infection among free-ranging white-tailed deer in the northeastern Lower Peninsula (NELP) of the state. The primary disease control measures applied during this period have been to reduce deer abundance through increased hunting pressure, and to restrict supplemental feeding and baiting activities that encourage deer to congregate. By 2003 these two measures had, in combination, significantly reduced the prevalence of Tb among deer within Deer Management Unit 452 (DMU 452) These measures have not, thus far, been able to achieve the TB management program's goal of eradication of the disease from Michigan wildlife.

More aggressive reductions in overall deer population abundance would be likely to further reduce Tb prevalence, but this course of action is unacceptable to hunters and landowners. In 2003, therefore, discussions were held among personnel from the Michigan Department of Natural Resources (MDNR), Landcare Research Institute Ltd. in New Zealand, Michigan State University (MSU) and United States Department of Agriculture's Animal and Plant Health Inspection Service - Wildlife Services (USDA-WS) regarding ways that targeted reductions in the number of infected deer might be achieved. It was considered that targeted culling of sick deer would be far more acceptable to hunters and landowners than would intensified harvesting of the deer population as a whole.

The outcome of these discussions was an agreement to pilot-trial a potential new bovine tuberculosis control strategy based on live-trapping and Tb-testing of wild deer. Deer suspect for Tb based on blood test results would be culled while test-negative deer would be released. This report summarizes the results of a 1-year pilot trial of this strategy, undertaken by MDNR staff in the winter of 2003 in a township within DMU 452 that had a relatively high prevalence of bovine Tb in the resident deer population.

BACKGROUND

Over the past 8 years, the MDNR has utilized recreational hunting to substantially reduce the number of white-tailed deer and has restricted the use of bait and feed in the NELP. The intent of these regulatory changes was to reduce prevalence and prevent further spread of Tb. These strategies appear to have been at least somewhat successful, because a major increase in Tb prevalence in the NELP predicted by an early model of Tb infection in white-tailed deer has not been documented (McCarty and Miller 1998). Instead, a modest but significant reduction in Tb prevalence has been achieved (O'Brien et al. 2002). However, it may not be possible to sustain, let alone increase hunting pressure in order to drive deer densities lower in the near future (Frawley 2002), so it is anticipated that deer densities will remain at or above current levels during the coming decade. In some townships, these densities are thought to be close to, or above, the hypothesised threshold level at which Tb is likely to be maintained in the deer population. Under current management, therefore, it is unlikely that Tb will reach undetectable levels in the deer population within the next decade (Hickling, 2002).

Current infection levels are maintained by the number of infectious contacts between deer, which depends in part on the density of deer and also on the proportion of the population that is infected. Further reduction in overall deer densities does not appear to be a socially acceptable option in Michigan (de Lisle et al. 2002), and the functional response between effort

to remove deer and deer density suggests that continued reductions would require even greater effort than has been expended in the past (VanDeelen and Etter 2003). A less efficient (i.e. more costly) but potentially more socially acceptable alternative would be to reduce the proportion of infected deer by live capture, Tb-testing and removal of Tb-suspect deer. This approach is already under investigation at Riding Mountain National Park in Manitoba, where Tb infection is persisting at low prevalence among elk and deer.

GOAL AND OBJECTIVES

The overall goal of the pilot study was to demonstrate the technical feasibility of the approach and obtain preliminary estimates of the likely time and cost required to implement the strategy. For the strategy to succeed five criteria need to be met, so an assessment of these criteria represented the specific objectives of the pilot study:

1. To assess the acceptability of the proposed method to landowners and the general public (particularly with regard to the MDNR's ability to gain access onto private land for trapping purposes);
2. To demonstrate the technical capacity to live-trap and blood-sample substantial numbers of deer in targeted areas over a 2-3 month period in winter;
3. To identify a blood test procedure that is acceptably accurate and comparatively superior to others available. Ideally, such a test would be capable of detecting at least half of all TB-positive wild deer in a live-trapped sample.
4. To demonstrate that sharpshooters can efficiently locate and kill Tb-suspect deer by tracking down the radio-collars fitted to those deer;
5. To demonstrate that radio-collars fitted to non-Tb suspect deer will release correctly after a specified period of time, thereby allowing for their recovery and re-use.

METHODS

We targeted infected deer, particularly does, in a high-risk township within DMU 452 where Tb prevalence is considerably higher than the average for DMU 452 as a whole. Deer in the study area were trapped using approximately 60 'clover' traps set over the period January 15 to March 15, 2004. The use of clover traps helped minimize capture of adult bucks, which we deliberately sought to avoid. Our intention was that each deer captured would have had a blood sample taken, be fitted with eartags and a radio-collar attached using a 90 day self-releasing device, and be released immediately.

A whole blood sample was submitted to MSU for tuberculosis testing using the gamma interferon test (Cervigam®). Additional serum from each deer was stored for follow-up analyses using other test methods. Deer that were suspect for Tb based on the blood test results were relocated by radio-tracking and shot by USDA-WS staff. Fawns with suspect blood test results were euthanized opportunistically when recaptured in the traps. The carcasses of all deer killed or found dead were submitted for post-mortem examination and culturing of lymph nodes for confirmation of Tb infection. Lymph nodes were divided into cranial, thoracic and abdominal pools for each deer and cultured as separate samples. The automatically detached radio-collars of any surviving deer were retrieved to be refurbished and re-used.

Unanticipated technical problems with the Cervigam® test emerged late in the field sampling period and so the stored serum was sent for analysis using a variety of alternative Tb tests. USDA Agriculture Research Service's National Animal Disease Center (ARS-NADC) performed Western Blot and ELISA tests, and Chembio Diagnostic Systems, Inc. undertook Multi-Antigen Print Immuno-Assays (MAPIAs)

and Rapid Test (RTs). Other serum samples were shipped to collaborating scientists at the University of Otago's Disease Research Laboratory in New Zealand for use in another experimental Tb test.

RESULTS

Acceptability of the Proposed Method

One public meeting was held to discuss the project, and there were numerous items of media coverage. The general response to the proposed field trial was very positive. The majority of landowners who were contacted to ask permission for MDNR personnel to trap on their land were agreeable to the request, although there were some who did not want to be inconvenienced with the project. Supporters frequently and favorably cited the concept of removing Tb positive deer from the population while allowing Tb negative deer to survive.

Trapping Costs and Success

The total cost of the pilot project was $179,000, with $83,000 for clover traps and radio-collars that can be reused. Labor and travel amounted to $96,000, which provides an estimate of the amount it would cost to repeat the project now that the necessary equipment is available.

The data on trapping effort and success – which were recorded in slightly different ways by the two field teams – suggest that deer were captured 248 times over 1,382 trap days (calculated as the number of traps used multiplied by the number of days each trap was set). A total of 130 different deer were captured, with a further 118 capture events (47.6%) being recaptures. Two mortalities occurred in traps, and several other captured deer had been found dead by the end of the study. Ten deer were captured and released without a blood sample being obtained. The catch rate estimates were: 5.6 trap days expended per capture event; 10.6 trap days per individual animal captured; and 11.6 trap days per animal captured from which a blood sample was collected.

Of the 119 deer that were captured from which a blood sample was collected (Table 1), 60 (51.4%) were adults (≥1.5 years old) and 59 (49.6%) were fawns (6-9 months old). The majority (78.9%) of the captured deer were female. As expected, only a few adult bucks were captured by the clover traps (N = 8; 6.7% of captures).

Table 1. Age- and sex-composition of the 119 deer trapped and blood-sampled during the pilot study (10 other deer were captured without a blood sample being obtained).

	Male	Female	Total
Fawns	17	42	59
Adults	8	52	60
Total	25	94	119

The overall catch rate declined slightly over the course of the study, although the rate did show an improvement when additional traps were established at new sites part-way through the trial. During the first 15 days of trapping most of the deer in the traps were new captures, but thereafter the majority of deer in the traps were recaptures (Figure 1).
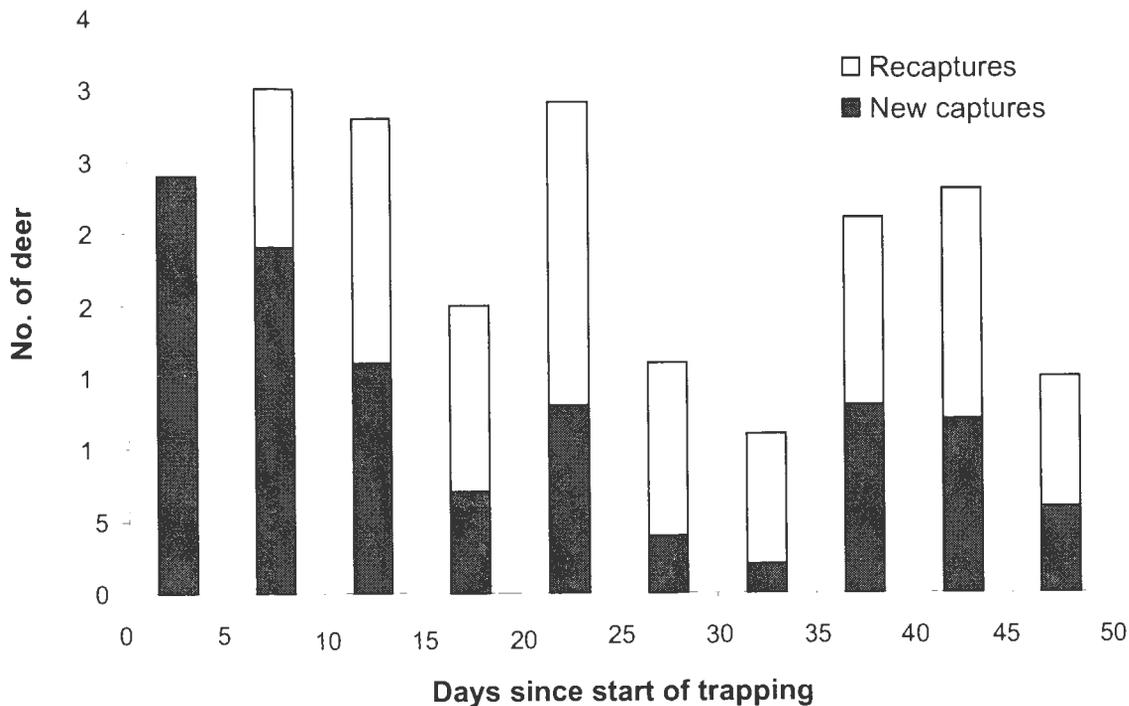
Figure 1. Numbers of newly captured, and recaptured, deer found in traps at 5-day intervals after the start of trapping on January 16, 2004.

Tb-Testing Success

The results of the Cervigam and four serum based tests are presented in Table 2 (results from the New Zealand testing are not yet available). A total of 31 deer were assessed as Tb-suspect, having tested positive by at least one of the five tests. However, there was a low level of agreement among tests. The testing produced 14 suspects based on their positive response using the MAPIA test, 13 using the RT test, 8 using the ELISA test, 5 using the Western Blot test, and 2 using the Cervigam test.

The technical deficiencies with the Cervigam test were not discovered until two weeks before the radio-collars were scheduled to drop off as programmed. As a result of some subsequent delay in requesting and conducting the alternative serum tests, only six of the 31 Tb-suspect deer could be culled by sharpshooters before their radiocollars released. Thereafter, the animals could no longer be located. The six Tb-suspects that were recovered were necropsied and the lymph nodes of each cultured for bovine tuberculosis in separate cranial, thoracic and abdominal pools. *Mycobacterium bovis* was cultured from head, chest and abdominal lymph nodes of one of the six deer, a 9-year old female, but the other 5 were negative on culture.

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No	Right Eartag	Left Eartag	Age *	Sex	Cervigam	MAPIA	RT	Western Blot	Elisa	Culture
1	131	132	0.5	F	-	-	-	+	-	NT
2	133	135	0.5	F	-	-	+	-	-	NT
3	151	157	0.5	F	-	MPB83+ 16/83+	-	+	-	NT
4	203	204	0.5	F	-	E6+	-	-	-	NT
5	211	217	0.5	M	-	16/83+, MBCF+	++	+	-	NT
6	236	238	0.5	F	+	NT	NT	NT	-	-
7	256	260	0.5	F	-	E6+	-	-	-	NT
8	263	265	0.5	M	-	-	-	-	+	NT
9	264	262	0.5	F	-	16/83+	+	-	-	NT
10	281(250)	241	0.5	M	-	E6+, E6/P10+	+/-	-	-	NT
11	1001	1002	3.5	F	+	-	-	+/-	-	-
12	1009	1	A	F	-	E6+, MBCF+	-	-	+	NT
13	1030	1222	A	F	-	MPB83+, 16/83+, MBCF+	-	-	+	NT
14	1097	219	A	F	-	-	-	-	+	NT
15	1130	1223	A	F	-	-	+	-	+	NT
16	1131	1424	A	F	-	E6+	-	-	-	NT
17	1208	1314	7.0	F	-	-	+	+	-	-
18	1211	1606	9.0	F	-	-	-	+	-	-
19	1214	14	A	F	-	E6/P10+	-	-	-	NT
20	1229	1228	A	F	-	E6+, E6/P10+, 16/83+	-	-	-	NT
21	1304	1305	A	F	-	-	+	-	-	NT
22	1306	1402	A	F	-	E6+, MBCF+ (Pr. L)	+	-	-	NT
23	1307	1502	2.5	F	-	-	+	-	-	NT
24	1308	1602	A	F	-	-	-	-	+	NT
25	1408	1115	A	M	-	-	-	-	+	NT
26	1410	1411	A	F	-	MBCF+	+	-	-	NT
27	1413	1607	9.0	F	-	MBCF+	-	+/-	+	+
28	1505	1104	A	F	-	-	+	-	-	NT
29	1525	1524	6.0	F	-	-	+	-	-	-
30	1530	1226	A	F	-	-	+	-	-	NT
31	1622	1227	3.5	F	-	-	+	-	-	NT
TOTAL POSITIVE					2	14	13	5	8	1

* A = adult (2 and older)

Relocation and Culling of Tb-Suspect Deer

By tracking each deer's unique radio signal, USDA-WS personnel were able to locate and kill all 6 deer that were determined to be Tb-suspect by blood test prior to their radio-collars dropping off. The USDA-WS team was also successful in locating, and approaching within rifle range, seven additional Tb-negative deer that were randomly selected as 'targets' to help assess the difficulty of culling specific individuals out of the deer population. All target animals were located within 1 – 2 days, with the process being most efficient when 2 -3 individuals were contributing to the tracking effort

Release and Recovery of Radio-Collars

The radio-collars were programmed using a computer to self-release at a future time (90 days). All collars dropped off as planned, were successfully retrieved, and were sufficiently undamaged to allow for refurbishment with a new self-release device.

DISCUSSION AND RECOMMEND-ATIONS

This pilot study provided useful new information on all five of the key issues that need to be evaluated when considering the likely success, or otherwise, of the 'trap and test' strategy.

First, we confirmed that there is considerable interest and support for the concept among hunters, farmers, state and federal agriculture agencies, the media and the general public. In particular, these individuals and groups approved of the idea of removing Tb positive deer from the population and allowing Tb negative deer to survive. We need to ensure, therefore, that stakeholders understand that it is not possible at present to guarantee that *only* Tb positive deer will be culled, because the current Tb blood tests are not 100% specific.

Similarly, we cannot promise that *no* tuberculous animals will be inadvertently released, as the current tests are not 100% accurate.

Second, we confirmed that it is possible to capture reasonable numbers of deer from a targeted area. Based on past experience, we had anticipated that two trap teams might be able to trap and blood-test 100 yearling and adult deer in a 2-3 month winter period, and that ~100 fawns would be caught in the process. Our trapping was moderately successful, and resulted in 60 adults and 59 fawns being tested. Only 130 deer were captured in total, primarily because 1) logistical constraints prevented all available traps from being set throughout the entire 2 month period; and 2) traps were maintained in existing sites after the proportion of recaptured deer rose, instead of being moved to new, as yet untrapped, locations.

Assuming the male:female ratio in the deer population is 1:1 for fawns and 1:1.85 for adults (G. Hickling, unpublished data), the trap ratios in Table 1 indicate that traps were 2.5 times as likely to catch female fawns than male fawns, and 3.5 times as likely to catch female adults than male adults. Adult males are the cohort most likely to be infected with Tb (O'Brien et al. 2002), but they are also the cohort most sought after, and likely to be shot, by hunters. Thus trapping complements hunting, by targeting the Tb-infected females in the deer population. However, this differential capture rate by sex suggests that any estimates of Tb prevalence among males based solely on trapping results are likely to be biased low.

Over the 50-day trapping period, the number of new captures and recaptures was similar. Although recapture of deer was identified as a potential complication, the 47.6% recapture rate that we measured was unknown prior to the field trial. This high rate of recapture was probably due, in part,

to the use of a much higher density of traps than has been used during previous capture efforts in this region (Sitar 1996, Garner 2001, Muzo 2003). Data on the rate and distribution of recapture by date and trap location, together with information on the relative costs of rechecking versus moving traps, will allow evaluation of the most cost-effective distribution and duration of trapping effort that will produce a high rate of new captures. Results relative to trapping date (Figure1) suggest that operations should focus on concentrated trapping sites-for approximately 2 weeks and that the traps then be moved to new locations. Trapping teams therefore must be mobile and prepared to move traps frequently to reduce the proportion of deer being recaptured. Fresh trapping areas need to be identified and prepared in advance for the trappers, for example by plowing roads and arranging landowner permission. This will likely be more effective than simply attempting to reduce recapture rate by distributing the traps over a broader area, particularly due to the emphasis on operating within regions of comparatively high Tb prevalence, but full analysis of the existing data has not been completed at this time.

The third criterion for a successful strategy was the availability of an acceptably accurate Tb blood test. Based on prior prevalence data for the selected township, we had predicted that a sample of 100 trapped adult deer should include, on average, five tuberculous individuals. Since the Cervigam test was thought to be at least 65% sensitive (S. Boulin, personal communication), we calculated that we had a >95% probability of detecting at least one

of these. In fact, the Cervigam test failed to detect the only Tb culture positive animal, while designating two Tb-culture-negative animals as Tb suspects. While sample sizes were small, these results clearly suggest the Cervigam test, at least under the conditions of the field trial, was inaccurate and thus unlikely to be acceptable as the sole test in a trap-and-cull Tb control strategy.

An adequate assessment of the accuracy of the serum tests used will require a more rigorous assessment than was possible under the circumstances prevailing at the end of this year's pilot study. In particular, we could not determine test accuracy due to our inability to kill, necropsy and culture all 31 suspect deer.

The limited data available on blood test performance for the six Tb suspect deer are summarized in Table 3. The results of the Rapid test were as disappointing as those of the Cervigam test: the culture positive deer tested negative and two culture negative animals were considered positive. The Western Blot test performed only marginally better, giving equivocal results for the culture positive animal and one culture negative deer, while marking two culture negative deer as suspects. Both the MAPIA and ELISA tests correctly categorized the culture positive deer as Tb suspect, while designating the culture negative deer as non-suspects. The fact that 31 of 119 deer were positive on at least one blood test is a clear indication that some of the tests were producing significant numbers of false positives. In short, whether one or more of these tests might perform satisfactorily in an adequately conducted evaluation remains to be determined.

Table 3. Agreement of blood test results with true Tb status (as determined by culture) for Tb suspects for which complete necropsy/culture results were available. κ is a measure of agreement among tests.

Test	n	Sensitivity [%] (95% CI)	Specificity [%] (95% CI)	κ	Agreement [a]
Cervigam	6	0 (0,0)	60 (17,100)	-0.29	LTC
MAPIA	5	100 (100,100)	100 (100,100)	1.0	Complete
Rapid Test	5	0 (0,0)	50 (1,99)	-0.36	LTC
Western Blot [b]	5	100 (100,100)	25 (0,67)	-0.12	LTC
Western Blot [c]	5	0 (0,0)	50 (1,99)	-0.36	LTC
ELISA	6	100 (100,100)	100 (100,100)	1.0	Complete

[a] After Thrusfield, 1995. LTC = Agreement less than would be expected purely by chance.
[b] Equivocal results interpreted as positive.
[c] Equivocal results interpreted as negative.

The blood tests are clearly an area where improved performance will be needed for the 'trap and test' strategy to be viable. The promising performance of the MAPIA and ELISA tests on this very limited sample suggests that improvements are indeed possible. Riding Mountain National Park in Manitoba is using a test and cull strategy to remove Tb positive free-ranging elk from the Park. Thus far, 38% of the elk that have been recorded as Tb suspect based on blood tests have subsequently proven to be culture-positive for Tb.

Our results suggest that the fourth criterion for this strategy – that radio-collared animals identified as Tb suspects could be relocated and shot – could be met at realistic cost and effort. Only a small number of Tb-suspect deer were removed, hence our decision to have the USDA-WS staff simulate removal of additional deer to gain extra data on the effort required. In a situation where numerous Tb-suspects needed to be removed, the effort required to track down each successive animal is likely to increase, especially in areas where populations have already been reduced through recreational hunting (Rudolph et al. 2000). This will be an important point to consider further when we evaluate the feasibility of implementing this the new strategy on a larger scale.

The fifth criteria for evaluation, determining whether the self-releasing collars would transmit reliably over an adequate range, would detach, and could be recovered for refurbishment and reuse, was well demonstrated during this project.

RECOMMENDATIONS

Where do we go next with our evaluation of the new strategy? We know that the work involved with the project is difficult, expensive, and as yet it is not proven that the approach would reduce the prevalence of Tb to undetectable levels more quickly than density reduction and baiting and feeding restrictions alone. Nevertheless, the results of this year's pilot are cause for optimism on a number of fronts. The project was well-received and supported by the public. Appreciable numbers of deer were captured with reasonable efficiency and low mortality. Tracking and removal techniques worked well. While the Cervigam test did not perform as expected, the capture, handling and tracking techniques nevertheless provided us with a field-tested protocol for obtaining deer for other diagnostic tests or future control projects. For example, should a suitable Tb vaccine ever be developed, it could be delivered by this protocol. Each of these factors would of course become more complicated with any expanded application of this strategy.

In a broader context, the new strategy is valuable in that it helps demonstrate the continuing commitment of the MDNR, hunters and northeast Michigan

landowners to work together to be good stewards of the state's wildlife resources.

With those thoughts in mind, we recommend that the pilot project be repeated in an effort to develop a more accurate[1] blood testing procedure. In an extension of the study, the five serum-based Tb tests used last year (and perhaps some additional tests) would be used to determine if trapped deer were Tb suspect. All blood test suspects would be killed, necropsied and cultured for *M. bovis*, with accuracy and inter-test agreement assessed critically. Through this process, one or more tests may emerge as acceptably accurate for the purposes of the new strategy.

Increased emphasis should be placed on accurate recording of appropriate trapping and effort data to enable spatial analysis of the distribution of trapping effort and success. USDA-WS personnel should repeat simulated removal exercises with additional deer to continue assessment of variability in effort required with a larger pool of animals. Additional data analysis on the size and distribution of properties in the region may be necessary in order to consider how logistical constraints would vary with any future application of this strategy in new areas.

In addition, we recommend that blood from a sample of hunter-harvested deer in comparatively high prevalence townships should be collected by hunters at the time of harvest. These blood samples would be tested by the candidate serum tests, with results compared to Tb culture results from the heads to help validate the blood tests.

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[1] *Sensitivity* refers to the proportion of genuinely-infected animals that are detected by a test. *Specificity* is a measure of how often healthy animals are misdiagnosed as infected (i.e 'false positives'). The overall *accuracy* of a test is determined by both its sensitivity and its specificity.

Third, we recommend that deer heads categorized as Tb-suspect through routine examination of lymph nodes by MDNR Wildlife Disease Laboratory personnel be tested using the RT. The RT reportedly can be performed using any fluid containing antibodies. Such fluids include whole blood, plasma, serum and aqueous humor from the eye. Aqueous humor would be tested using the RT and compared with Tb culture results of the head lymph nodes. If accurate, Tb testing of aqueous humor of dead deer could be a quicker, more efficient means of mass surveillance in the future. Although the Rapid Test performed poorly on limited samples in this year's pilot, its ability to use small quantities of whole blood and provide near instant results (which could obviate the need to collar, release and track down suspect deer, resulting in substantial cost and labor savings), justify a more rigorous evaluation of its potential application in a test-and-cull strategy.

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