2010-11 Elk Brucellosis Surveillance

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Introduction:

Brucellosis surveillance in Montana elk herds has taken place since the early 1980's with most of the effort focused on the Greater Yellowstone Area (GYA). Surveillance consists primarily of testing blood collected from hunter-harvested elk or elk captured for research purposes. The primary goal of the surveillance program since 2008 has been to determine the geographical distribution of brucellosis in Montana elk populations with secondary goals of: 1) estimating seroprevalence in areas where brucellosis has been documented, 2) collecting tissue for culture to increase the number of *B. abortus* isolates enhancing our ability to identify the relationship between brucellosis cases in cattle and other elk populations, 3) obtaining information to help evaluate current diagnostic tests utilized to determine brucellosis exposure, 4) obtain information to help evaluate potential causes for increases in seroprevalence and the geographic distribution of brucellosis, and 5) provide information to inform management decisions related to brucellosis management in elk and domestic livestock.

Within the last 5-10 years the proportion of blood samples testing positive for exposure to brucellosis (seroprevalence) has increased in some areas of the Montana GYA (Anderson and Williams 2008, Anderson et. al. 2009, Anderson et. al., 2010). The cause for this increase is unknown, but as early as 2008 MFWP expressed concern that changes in elk distributions resulting in larger group sizes on winter range may be contributing to increased *B. abortus* exposure rates among elk. Cross et al. (2010) suggested that increases in seroprevalence in Wyoming elk could be linked to increases in elk density on winter range. Proffitt et al (in review) also theorized that increasing seroprevalence in elk utilizing the eastern Gravelly Mountains may be related to increases in group density associated with higher population levels of elk. Montana Fish, Wildlife and Parks is currently evaluating the relationship between group sizes of elk on winter range and seroprevalence in other areas of the Montana GYA.

Brucellosis seroprevalence in Montana is based on a panel of standard tests run at a diagnostic laboratory. Determination of a reactor (a sample considered positive for exposure to *B. abortus*) is based on a positive reaction on two or more standard serologic tests. The results of these tests are evaluated according to the Uniform Methods and Rules for Cervidae (2003) as published by USDA, APHIS. Other bacteria, similar in biochemical makeup to *B. abortus*, may cause a cross-reaction or false-positive, complicating interpretation of these test results. The only test currently available to aid in determining whether a cross-reaction has occurred is the western blot assay. However, this test has not been validated for elk and is considered a research tool. Recent information from paired (i.e. samples from the same animal) blood and culture information for elk in Montana suggests that the western blot test is not 100% accurate for discerning between *B. abortus* and other bacteria (Anderson et al. 2009, Anderson et al 2010). As a result MFWP has taken the stance of reporting serologic data for brucellosis in elk both with and without utilizing western blot results. MFWP, Idaho Fish and Game, and Wyoming Game and Fish are currently working together in efforts to obtain guidance from the United States Animal Health Association Scientific Subcommittee on Brucellosis regarding the proper application of western blot results for interpreting serologic status in elk.

Since 2008 MFWP has tested elk within 30 hunting districts in southwestern Montana to determine the geographic distribution of brucellosis. Based on limited serologic data from historic surveys and more recent information, it is believed that the geographic distribution of brucellosis has expanded. Surveillance since 2008 has focused on determining the extent of expansion and establishing the current distribution of the disease in Montana elk populations. Hunter harvested elk were the primary source of blood samples from 2008 through the fall of 2010. Additional samples were obtained through research efforts within the survey area. However, inadequate sample sizes have restricted our ability to determine the actual geographic distribution of brucellosis in elk and estimate the level of exposure. As a result, MFWP elected to capture elk in an area of concern (targeted surveillance) to help bolster sample size and improve our ability to detect brucellosis should it be present. Radio collars and vaginal implants were utilized to learn more about population movements and interchange, and the risk seropositive female elk may pose in transmitting brucellosis.

Survey Area:

The general brucellosis survey area consists of 30 hunting districts (HD) in southwestern Montana. There area has been consistent since 2008 with an increased focus on hunting districts in areas adjacent to elk populations demonstrating exposure to brucellosis in past surveillance activities. Targeted surveillance focused on HD 326 east of Dillon, MT. The area was chosen due to the limited amount of information gained from hunter-harvested samples, its proximity to areas of known brucellosis exposed elk, and changes in elk distribution that have resulted in large groups of elk wintering farther west than historically observed (Figure 1). Additionally, in 2010-2011 we collected samples from female elk in the upper Bitterroot Valley as part of another MFWP research project.

Figure 1. Survey area for the 2010 general surveillance (hunter harvest) and targeted surveillance (elk capture) efforts.

Methods:

General Surveillance - Hunter harvest:

Blood collection kits were mailed to hunters receiving antlerless elk "B" tags within the survey areas. Additionally, kits where provided to cooperating landowners and businesses, and were available at the MFWP Regional office in Bozeman. Information regarding proper blood collection and shipping protocols was included with each kit. Blood collected by elk hunters could be returned to the MFWP wildlife lab through the mail or by submitting the sample to an area game check station or the Regional office in Bozeman. Blood was processed at the MFWP wildlife lab to determine quality and collect serum for testing. Serum from blood samples deemed suitable for submission was submitted to the MT Dept of Livestock Diagnostic Laboratory (Diagnostic Lab) for testing. The BAPA, Rivanol, Flourescence Polarization Assay (FPA), and Standard Plate Test (SPT) were used to screen serum for antibodies against *B. abortus*. A sample was called a reactor if a positive result was observed on any two of the tests. All reactors were then submitted to Louisiana State University for western blot (WB) analysis to evaluate whether a cross-reaction to other bacteria may have occurred. Reactors on the screening tests were also additionally tested with the Card and Complement Fixation test. Seroprevalence was defined as the percentage of elk classified as reactors to brucellosis on standard serologic tests. WB-prevalence was defined as the number of elk classified as reactors on standard serologic test and testing positive for exposure to *B. abortus* on the WB assay.

Tissue samples from hunter-harvested elk were collected opportunistically in an effort to culture *B. abortus*. Retropharyngeal lymph nodes, supramammary lymph nodes, amniotic fluid, cotyledons and reproductive tracts were collected as available from carcasses at check stations and by backtracking to kill sites. Tissue samples were submitted to the National Veterinary Services Laboratory (NVSL) for culture.

Targeted Surveillance – Elk capture

One hundred adult (≥ 1 year old) female elk were captured within the targeted surveillance area (Figure 1). A blood sample was collected from the jugular vein via venipuncture and centrifuged in the field to collect the serum. Serum samples were tested in the field utilizing the Card test. Elk that tested positive for exposure to brucellosis on the field Card test were checked for pregnancy by rectal palpation. All Card positive elk, as determined in the field, were fitted with a radio collar and all Card positive pregnant elk also received a vaginal implant transmitter (VIT), designed to be expelled during a birth/abortion event. Additional GPS collars were placed on randomly selected seronegative elk, bringing the total number of elk collared (seropositive + seronegative) to 31, with one elk receiving a standard VHF collar. Additional serum from each elk was submitted to the MT Dept. of Livestock Diagnostic Laboratory after the capture operation was completed and tested using the methodology described above for general surveillance. Reactors on standard serology were submitted for additional testing utilizing the CF and western blot assay. Serum samples from radio collared elk were submitted for pregnancy specific protein B testing conducted by Bio Tracking LLC, Moscow, ID.

Elk implanted with a VIT were tracked continuously by ground crews after capture until parturition, with the goal of locating individuals at least twice a week. All collared elk were also monitored from the air approximately twice a month. When a VIT was expelled it was located and swabbed to determine if *B. abortus* could be cultured. The area surrounding the expelled VIT was searched in an attempt to detect a birth or abortion site and collect tissues if found. Swabs were submitted to the Wyoming State Diagnostic Laboratory for culture in an effort to determine if *B. abortus* was shed during the birth event. For the purpose of this project, elk that lost VITs during the typical parturition period for elk (late May thru mid June) were considered to have carried their calf to full term.

GPS collars are scheduled to blow off automatically in January 2012. Data from the GPS collars will be downloaded and used to evaluate elk movement patterns. Bi-monthly aerial locations were plotted on a map to track animal movements, determine mortality status, and assist with ground tracking activities.

Bitterroot Research

Blood samples from 42 adult female elk (≥ 1 yr old) captured during a predator/prey study in the Bitterroot Mountains (HD's 250 and 270) were also tested for exposure to brucellosis. Blood was collected by jugular venipuncture. Serum samples

were screened for exposure to brucellosis at the Montana Department of Livestock Diagnostic Laboratory utilizing the BAPA, Rivanol, SPT, and FPA tests.

Results:

Hunter harvest

One hundred and ninety-six samples from hunter-harvested elk were submitted for serologic testing during the 2010 general hunting season. Adult females ($\geq$ 1 year of age), adult males and calves comprised 69.9% (n = 137), 17.3% (n = 34), and 9.7% (n = 19) of the samples tested, respectively. The sex or general age was not provided on 7 (3.6%) samples. One sample, for which age was not reported, was a reactor on serologic tests but negative on western blot for brucellosis exposure. This elk was harvested in HD 362 but excluded from further analysis due to lack of age information. All 19 calves and 34 adult males were negative for exposure to brucellosis based on standard serology. Only adult females were considered in calculations of exposure rates. Sample sizes, exposure rate estimates based on standard serology (seropositive), and exposure rates after WB results were applied (WB-prevalence) by HD are listed in table 1. Prevalence estimates and binomial confidence intervals were calculated, but little inference can be made about seroprevalence or WB-prevalence in relation to previous test results due to the small samples sizes and large confidence intervals.

Tissue samples from 84 female elk were submitted for culture. Adult female elk comprised the largest proportion of the sample (n = 82). One sample was from a calf and the age was not known for the remaining sample. *B. abortus* biovar 1 was isolated from one adult female collected in hunting district 311 (Table 2). All remaining samples were culture negative.

Targeted surveillance and research

One hundred and one elk were captured, utilizing a net gun fired from a helicopter. Capture mortality rate was 1% due to one mortality, the result of a leg fracture. Field tests utilizing the Card test identified eight possible reactors out of the 100 adult female elk tested. Seven of the eight Card positive elk were fitted with GPS collars. In total, GPS collars were placed on 7 seropositive and 23 seronegative elk. One seropositive elk received a VHF collar due to sizing concerns with the GPS collars. Of the field positive elk, six were determined to be pregnant based on rectal palpation and implanted with a VIT. Samples from all 100 elk were submitted to the Diagnostic Lab after capture and screened for exposure to brucellosis utilizing the Card, BAPA, FPA, Rivanol, and SPT. Reactors were further tested using the CF and Western Blot tests.

Table 1. Test results from hunter-harvested adult female serum samples collected during the 2010-11 hunting season. Samples were tested for exposure to *B. abortus* utilizing standard serologic tests (Seropositive) and the western blot assay (WB-Positive).

HD	Sample Size	Seropositive	95% CI – Seroprevalence	WB-Positive	95% CI- WB Prevalence
300	13	0	0-22.8	0	0-22.8
302	7	0	0-35.4	0	0-35.4
311	13	2	4.3-42.2	1	0.4-33.3
312	1	0	0-95	0	0-95
313	7	0	0-35.4	0	0-35.4
314	31	1	0.2-16.2	1	0.2-16.2
315	2	0	0-65.8	0	0-65.8
317	12	2	4.7-44.8	0	0-24.2
320	2	0	0-35.8	0	0-35.8
323	1	1	5.1-100	0	0-95
329	1	0	0-95	0	0-95
333	1	0	0-95	0	0-95
360	16	6	18.5-61.4	0	0-19.4
361	2	0	0-65.8	0	0-65.8
362	20	4	8.0-41.6	0	0-16.1
393	4	0	0-49	0	0-49
520	2	0	0-65.8	0	0-65.8
560	1	0	0-95	0	0-95

Table 2. Hunting districts where tissue collections occurred during the 2010-11 surveillance period.

HD	Sample Size	Culture Results	Isolate
Unknown	1	Negative	
310	2	Negative	
311	10	1 Positive, 9 Negative	*B. abortus* biovar 1
313	4	Negative	
314	37	Negative	
317	13	Negative	
320	2	Negative	
324	1	Negative	
325	2	Negative	
327	2	Negative	
330	1	Negative	
333	1	Negative	
360	4	Negative	
361	2	Negative	
362	2	Negative	
Total	84	1 Positive, 83 Negative	

Screening tests performed at the Diagnostic Lab detected a total of 12 reactors based on standard serologic tests, four more than we detected in the field using just the Card test, for a seroprevalence of 12%. Two of the twelve seropositive elk were also positive on WB for a WB- prevalence of 2%. Both of the WB positive elk were Card positive in the field and fitted with GPS collars. One was pregnant, implanted with a VIT and tracked through calving. The second was not pregnant and therefore did not receive a VIT. WB test results suggested that exposure to a similar bacteria (*Yersinia*) had resulted in a cross-reaction or false-positive on standard serologic tests on the remaining 10 samples.

A blood test (for pregnancy specific protein B) to determine pregnancy was completed on the eight field positive elk to confirm pregnancy. One elk believed to be pregnant on rectal palpation was determined to be not pregnant on the blood test. Within two weeks of capture four of the VIT's had been prematurely expelled or were pulled out by the elk including the elk believed to be pregnant by rectal palpation but non-pregnant on blood testing. The GPS collar also failed on one of the field positive elk that lost its VIT. The two field positive and pregnant elk that we were able to locate via telemetry were recaptured and re-implanted with a VIT. No evidence of an abortion event was apparent when the VIT's were originally recovered in the field or on examination of the elk after being recaptured. In total, four seropositive pregnant elk were tracked through the winter and spring.

On average VIT implanted elk were relocated 1.6 times per week from the ground with additional locations coming from flights. All four elk carried their VIT until late May/early June, which is the typical calving period for elk in Montana. VITs were recovered from 1 to 21 days from the last time the VIT was confirmed being present within the female. Weather conditions and remote terrain hampered our ability to locate two of the VITs quickly. Culture results from swabs of the VITs, collected after recovery in the field were negative for *B. abortus.* All of the VITs were recovered on public land with no evidence of domestic livestock within ¼ mile from the VIT location.

Radio collared elk demonstrated relatively limited movements during winter. However some movement between the survey area and the foothills of Blacktail Ridge, southwest of the study area was documented. There was no evidence that these elk crossed over Blacktail Ridge, and all remained in the targeted survey area until spring migration. Elk migrated primarily to summer ranges in the Gravelly Mountains, Centennial Mountains and Henry's Lake area (Figure 2).

Bitterroot Research:

All 42 adult female elk tested for exposure to brucellosis in conjunction with the Bitterroot research project were negative on the standard serologic screening tests performed at the Montana Department of Livestock Diagnostic Laboratory. No additional brucellosis testing was done.

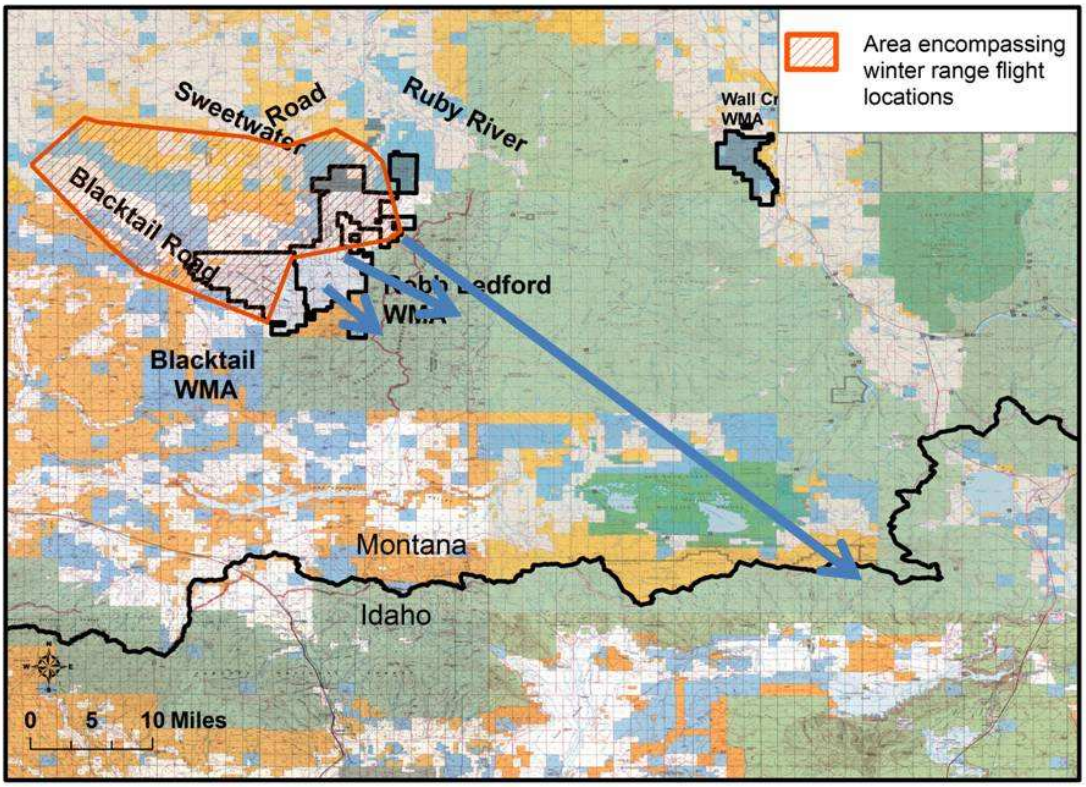

Figure 2. Area occupied when elk were on winter range and general movement patterns for elk migrating to summer ranges. Locations are based on collared elk locations recorded during relocation flights (February thru July, 2011).

Seroprevalence 2008-2010

Since 2008, when MFWP expanded its surveillance area to 30 hunting districts in south western Montana, 1076 adult cow elk have been tested for exposure to *B. abortus* within the survey area. Pooling samples across all three years, reactors to standard serology were detected in 10 hunting districts within the survey area with seroprevalences ranging from 5% to 30.8% (Table 3). When reactors were retested using western blot in efforts to determine if cross-reactions had occurred on standard serology, that number was reduced to 6 hunting districts with WB-prevalences ranging from 1.9% to 7.6% (Table 3).

Table 3. Brucellosis samples and test results from adult female elk tested for exposure to brucellosis, pooled during 2008-2010. Seroprevalence is based on the number of reactors on standard serologic tests. WB prevalence is based on both standard serology and WB results. Samples collected through hunter harvest and research activities were pooled.

HD	Samples	# Reactors (Seroprevalence)	Seroprevalence 95% CI	# WB Positive (Prevalence)	WB 95% CI
300	45	0	0-7.8	0	0-7.8
301	2	0	0-65.8	0	0-65.8
302	19	0	0-16.8	0	0-16.8
309	1	0	0-95	0	0-95
310	1	0	0-95	0	0-95
311	29	2 (6.9%)	1.9-22.0	1 (3.4%)	0.1-17.2
312	6	0	0-39.0	0	0-39.0
313	118	17 (14.4%)	9.2-21.9	9 (7.6%)	4.0-13.9
314	230	13 (5.6%)	3.3-9.4	5 (2.2%)	0.9-4.9
315	28	0	0-12.1	0	0-12.1
317	21	4 (19.0%)	7.7-40.0	0	0-15.5
320	15	0	0-20.4	0	0-20.4
322	12	0	0-24.2	0	0-24.2
323	13	4 (30.8%)	12.7-57.6	0	0-22.8
324	26	3 (11.5%)	4.0-28.9	0	0-12.9
325	9	0	0-29.9	0	0-29.9
326	106	14 (13.2%)	8.0-20.9	2 (1.9%)	0.5-6.6
327	20	1 (5.0%)	0.3-23.6	0	0-16.1
328	8	0	0-32.4	0	0-32.4
330	16	0	0-19.3	0	0-19.3
333	13	0	0-22.8	0	0-22.8
360	105	14 (13.3%)	8.11-21.1	2 (1.9%)	0.5-6.7
361	8	0	0-32.4	0	0-32.4
362	137	23 (16.8%)	11.5-23.9	5 (3.6%)	1.6-8.2
393	61	0	0-0.6	0	0-0.6
502	0	0	NA	0	NA
510	0	0	NA	0	NA
520	11	0	0-25.9	0	0-25.9
560	12	0	0-24.2	0	0-24.2
575	4	0	0-49.0	0	0-49.0
Total	1076	95 (8.8%)	1.5-3.3	24 (2.2%)	1.5-3.3

Discussion:

Collection of samples from hunter-harvested elk continues to be a challenge. The estimated 2010 general season cow elk harvest for hunting districts within the survey area was approximately 6958. An estimated 895 cow elk were harvested on B licenses within the survey area; the remainder was harvested in areas where cow harvest was allowed on a general hunting license (MFWP unpublished data). Hunters submitted 259 blood samples with 75.7% being suitable for testing. MFWP received blood from 3.7% of the estimated cow harvest. Sample sizes for individual hunting districts varied from 1 to 31, being greatest in districts where FWP biologist and technicians actively collected blood, often in association with tissue collection efforts, and where landowners participated in dispensing kits to hunters.

MFWP focused targeted surveillance efforts in HD 326 following concerns that: 1) brucellosis could be present in the elk population there because of its proximity to herds with documented exposure, 2) observed changes in elk distribution with large groups forming in areas west of traditional winter range may influence risk to livestock producers and elk-to-elk transmission, and 3) limited recent information of brucellosis presence or absence was available. Seroprevalence was estimated to be 12% for elk wintering within the survey area during the winter of 2010-11 and 13.2% when pooled with hunter-harvest samples from 2008-2010. If the WB assay was used as a definitive test for determining brucellosis exposure rates, the prevalence would be reduced to approximately 2% for both the 2010 targeted surveillance and the 2008-2010 pooled samples. Although seropositive elk were detected during research activities in the Gravelly-Snowcrest Mountains from 1984-1995, seroprevalence was estimated to be 0.44% (Hamlin and Ross 2002, MFWP 2005). However that estimate included both sexes and all age classes, and the WB assay was not being used to evaluate potential cross reactions. Information gained from MFWP's surveillance and research projects on elk will be used to help evaluate the WB assay and its ability to differentiate between *Brucella* and *Yersinia* exposure.

The four implanted elk were tracked both from the ground and the air to determine status of the VIT. All four carried their calf to full term expelling the VIT during the typical elk calving period, which is late May to mid June. *B. abortus* was not isolated by culture from swabs of VITs and no evidence of an abortion or still birth event was detected. Although this is encouraging, caution should be taken in drawing the conclusion that *B. abortus* was not shed into the environment. Due to extreme environmental conditions and rugged terrain, our recovery of two VITs took two weeks or more. This reduced the likelihood of successfully culturing *B. abortus*, should it have been present. A calf was observed near one cow elk for which it took two weeks to recover her VIT, suggesting a live birth. All of the VITs were recovered on federal or state land and no livestock were observed within ¼ mile of an expelled VIT. The eight seropositive elk that received radio collars will be recaptured and retested in the winter of 2011-12 if they are still alive and can be located. Pregnant individuals will again be implanted with VITs to monitor birth/abortion events.

Although utilizing elk hunters to collect samples allows FWP to obtain samples from a large area and may help supply information on seroprevalence in areas where brucellosis is known to exist, the small number of samples collected during the 2010 hunting season did little to enhance our understanding of the geographic distribution of brucellosis in elk. Even evaluating seroprevalence for a given area becomes challenging if the samples are collected primarily in the fall, when Montana's general hunting season takes place. The primary risk period for transmission of brucellosis occurs during the third trimester of pregnancy (between January and mid June) when elk are mostly on winter range or en route to calving grounds. It can be difficult to assign a winter range location for elk harvested in the fall as many elk populations tend to disperse once they leave winter ranges and may not have moved back to winter range during the fall hunting season. Samples collected during the fall hunting season may not provide information on seroprevalence or transmission risk in the general location where the harvest took place, if that elk winters in a completely different location.

There may also be differences in observed seroprevalence between hunter-harvested elk sampled in the fall and seroprevalence during the winter high risk period. Cross et al. (2010b) detected a nearly two fold difference between hunter-harvest samples collected in the fall and those of elk capture on feedgrounds in the winter, even though the elk were from the same hunting unit. Movement data from various research projects within Montana suggest that, in many areas, migratory movements result in some mixing of elk both on summer range and in transitional areas (MFWP unpublished data), and it can be difficult to predict where an elk harvested in the fall may winter. This is emphasized by the elk movement patterns observed in the targeted surveillance area. Although these elk demonstrated relative site fidelity during the winter, many started to disperse just prior to calving, and as of mid July were found in Hunting Districts 323, 324, 327 and in Idaho. Should these animals be harvested in the fall in the current district they reside, it will provide little information on brucellosis transmission risk for the area of harvest. A better understanding of seasonal elk movements is needed to improve evaluation of transmission risk to livestock and to wintering elk populations, particularly when surveillance activities rely primarily on samples obtained during fall hunting seasons.

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