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Assessment of Pathways for the Introduction and Spread of *Mycobacterium bovis* in the United States, 2009

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Table of Contents

List of Figures

List of Tables

Acronyms

AAZA	American Association of Zoos and Aquariums
AF	Accredited free
AP	Accredited preparatory
APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Service
CCT	Comparative cervical tuberculin test
CFR	*Code of Federal Regulations*
CFT	Caudal fold tuberculin test
CWD	Chronic wasting disease
EMRS	Emergency Management Response System
FDA	Food and Drug Administration
FSIS	Food Safety and Inspection Service
MA	Modified accredited
MAA	Modified accredited advanced
NA	Non-accredited
NAHMS	National Animal Health Monitoring System
NAHSS	National Animal Health Surveillance System
NASPHV	National Association of State Public Health Veterinarians
NASS	National Agricultural Statistics Service
NVSL	National Veterinary Services Laboratories
OIG	Office of the Inspector General
PPD	Purified protein derivative
RFLP	Restriction fragment length polymorphism
RMEA	Riding Mountain Eradication Area
SCT	Single cervical tuberculin test
UM&R	Uniform Methods and Rules
USAHA	United States Animal Health Association
VS	Veterinary Services
WS	Wildlife Services
γ-IFN	Interferon gamma assay

Executive Summary

Bovine tuberculosis (TB) was responsible for more losses among U.S. farm animals in the early 20th century than all other infectious diseases combined. The Cooperative State-Federal Tuberculosis Eradication Program (established in 1917 and administered by APHIS, State animal health agencies, and U.S. livestock producers) has nearly eradicated bovine TB from the nation's livestock population. However, despite the many accomplishments of the program, bovine TB remains a serious and costly disease of livestock in the United States. In 1992, VS conducted an assessment to identify pathways for the introduction and spread of bovine TB, in order to develop the most effective strategies for controlling the disease. Several changes have occurred in the livestock industry since 1992, and new pathways for the spread of bovine TB have been identified. The goal of this assessment is to describe the current pathways for bovine TB introduction and spread.

The pathways considered for the 2009 assessment are:

- Legal and illegal importation of cattle into the United States
- United States cattle industry practices
- The U.S. captive cervid industry
- Wildlife
- Zoo and other nontraditional species

Retrospective epidemiologic analyses of outbreaks in four States (California, Michigan, Minnesota, and New Mexico) identified several risk factors for the introduction and spread of bovine TB. In California and New Mexico, molecular fingerprinting techniques revealed several strains of *Mycobacterium bovis* (*M. bovis)*, indicating multiple sources of introduction. Risk factors for California and New Mexico included the importation and commingling of Mexican-origin steers, management and biosecurity practices used by calf-raisers for dairy replacement heifers, and a large influx of purchased additions. In States with similar practices and risk factors, both beef and dairy herds are at risk for exposure to *M. bovis*.

Conversely, Michigan and Minnesota each had just one strain of *M. bovis*, indicating a point source of introduction and local area spread. The same strains were identified in the wildlife of each State, making cattle contact with infected white-tailed deer (especially contact with feed contaminated by deer) an important risk factor for the introduction and spread of bovine TB in those States and other areas in which infected wildlife reside.

Human-to-cattle contact has not been fully investigated in the four States analyzed, but cannot be ruled out as a risk factor. It is difficult to determine the original sources of these bovine TB cases because evidence is sparse as to whether these events were new introductions of disease or established cases that were undetected in wildlife or cattle.

The importation of Mexican cattle continues to be a risk factor for the introduction of bovine TB into domestic livestock herds, especially when commingling occurs outside of slaughter channels. From 2003-2008, the number of cases of Mexican-origin cattle identified at slaughter has decreased. This is consistent with the decrease in number of animals imported. Each year 1-2 infected animals per 100,000 animals imported from Mexico are identified through slaughter detection or epidemiologic investigations.

Management practices play an important role in increasing or decreasing the risks of *M. bovis* infection and other pathogens in dairy and beef cow-calf operations. The introduction of new cattle to an operation, exposure to wildlife, and commingling with cattle from other operations increase the risk of introducing bovine TB to a herd. Methods for decreasing the risk of introducing bovine TB to an uninfected herd include minimizing the number and sources of incoming cattle, knowledge of the bovine TB status of source herds, testing new additions before exposing the home herd, decreasing

cattle and feed contact with free-ranging and captive cervids, and limiting exposure of cattle to animals from other operations with unknown disease status.

M. bovis may also be introduced by contact between captive cervids and wildlife. The development of the 1999 TB Eradication Uniform Methods and Rules (UM&R) and the inclusion of captive cervids in Title 9 of the *Code of Federal Regulations* are regulatory actions taken to decrease the risk of introducing or spreading *M. bovis* in captive cervids. However, limited surveillance of these animals negates reliable data on the true prevalence of bovine TB in captive cervids.

Exotic game ranching is a rapidly growing segment of U.S. animal agriculture. Little is known about the disease status or movement of animals in this industry, which may pose a risk for introducing bovine TB and wildlife diseases to cattle and other animals.

The re-emergence of bovine TB in wildlife and cattle highlights the importance of wildlife surveillance. The true prevalence of disease in wildlife species is not known because bovine TB surveillance and control in this population is inconsistent and difficult. Feral swine and supplemental feeding or baiting of free-ranging cervids have been factors in the propagation and persistence of *M. bovis*. The development of TB vaccines for wildlife may help eradication efforts by decreasing disease transmissions among wildlife.

The risk of *M. bovis* transmission to cattle from zoo animals is low because of limited contact; however, zoo species may pose a risk to humans. Game parks and other areas where animals roam freely or have fence-line contact with cattle or other livestock could contribute to *M. bovis* transmission. Hay and other fomites are also pathways for bovine TB given the right environmental conditions. Dogs, cats, wild birds, humans, and other species may act as hosts for TB and should be considered during epidemiologic investigations. Although bison are included under the same regulations as cattle, they may not enter routine slaughter channels, thereby limiting the availability of surveillance information. Additional research is needed to determine the true status of bovine TB in the captive bison industry.

Eradication of bovine TB continues to be a challenge. The pathways outlined in this assessment are intended to help APHIS-VS identify new measures for controlling and preventing spread of this disease in the United States.

1. Overview of the Current Bovine TB Program

1.1 Introduction

M. bovis is the causative agent for bovine TB, a disease found in many species worldwide. Cattle and other bovids are thought to be reservoir hosts, but other species may also play a role in the epidemiology of bovine TB. Many industrialized countries have bovine TB eradication programs in place because of the economic and public health impact of the disease; however, only a few countries have been successful in achieving eradication (Spickler and Roth, 2006).

In the United States, the Cooperative State-Federal Bovine Tuberculosis Eradication Program has been underway since 1917 and has reduced the prevalence of *M. bovis* infections in humans and cattle. Despite the successes of the program, affected herds continue to be identified. In 1992, an assessment was conducted to identify pathways for the introduction and spread of bovine TB to help develop the most effective strategies for controlling the disease. Several changes have occurred in the livestock industry since 1992 and new pathways have been identified for bovine TB spread. In January 2009, VS Centers for Epidemiology and Animal Health's (CEAH) Center for Animal Health Information Analysis (CAHIA) and National Animal Health Monitoring Systems (NAHMS) were asked to update the assessment conducted in 1992.

The objective of this qualitative assessment is to identify the pathways responsible for the recent introduction and spread of *M. bovis* in the United States as well as other pathways that might play a role in disease spread. Epidemiologic investigations from four States will be used to identify specific risk factors.

1.2 Epidemiology of *M. bovis*

M. bovis is a slow-growing, acid-fast, gram-positive, rod-to-filamentous-shaped bacterium. It has a very broad host range and can infect all warm-blooded vertebrates, including humans. *M. bovis* is an intracellular pathogen of macrophages (de la Rua-Domenech et al., 2006) and does not multiply outside the host except in cultured media. The survivability of *M. bovis* outside of the host depends largely on environmental conditions. Survival time is increased in moist environments, particularly those in which oxygen and organic matter are present. Sunlight, low pH, other microbes, and rising temperatures may decrease survival time (Morris et al., 1994).

Cattle and other bovids are thought to be reservoir hosts of bovine TB and several wildlife species in many countries have been identified as reservoir hosts. Wildlife hosts include the possum in New Zealand, badgers in Ireland and Britain, and cervids in the United States (Brown et al., 1994; Corner, 2006). Other species have been identified as spillover hosts, such as humans, coyotes, and cats. After an infection is established in reservoir hosts, it can persist in the population without any outside source of introduction and may also be transmitted to other species. In spillover hosts, infection in the population cannot persist indefinitely unless there is re-infection from another species or a change in the population that enhances interspecies transmission (Corner, 2006).

The disease dynamics of *M. bovis* are not well understood despite the long history of disease recognition. The incubation period for bovine TB may last for several months or longer. During the course of infection, some animals may be asymptomatic, but disease may progress rapidly in others. Clinical signs (that often include progressive emaciation and weakness) may appear with stress or age and are dependent on the location of lymph node involvement. Enlarged lymph nodes may lead to abscesses. Signs of respiratory involvement include coughing, dyspnea, or exercise intolerance. Animals with gastrointestinal involvement may have diarrhea or constipation.

Transmission of *M. bovis* can occur through various mechanisms based on the route of exposure and location of infection. A single bacillus in a droplet may be sufficient to establish infection (Morris et al., 1994.). Some species, such as possums, badgers, buffalo, deer, and cattle, excrete bacilli through droplets aerosolized from respiratory infection. These species are able to maintain infection in the

population by spreading it to each other through nose-to-nose contact (Corner, 2006). Aerosolization is thought to be the most infectious route of transmission, accounting for 80 to 90 percent of infections in cattle (Menzies et al., 2000).

Milk contaminated by bacilli from mammary infections may serve as a source of infection. In humans, infections acquired by milk have decreased with the introduction of pasteurization in the early 1900s. Animals have also become infected through wounds and exposure to contaminated urine (Corner, 2006). Vertical transmission has been documented. Spillover hosts (such as ferrets, pigs, cats, and dogs) that feed on infected carcasses can acquire infection in the gastrointestinal tract and are more likely to shed bacteria in feces.

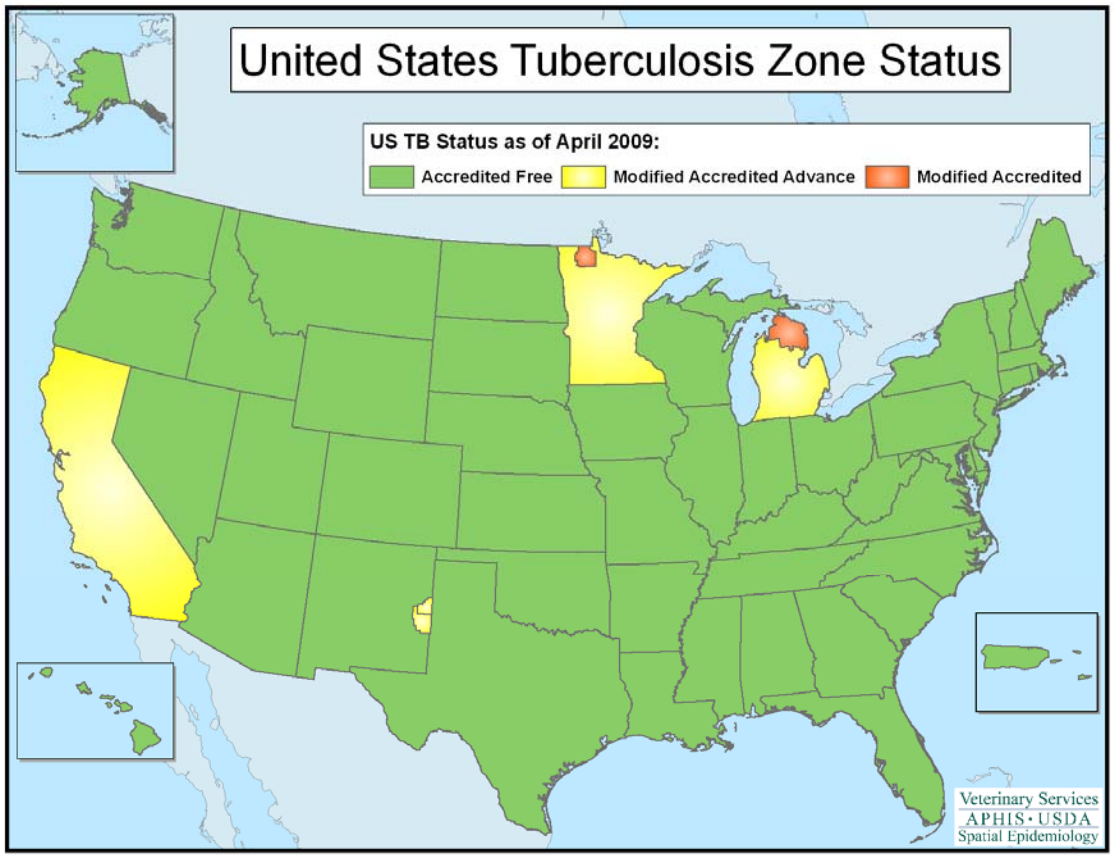
The risk of exposure is greatest in enclosed areas, such as barns. However, livestock can become infected if they share a common watering or feeding place contaminated with saliva and other discharges from infected deer or other animals. In cattle, infection usually takes months to develop. In some instances, the organisms lie dormant within the host's body for its lifetime without causing progressive disease. Communicability may be as early as 10 days post-exposure but typically occurs by day 87 (Neill et al, 1992: Morris et al., 1994). Excretion of bacilli may also occur in animals negative on a tuberculin test (Neill et al., 1992) and may be recovered as early as 3 days after experimental infection (Cassidy et al., 1998).

Transmission rates within a herd may range from 0 to 40 percent with lesions identified in 0 to 10 percent of the infected animals (Costello et al., 1998; Spickler and Roth, 2006). Typical postmortem lesions include tubercles or granulomas where bacteria have congregated. These granulomas are encapsulated and caseous or calcified. Lesions may be found in the lymph nodes, lung, visceral surfaces, or other locations. The size of the lesions in cattle is not an indicator of infectiousness. Animals in the early stages of disease may have no visible lesions but produce substantial amounts of aerosolized bacilli (Neill et al., 1992; Morris et al., 1994). In experimental infections, lesions have been detected as soon as 14 days after exposure (Cassidy et al., 1998).

1.3 *M. bovis* activity in the United States

The current objective of the TB program is to eradicate the disease so that it no longer poses a threat to livestock, wildlife, and public health (USDA-APHIS-VS, 2001). In 2001, the TB accreditation status for all States except Michigan and a portion of Texas was accredited-free (AF). As of April 2009, Texas had regained its AF status, and four States (California, Michigan, Minnesota, and New Mexico) were, or had portions of their State, designated less than AF (Figure 1.1).

Figure 1.1 Bovine TB status in the United States - April, 2009



1.3.1 Surveillance

New cases of bovine TB must be quickly identified through surveillance systems and contained by traceability of infected sources and control measures. The current goals of surveillance for bovine TB are to:

- Detect the last cases of infection in domestic ruminants
- Measure the progress and effectiveness of the eradication program
- Demonstrate disease freedom or low risk for trade
- Rapidly detect bovine TB in the event that it is introduced into the United States

Slaughter surveillance: The primary case[1]-finding tool for bovine TB in the United States is slaughter surveillance. Slaughter surveillance should be sufficient to detect a 0.05 percent or lower prevalence with 95 percent confidence. To validate slaughter surveillance, slaughter plants are required to submit suspicious lesions from at least 1 in every 2,000 adult cattle slaughtered. Financial incentives are offered to inspectors when lesions are culture positive for *M. bovis.*

Since 2001, the number of granuloma submissions increased from 2,030 in fiscal year (FY) 2001 to 10,666 in FY 2008. In FY 2007, 95 percent of adult cattle slaughtered went through slaughter plants that met or exceeded the 1 per 2,000 submission standard. A complete evaluation of surveillance in AF States determined that the slaughter submission rate from adult cattle under a valid slaughter inspection was higher than 11 percent. This submission rate was sufficient to detect 0.0002 percent prevalence with 95 percent confidence (USDA–APHIS–VS, 2009).

[1]A case is defined as an individual animal culture positive for *M. bovis*.

Antemortem testing: Antemortem detection of *M. bovis*-infected animals currently relies on a cell-mediated immune response of T-lymphocytes. Only in advanced stages of infection are antibodies detected (Neill et al., 2001). Therefore, tests that rely on cellular immunity have a greater sensitivity than antibody-based assays. Results of diagnostic testing may be influenced by the stage of disease, cross-reaction of other organisms, test interpretation, and other factors. (Table 1.1) (Norby et al., 2004).

Four tests are approved for antemortem use in the United States:

- Caudal fold tuberculin test (CFT)
- Comparative cervical tuberculin test (CCT)
- Single cervical tuberculin test (SCT) (cervids only)
- Bovine interferon gamma assay (γ-IFN) (cattle only)

The first three tests are intradermal, which is the international standard for detection (OIE, 2004). Intradermal tuberculin tests detect a delayed-type hypersensitivity that develops between 3 to 6 weeks after infection, though reactions have been noted sooner in experimental infections (de la Rua-Domenech et al., 2006).

Table 1.1 Factors associated with test misinterpretation

False negative results	
Tuberculin	Tuberculin expired or improperly stored
	Desensitization from previous tuberculin test
	Administrator error (e.g., incorrect administration, interpretation or testing bias)
Tuberculin and γ-IFN	Corticosteroids (natural or drug administration)
	Too early in infection for reaction to develop
	Co-infection and hypersensitivity to *M. avium*
Culture	Early stage of infection, lesions may not be seen
	Overgrowth with microbial contaminants
False positive results	
	Infection with other *Mycobacterium* sp.
	Johne's vaccination in calves
	Vaccination with *M. bovis* BCG
	Environmental mycobacterial sp. (e.g., soil)
	Other nonmycobacterial agents

Source: Adapted from de la Rua-Domenech (2006).

The CFT is often the screening test of choice and can be administered by accredited veterinarians or approved State or Federal veterinarians. Cattle are injected intradermally in the caudal fold of the tail

with purified protein derivative (PPD) and the injection site is palpated 72 hours later for any swelling, indicating an inflammatory response.

The reported sensitivity of the CFT varies greatly (Table 1.2). Cross-reaction (which may vary by geographic region) is possible with other organisms that share antigenic properties. Additionally, the CFT is subject to the interpretation of individual veterinarians. In an effort to minimize this subjectivity, the 2005 Bovine Tuberculosis UM&R provides guidelines for States to monitor response rates reported by veterinarians.

An animal with a suspicious reaction to the CFT is classified as a responder and its herd is quarantined. The animal must then undergo additional testing to rule out cross-reaction with other mycobacterial species or to rule out other reasons that might cause a false-positive response. CFT responders undergo additional testing by CCT or γ-IFN.

The CCT test may only be administered by approved State or Federal veterinarians who compare the response to injections of bovine PPD tuberculin and avian PPD tuberculin at separate sites in the mid-cervical area to determine the probable presence of *M. bovis.* Responses to the PPD tuberculins are recorded on a scatter plot and are the basis of CCT test classifications as negative, suspect, or reactor.

The γ-IFN test is a blood test that measures cell-mediated responses to *M. bovis, M. avium* PPD, and a negative control in an enzyme-linked immunosorbent assay (ELISA) (de la Rua-Domenech et al., 2006). This test may be used to classify CFT responders or in parallel with the CFT. The γ-IFN test is a cytokine released by T-lymphocytes and can be measured as early as 1 to 4 weeks after infection. The CFT is sometimes considered more sensitive than the γ-IFN for diagnosing bovine TB (Whipple et al., 1995; Gonzalez Llamazares et al., 1999). However, the γ-IFN has several advantages over the skin test, including a shorter time to detection, less animal handling, and antigen differentiation (vaccine vs. nonvaccine strains). The γ-IFN has been an approved test in the United States since 2001.

Table 1.2 Individual animal test sensitivity (Se) and specificity (Sp)

	Se		Sp	
Test	**Range (%)**	**Median (%)**	**Range (%)**	**Median (%)**
CFT	63.2–100	83.9	75.5–99	96.8
CCT	52.0–100	80	78.8–100	99.5
γ-IFN	73–100	87.6	85–99.6	96.6

Adapted from de la Rua-Domenech et al., 2006.

Some animals may be detected on one screening test and not the other. Combining the γ-IFN and CFT in parallel may improve sensitivity, but parallel testing is not standard practice in the United States. However, parallel testing may be conducted as part of an affected herd plan to implement a test-and-removal approach. Initial testing protocols for herds more commonly include testing in series, with only animals positive on the previous test advancing to the next test. In some instances, antemortem testing is inconclusive and the animal may be held for additional testing. Cattle with positive antemortem test results are sent to slaughter as “USDA Suspect.” A thorough postmortem exam is conducted, and all head and thoracic lymph nodes and suspicious lesions are collected and submitted for histopathology and culture (Food Safety and Inspection Service [FSIS] Directive 6240.1).

When testing a herd of unknown *M. bovis* status, testing in series reduces costs by increasing specificity, i.e. fewer false-positive animals are sent to slaughter. However, sensitivity is decreased and infected animals may be falsely identified as negative. For large herds in which all animals are tested, this effect may not be as significant as in smaller herds or individual animals. Consideration should be given to using the most sensitive technique for detecting infection in smaller herds, particularly high-risk herds. Table 1.3 describes the sensitivity and specificity for commonly utilized protocols. These values were estimated by a beta distribution derived from the sensitivity and specificity in Table 1.2. The beta distribution was applied to the model in Figure 1.2 for each testing in series protocol.

Figure 1.2 Possible test outcomes based on testing in series. (wp=within-herd prevalence, Se=sensitivity, Sp=specificity)

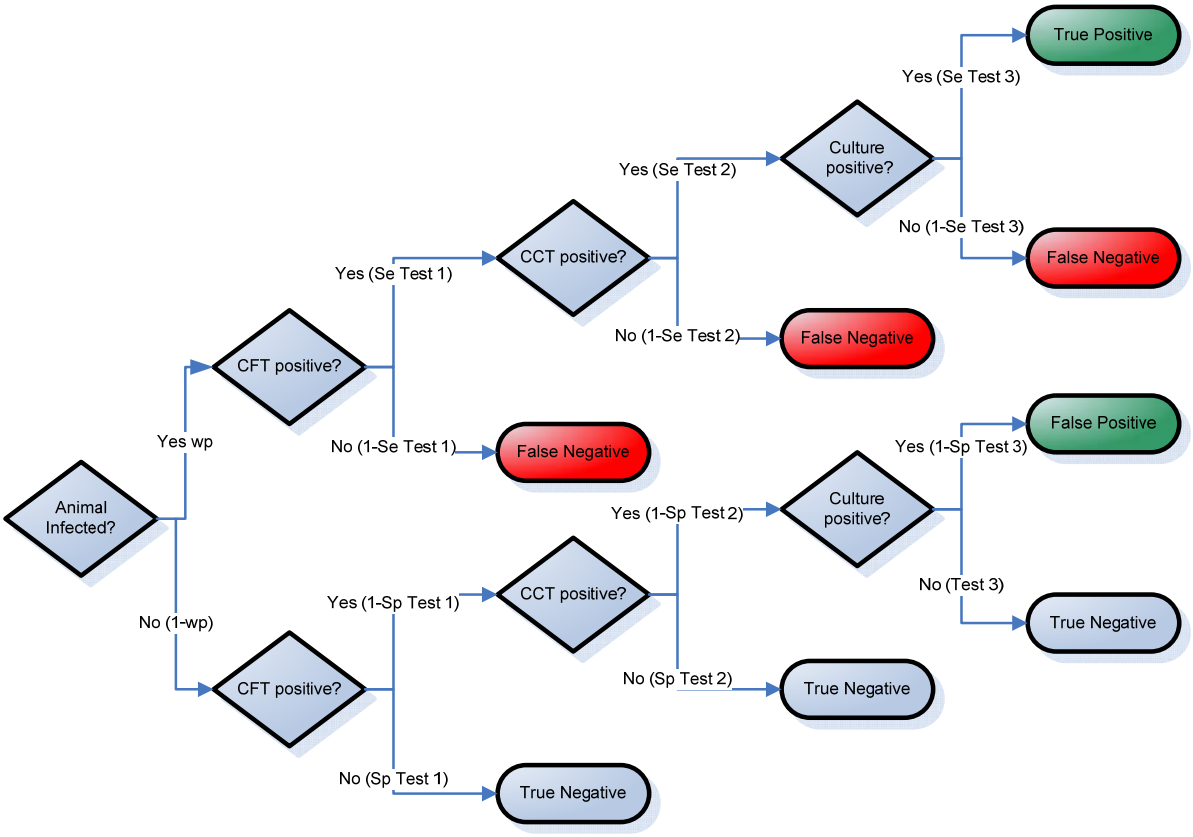


Table 1.3 Performance of common testing in series strategies

	Se		Sp	
Test protocol	**Range (%)**	**Mean (%)**	**Range (%)**	**Mean (%)**
CFT (responders)→ culture*	34–95	76	99–99.9	99.9
CFT (responders)→ CCT(reactors)→ culture*	20–86	57	99–100	99.9
CFT (responders)→ γ-IFN (positive)→ culture*	32–88	65	99–100	99.9

*Culture was presumed 95% sensitive and 99.9% specific

Molecular techniques: Molecular genotyping techniques identify the unique genetic profiles among *M. bovis* strains. These differences may result from genetic mutations over time; therefore, epidemiologic related strains may have similar genetic fingerprints. Several molecular techniques can be applied to *M. bovis* to identify differences or similarities between two strains. These differences, when identified during an outbreak, can help determine if a common source or unrelated source is contributing to disease introduction and spread. Examples include IS*6110* restriction fragment length polymorphism (RFLP), polymorphic G/C-rick sequence RFLP, spacer oligonucleotide typing (spoligotyping), and variable-number tandem repeat typing.

Spoligotyping (using the octal code) is the molecular technique used to rapidly assist epidemiologic analysis and is the method referred to most in this assessment. It has a shorter turnaround time than the RFLP and a higher degree of differentiation. Currently, all *M. bovis* isolates cultured at NVSL are spoligotyped. This method designates the genotype strains of TB belonging to the *Mycobacterium tuberculosis* complex (MTC), which includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canetti*, and *M. microti*.

Spoligotyping also detects variability in the direct repeat (DR) region in the MTC genome. The 36 base-pair DR regions are interspersed with spacer sequences. There are 94 spacer sequences, of which 43 are used for MTC strain differentiation. Clinical isolates of MTC bacteria can be differentiated by the presence or absence of one or more spacers. Spoligotyping is a polymerase chain reaction- (PCR) based method. Because it only requires a small amount of DNA to generate results, it is suited for rapid turnaround in the laboratory (Vitol et al., 2006). The presence or absence of the spacer regions is determined through a Southern blotting technique. The resulting hybridization patterns are scored in a binary fashion, with the presence of spacer regions designated with a value of 1 and the absence of spacer regions designated 0. This binary number system can be converted to one of several codes—such as the 15-digit numeric octal code—for ease of comparison between laboratories. Spoligotyping has the limitation of not identifying differences outside of a DR cluster; therefore, caution must be used when interpreting the results. The confidence of this test can be improved by coupling spoligotyping with additional fingerprinting techniques.

Of the 705 isolates identified from January 1991 through July 2008 that were typed by NVSL, 69 different spoligotyping patterns representing 10 different species were identified in 21 States, Mexico, and Canada. The strain identified in the recent TB outbreak in Minnesota appears to be isolated to that State. The outbreaks in California and New Mexico identified several different strains that were also found in several other States and Mexico. Superficially, it appears that local-area spread occurred in Minnesota, while the outbreaks in California and New Mexico appear to be a result of widespread animal movement and mixing among animals in Mexico and the Southwest.

Antemortem tests under development: New serologic tests intended to improve the speed and accuracy of bovine TB detection are being developed. When bovine TB is diagnosed by serologic testing, there are many different *M. bovis* antibodies that can be detected. To account for these

myriad antibodies, a multitude (cocktail) of antigens must be used. Multi-antigen print immunoassay (MAPIA) is a laboratory process that uses a cocktail of antigens for serological diagnosis. The sensitivity of detecting an antibody to one of the cocktail antigens using MAPIA is the same as detecting an antibody to a single antigen test (Lyashchenko, 2000).

Chembio Diagnostic Systems, Inc. has developed a serological *M. bovis* test for a variety of species, but the only tests currently licensed by USDA are the Primate and Elephant TB STAT-PAK (Chembio, 2009). The Bovid TB STAT-PAK, (currently under development) is a screening test that uses serum, plasma, or whole blood from bovids to detect the presence of *M. bovis* antibodies. The tests are small and portable and will yield a result within 20 minutes.

The *M. bovis* Fluorescence Polarization Antibody Test Kit produced by Diachemix is another bovine TB serologic test under development. This test is designed to screen large cattle herds or for slaughterhouse surveillance. The test is relatively rapid with results obtained within 60 minutes. According to Diachemix, the sensitivity is equal to existing test methods and the specificity is 99+ percent, higher than any existing TB test. These tests have not been approved by USDA or any other regulatory or governmental entities. They are available only for research evaluation i.e., not for retail sale (Diachemix, 2009).

PriTest's SeraLyte-Mbv is a rapid antibody test that can be completed in approximately 2 hours. This test uses a protein probe to locate antibodies against specific proteins found in the sera of cattle infected with *M. bovis*. The protein probe used is highly specific to *M. bovis, which* helps to significantly reduce false-positive results caused by cross-reaction with other antibodies of nonpathological mycobacterium (PriTest, 2009).

Another antemortem test currently under development for diagnosing bovine TB in cattle is the "electronic nose" (EN). The EN uses chemical sensors and a pattern recognition system to detect *M. bovis* using a serum sample from cattle or badgers. The chemical sensors produce electrical signals that are analyzed by pattern recognition software that search for an *M. bovis* pattern. It is not known exactly what the EN is detecting, i.e., the test may be sensing compounds released from *M. bovis* bacteria into the systemic circulation. The use of an EN is easy to perform, relatively inexpensive, can detect *M. bovis* within 3 weeks of infection, and would require only one trip to the premises instead of multiple trips, as is the case with CFT (Fend et al., 2005).

1.3.2 Traceability

Controlling bovine TB in the United States relies on rapidly detecting disease and identifying potential source herds (tracing). A trace may be initiated from an infected animal or an affected herd identified through slaughter surveillance, individual animal testing, herd testing for accreditation, or as a result of epidemiologic investigations. The identification of an infected animal or affected herd through any avenue initiates further epidemiologic investigation to determine the extent of infection. Table 1.4 lists the method of detection and the number of affected cattle herds for each of the past 8 years.

Table 1.4 Method for detection of affected cattle herds by fiscal year

Fiscal Year	Epidemiologic Investigation[1]	Other[2]	Slaughter	Total
2001	8	1	1	10
2002	6	1	3	10
2003	6	0	4	10
2004	4	0	0	4
2005	2	0	2	4
2006	2	7	0	9
2007	3	2	1	6
2008	5	7	1	13
Total	36	18	12	66

[1]Area testing, trace-in, trace-out, association with an affected herd.
[2]Movement, diagnostic, market, accreditation testing.

A trace is considered successful when it correctly identifies the source herd of infection. Epidemiologic investigations should also identify all individual cattle and herds that were exposed to the source or to infected animals. Because bovine TB is a reportable disease, all positive TB tests in cattle along with the official identification, age, sex, and breed of each animal must be reported to State and Federal officials. Appendix I describes tracing for bovine TB and how data are captured.

Figure 1.3 and 1.4 illustrate the discrepancy among the number of animals identified as culture-positive cases and the number of herds declared affected after successful traces. Many of the cases are identified at slaughter; therefore, some may be traced to Mexico. Of the 329 slaughter records available for *M. bovis* culture-positive cases from 2001 to 2009 (as of February 2009), 236 were traced to Mexico, 3 to Canada, and the remainder were within the United States (30), or unknown or unrecorded (63). Mexican origin cattle will be discussed in section 3.3 U.S. cattle imports from Mexico.

Figure 1.3 Number of NVSL culture-positive cattle by State and country (1986–2008)

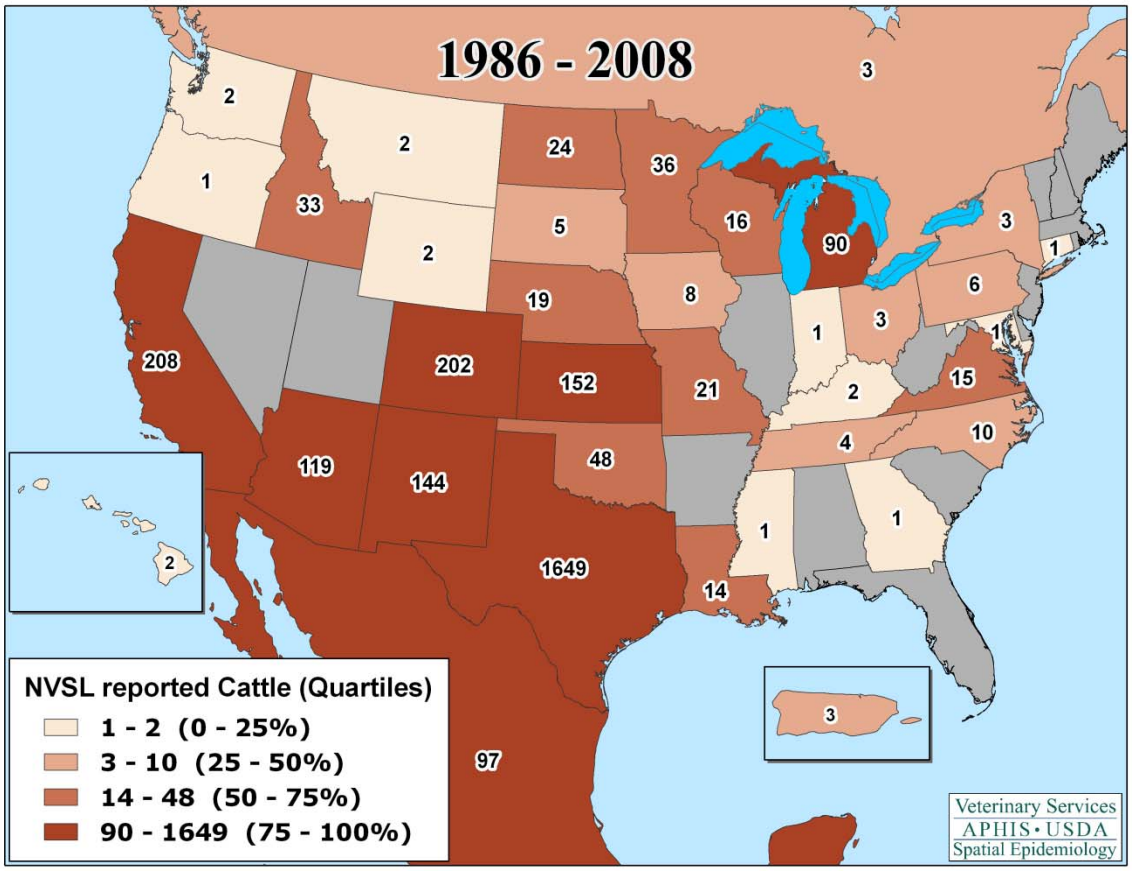
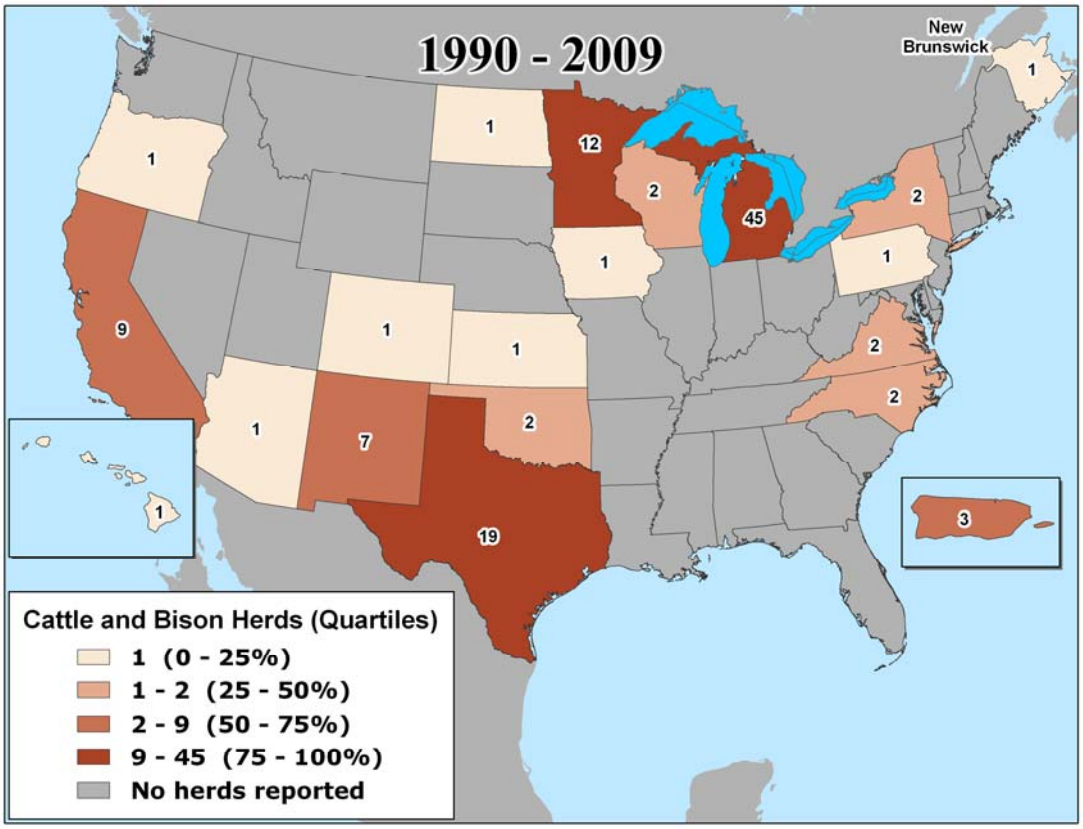


Figure 1.4 Number of confirmed positive cattle and bison herds (1990–2009)

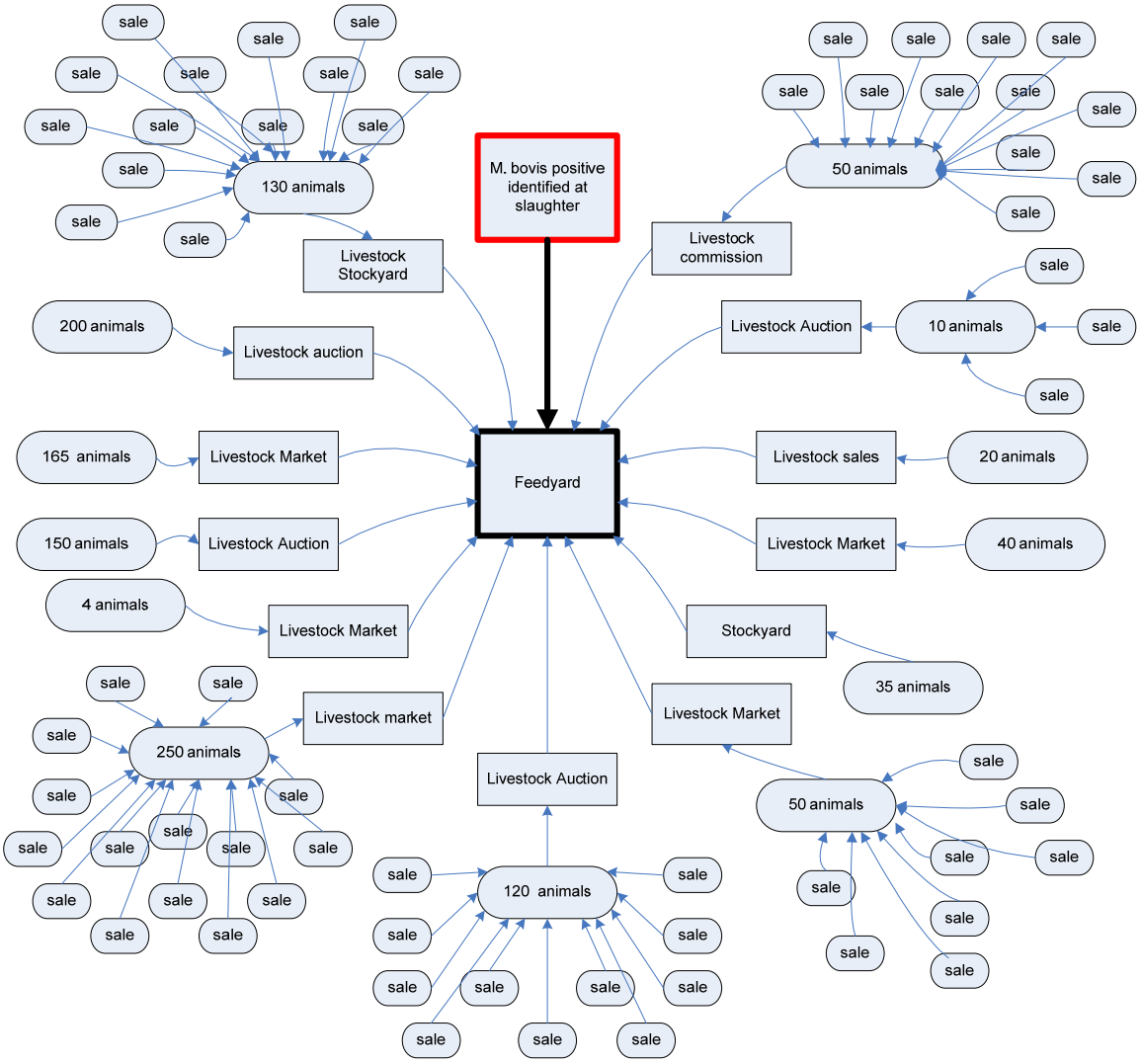


Slaughter traces: Individual bovine TB cases identified at slaughter undergo epidemiologic investigations to identify the herd-of-origin. According to the TB Uniform Methods and Rules (UM&R), these investigations must be completed within 90 calendar days of laboratory notification. Animals at slaughter are identified by ear tags or back tags to assist with traceability. Any dealer who purchases, deals, or sells cattle or bison must maintain records to facilitate the trace back of animals to the herd-of-origin. Many of these records exist only on paper, and compared to electronic records, may increase the time and resources needed for tracing.

Epidemiologic investigation: After an affected herd is identified, animal shipments into and out of the affected herd are typically traced back for the previous 5 years, although this may vary by situation. All associated cattle and bison herds must be quarantined. When an individual animal (traceout) is still a part of the herd it is sent directly to necropsy or tested; otherwise, the entire herd is tested. The UM&R suggests testing livestock and free-ranging wildlife around an affected herd within 6 months of diagnosis.

Affected feedlots also undergo epidemiologic investigations that focus on detecting disease spread from the feedlot. Figure 1.5 is based on an actual trace investigation and demonstrates the complexity of tracing out from a feedlot. Because of the movement of animals throughout the beef industry, it is difficult to identify a herd-of-origin because many operations do not meet the definition of a herd. A herd is defined in title 9 of the *Code of Federal Regulation*s, part 77.2 (9 CFR, 77.2) as any group of livestock maintained for at least 4 months on common ground for any purpose, or two or more groups under common ownership or supervision, geographically separated but all have an interchange or movement of livestock without regard to health status.

Figure 1.5 Trace investigation from a feed yard



1.3.3 Control

After suspect herds are identified, movement controls are implemented while the herds undergo testing to prevent further spread of disease. Wildlife in the vicinity of affected herds is also tested in situations when a known wildlife reservoir exists. When infection is detected in multiple herds, movement control occurs largely at the State level. States are classified by accreditation status (State status) based on the infrastructure in the State, compliance with the TB UM&R, and the prevalence of infection in the State (Table 1.5). Through the application of State status, movement controls and additional testing requirements may be implemented to prevent disease spread to additional States. A State animal health official may also request that a part of the State be classified differently than other areas of the State, a process known as zoning, regionalization, or split-State status.

Application for split-State status is outlined in VS Memo 552.44. States must demonstrate the authority to control movement outside of the affected zone and demonstrate adequate surveillance in all zones within the State. At the time of this report, Michigan, Minnesota, and New Mexico have met the criteria outlined in 9 CFR 77 for split-State status; however, other States have reserved the right

not to recognize this classification and have treated the entire State as being in the lower status classification.

Table 1.5 Bovine TB status classification

Status	Prevalence requirement[1] (%)	States (current as of 04/2009)
Accredited Free	0	46 States, MI (zone), Puerto Rico, U.S. Virgin Islands
Modified Accredited Advanced	<0.01	MN, MI, NM (zone), CA
Modified Accredited	<0.1	MI (zone), MN (zone)
Accredited Preparatory	< 0.5	None
Non-Accredited	>0.5	None

[1]Of the total number of herds.

Vaccination and treatment: A Bacillus Calmette-Guérin (BCG) vaccine for cattle exists that may offer some protection through oral or subcutaneous injection. However, the vaccine does not afford complete protection (Buddle et al., 2008) and is not approved for use in the United States. The γ-IFN may provide a means for distinguishing vaccine strains from nonvaccine strains (Sopp et al., 2008), but this could not be distinguished using skin tests. Further, a treatment for bovine TB with isonicotinic acid hydrazide in cattle exists but is also not allowed in the United States.

1.3.4 Emergency Actions

Despite the challenges in funding and the development of emerging issues for bovine TB control, progress has been made since the original pathways assessment in 1992.

In October 2000, the Secretary of Agriculture issued a declaration of emergency due to bovine TB (*Federal Register* 65 63227). The declaration identified risk factors for the spread of bovine TB in the United States including; the emerging threat that *M. bovis* in free-ranging deer presents to cattle, the recurring infection of dairy herds in the El Paso milkshed, and the decline of *M. bovis* surveillance. The declaration outlined several additional steps necessary for eradicating bovine TB, such as improving diagnostic tests, animal identification, and assisting Mexico with eradication efforts. To ensure that funds were available to carry out these measures, the emergency declaration authorized the transfer of $44 million from emergency funds to expand the TB eradication program in the United States.

This funding was intended to be part of a 4-year process to eliminate all TB from cattle, bison, captive deer, and elk herds in the United States. APHIS was successful in completing many of the goals set in the emergency declaration, yet new challenges arose that prevented total eradication (such as infection in large dairy herds in the Southwest). Through these funds, APHIS intended to enhance monitoring and surveillance in captive cervid herds and to work with States to remove affected herds. The voluntary buyout in the El Paso milkshed was funded and a buffer zone around the milkshed was created.

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2. Recent Bovine TB Outbreaks

2.1 Introduction

Because eradication of bovine TB in the United States is the goal, and most States are considered "free" of disease, the finding of one *M. bovis* culture-positive animal could be classified as an outbreak. For the purpose of this report, an outbreak refers to finding more than one affected herd in a State with a subsequent reduction in State status. As of May 2009, California, Michigan, Minnesota, and New Mexico had less than accredited-free (AF) status in all or portions of those States.

Data from multiple sources were used to describe these outbreaks (Appendix I.) In many instances, information (e.g., samples from wildlife, humans, and other fomites) were missing or not collected at the time of the epidemiologic investigation. The following summaries describe the source of disease introduction and its spread in each of these States.

2.2 Tuberculosis in California cattle herds, 2007–2009

2.2.1 Background

California tuberculosis program: Before December 2007, California had not had a case of TB in a dairy herd since January 2003. Three dairy herds were declared affected in 2003 that led to a change in the State's TB status from AF to modified accredited advanced (MAA). After a State is classified as MAA, it cannot regain its AF status until 2 years after the last affected herd is either depopulated or released from quarantine. In April 2005, California regained its AF status and in 2008, APHIS changed the State's status to MAA after more TB-positive dairy cattle were discovered.

Before 1994, the TB program in California consisted of area testing in which approximately 15 percent of the State's cattle population underwent antemortem testing each year. All herds in the State were tested every 6 years. This method was the primary tool used in the eradication effort until national prevalence levels of TB were greatly reduced. In 1994, California abandoned area testing for TB because the prevalence of disease in California (and overall in the United States) had reached such low levels that area testing was no longer efficient.

The primary surveillance method for TB in the United States has since shifted from area testing to slaughterhouse inspection. Slaughter surveillance in California includes examining every bovid carcass processed through Federal- or State-inspected slaughter facilities for characteristic signs of disease. Any carcasses with lesions suspicious for TB are held and undergo further testing to determine if bovine TB is present. If a carcass is found to be infected, a traceback investigation is initiated by the California Department of Food and Agriculture (CFA) to determine the origin of the animal.

Table 2.1 and Figure 2.1 highlight the slaughter surveillance program for California. Since 2002, the number of samples submitted for TB surveillance in California[2] has met or exceeded the goal of 1 sample per 2,000 adult cattle slaughtered or 0.05 percent.

[2] The number of animals slaughtered in California may not reflect the total number of California-origin animals slaughtered. Animals may come to California from other States for slaughter or leave California to be slaughtered in other States.

Figure 2.1 Number of cattle slaughtered and number of samples submitted for suspicion of TB in California (FY 2002–2008)

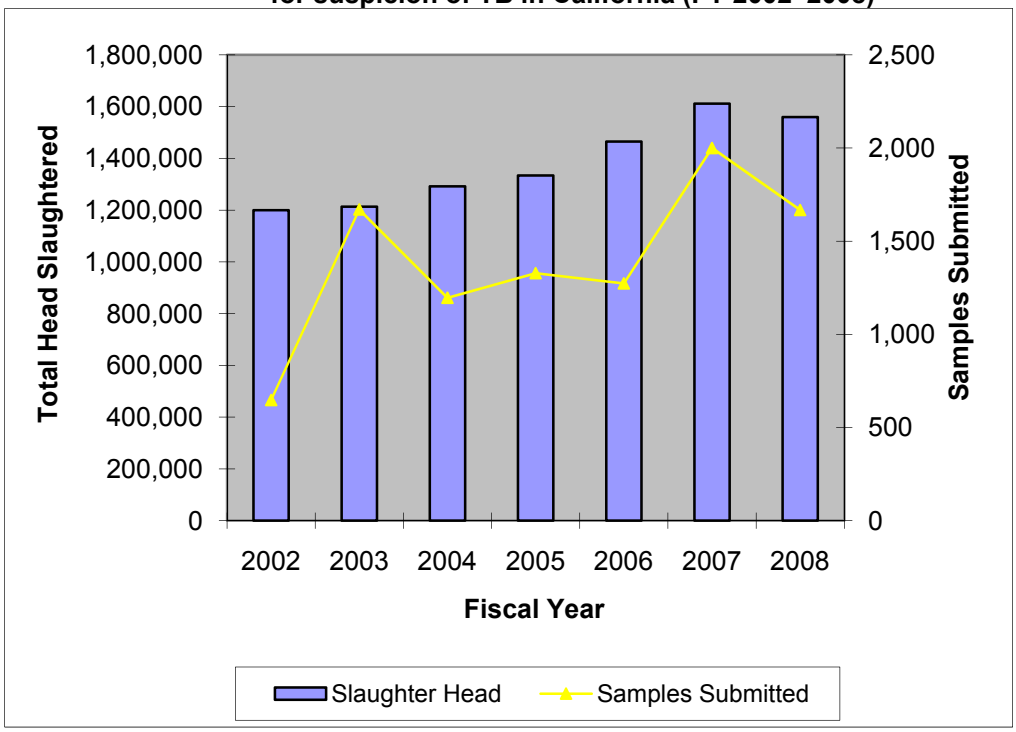


Table 2.1 Slaughter submissions for TB in California—All cattle (FY 2002–2008)

Fiscal Year	Slaughter Head	Samples Submitted	Percent Sampled	Number Infected	Percent Inf/Sampled	Percent Inf/ Slaughtered
2002	1,200,000	647	0.05	NA	NA	NA
2003	1,213,062	1,670	0.14	NA	NA	NA
2004	1,291,868	1,196	0.09	NA	NA	NA
2005	1,334,117	1,328	0.10	0	0.00	0.0000
2006	1,465,176	1,273	0.09	0	0.00	0.0000
2007	1,611,477	2,000	0.12	3	0.15	0.0004
2008	1,559,656	1,667	0.11	1	0.06	0.0001

Slaughter surveillance identified three infected animals in California in FY 2007. Two of these animals were feedlot steers of Mexican origin. The third animal was traced back to a newly affected dairy in New Mexico.

In 2008, a dairy cow from a California dairy was identified as being infected through slaughter surveillance and testing. Epidemiologic investigations from this cow identified four additional affected herds.

California dairy industry: In 2008, California led the nation in terms of number of dairy cows (1.844 million) and milk production (41,203 million lb) (Table 2.2). These numbers account for 19.8 percent

of U.S. dairy cows and 21.7 percent of all milk produced. Although the number of dairy operations in 2007 (2,200) was relatively low compared to other States, the average herd size in California was 968 cows; 50.0 percent of operations had 500 or more cows while only 5.0 percent of all operations in the United States had more than 500 cows.

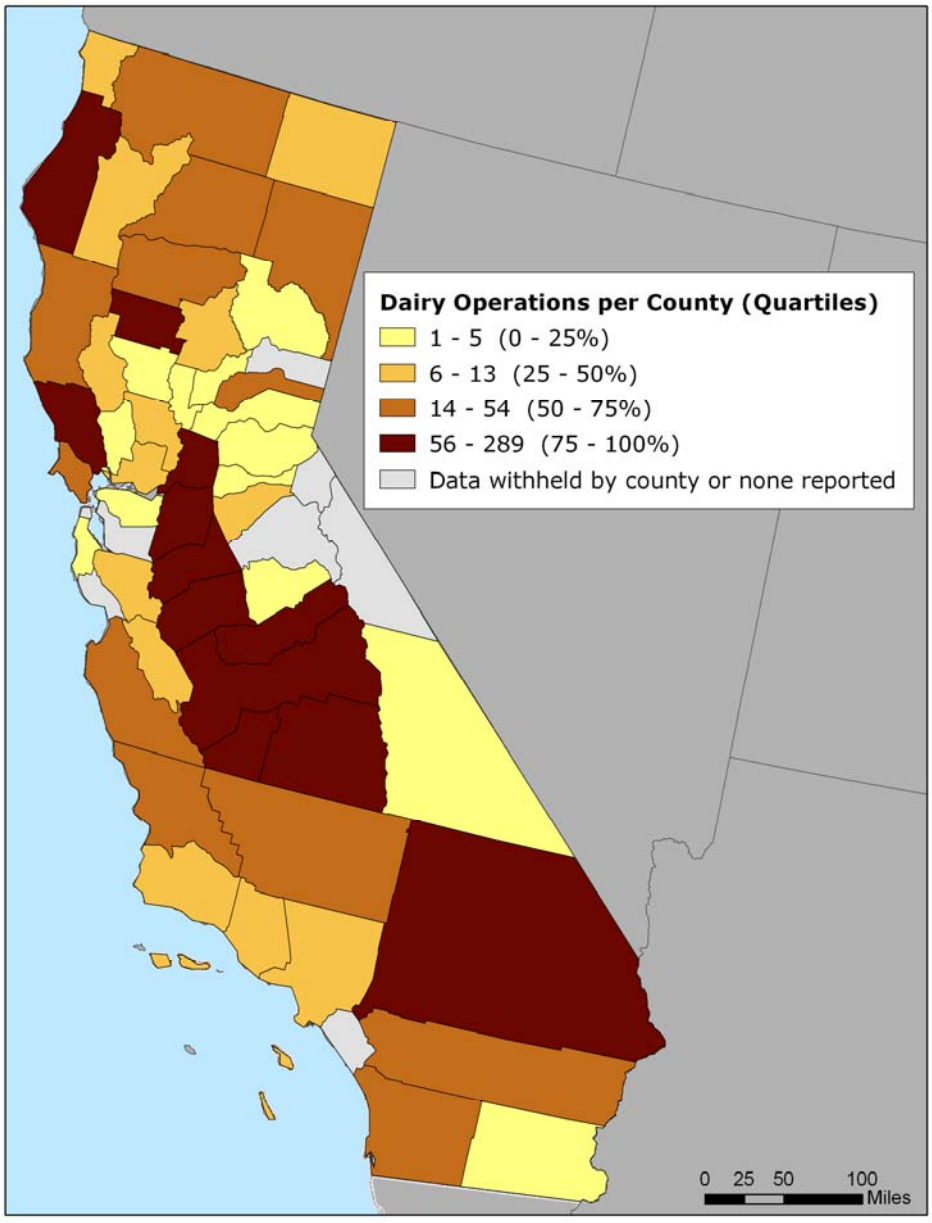
Table 2.2 California dairy industry, 2007

Population	Number	U.S. Rank	% of U.S.	U.S. Total
Dairy operations	2,200	10	3.3	67,000
Dairy cows (millions)	1.844	1	19.8	9.315
Average herd size	968	4		163
Average milk production/cow (lbs.)	22,334	6		20,396
Total milk production (million lbs.)	41,203	1	21.7	189,992

Source: NASS 2007 Census of Agriculture

In 2007, California had 2,165 dairy operations. At the county level, using 2007 Census of Agriculture Data, three California counties (Merced, Tulare, and Stanislaus) had more than 265 dairy operations each. Kings and San Joaquin counties had between 200 and 250 dairy operations. These counties make up the majority of California's Central Valley (Figure 2.2).

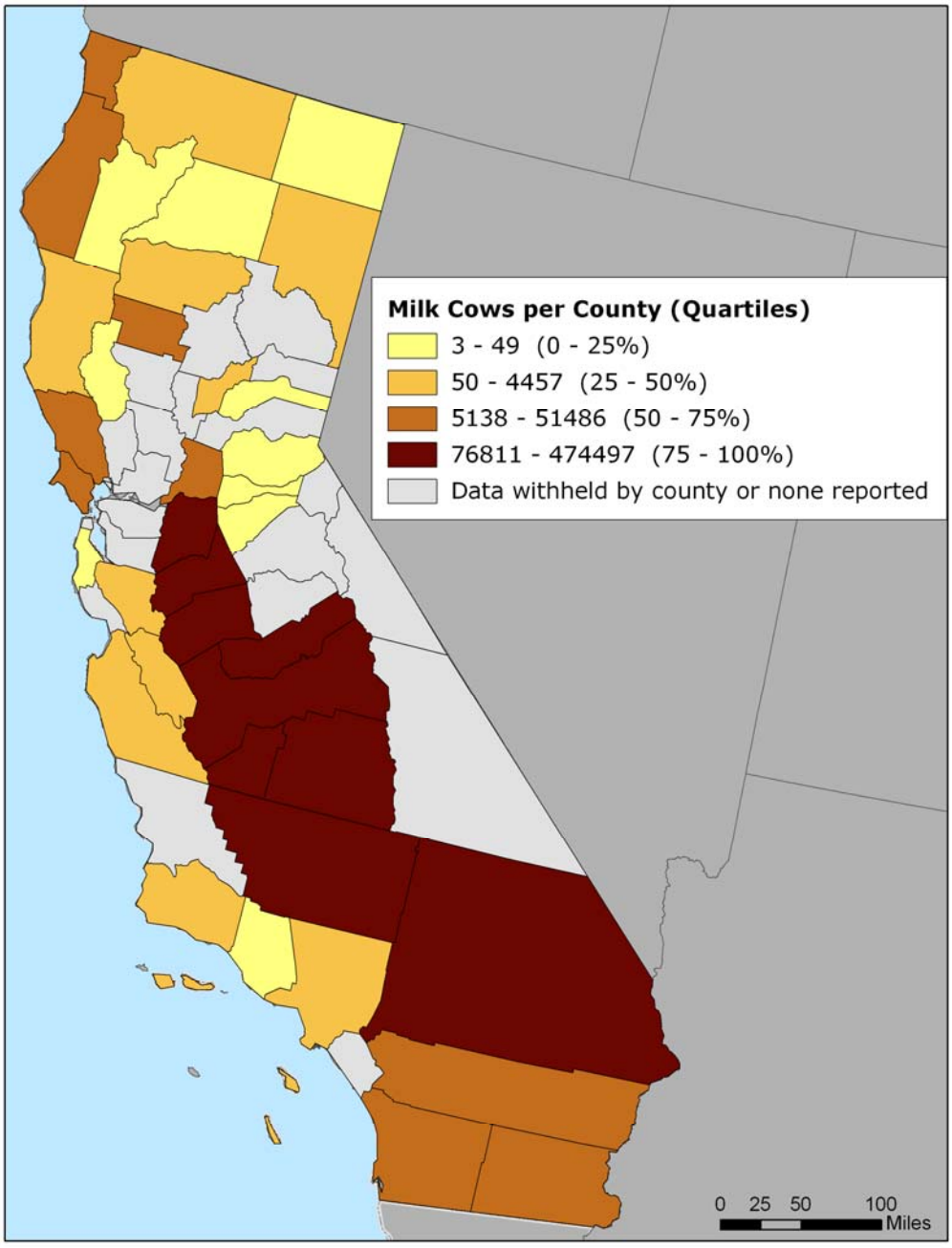
Figure 2.2 Number of California dairy operations per county, 2007



Source: NASS 2007 Census of Agriculture

California had the largest number of dairy cows of any State in 2007 with 1.813 million head. Tulare County had the largest number of cows with approximately 474,500 head, followed by Merced County with approximately 273,000 head (Figure 2.3).

Figure 2.3 Number of milk cows in California per county, 2007

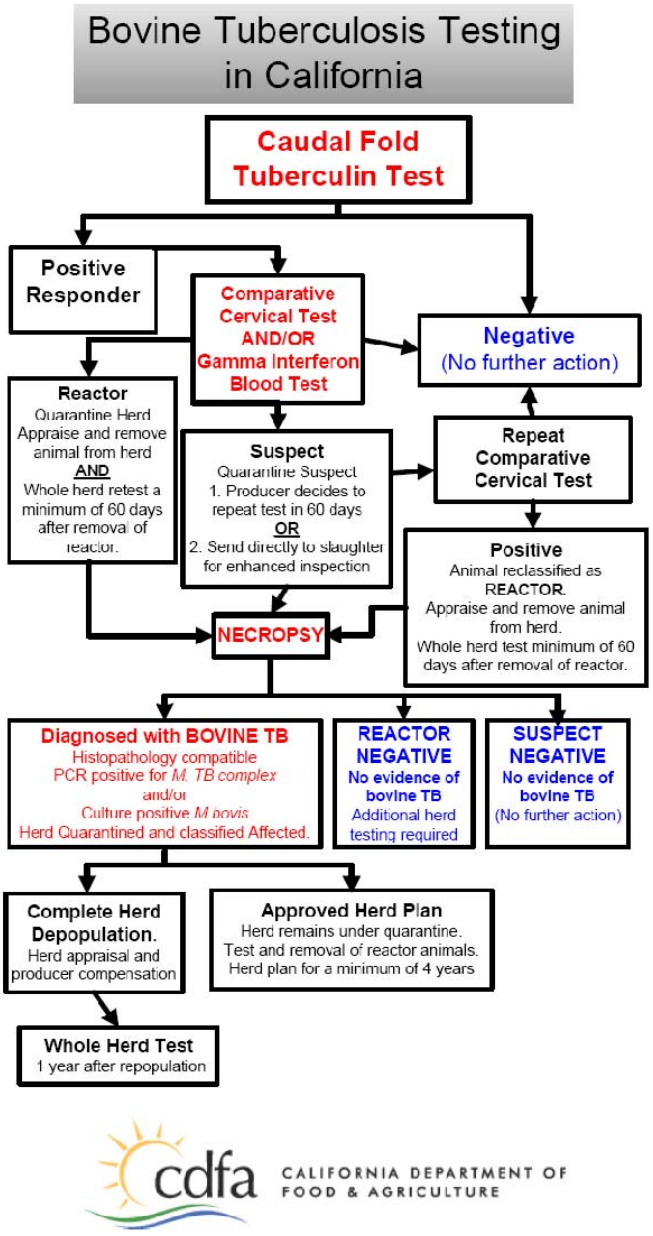


Source: NASS 2007 Census of Agriculture

California testing protocols: A flow diagram outlining California testing procedures is provided in Figure 2.4. Cattle that may have been exposed to infected cattle are purchased for necropsy or tested using the CFT. Further testing is not required if the animal does not respond to the tuberculin injection. Alternatively, if a response or swelling is observed, either a CCT or γ-IFN blood test is used to determine if the animal will be euthanized and samples taken at necropsy or slaughter. Cattle that originate from or reside in affected herds and respond to a CFT are taken to necropsy or slaughter. If a herd is determined to have bovine TB, quarantine is immediately placed on the herd. Owners of affected herds can choose to depopulate all animals or agree to a herd management plan that details

required testing and restrictions on moving animals from the herd. This management plan remains in effect until the herd is released from quarantine. Traditionally, depopulation has been the preferred control method because it is the only method that ensures elimination of the disease. However, as dairy herds become larger, depopulation may become too costly.

Figure 2.4 Bovine tuberculosis testing in California



2.2.2 Description of the outbreak

Index case: A 5-year-old Jersey cow slaughtered on December 6, 2007, had lymph nodes sampled due to suspicious lesions found during routine slaughter surveillance. The cow was confirmed infected based on positive results from histopathology, PCR and culture. The herd from which the infected cow originated (Herd A) was immediately quarantined.

Specific information from the index case and the additional seven infected cows found is shown in Table 2.3 and Figure 2.5.

Table 2.3 *M. bovis* infected dairy cattle in California outbreak, 2007–2009

Herd	Sex	Breed	Age (yr)	Date of Birth	Date of Detection	Origin
A[1]	Fe	Jersey	5	3/2002	12/2007	Purchased 6/25/07 from Herd B via auction
B[1]	Fe	Jersey	2	4/2005	1/2008	Home-raised
B[1]	Fe	Jersey	3	1/2005	1/2008	Home-raised
B[1]	Fe	Jersey	3	2/2005	1/2008	Home-raised
B[1]	Fe	Jersey/ Holstein	2	4/2006	1/2008	Home-raised
C[1]	Fe	Jersey	3	1/2005	4/2008	Purchased 8/20/07 from Herd B via auction
D[2]	Fe	Holstein	8	1/2000	4/2008	Home-raised
E[3]	Fe	Holstein	4	12/2004	1/2009	Home-raised

[1, 2, 3] Denotes different *M. bovis* strains identified through spoligotyping

Figure 2.5 California outbreaks by county (USDA-APHIS-VS, May 2009)

Herd A: The dairy operation in Madera County milked approximately 1,800 Holstein and Jersey cows. Calves were raised offsite and usually returned at approximately 120 days-of-age. Calves were fed powdered colostrum before leaving for the calf ranch and were identified with bangle tags before leaving the dairy. Calves are normally segregated at the calf ranch, but it cannot be determined if segregation was occurring prior to finding the infected cow.

The index cow was a Jersey born in March 2002 (5-years-old). Dairy A purchased the cow approximately 6 months before detection from Dairy B at a local auction yard. Dairy A had purchased 85 cows from Dairy B between June 2005 and June 2007. Three of these cows were re-sold to two local dairies. Dairy A was quarantined in December 2007. Testing at Dairy A revealed no other

infected cows. Furthermore, cows originating from Dairy B and present on Dairy A were purchased and necropsied with no additional infection found. The CFT responder percentage was 2.5 percent (62/2450). Of the 29 cows that were CFT responders, 17.2 percent were γ-IFN positive. Quarantine was removed on April 15, 2008.

Herd A became infected by purchasing additions, specifically cull dairy cattle. Other potential risks of TB introduction include commingling calves at the calf ranch. No previous TB herd testing was reported; however, because no other infected animals were found in the herd, it was unlikely that bovine TB had been present previously.

Herd B: Herd B dairy was located in Fresno County and milked approximately 2,600 Holstein and Jersey cows. Heifers were home-raised until June 2006, and then sent to a feedlot in Nevada. At the time that the infected cows were sold to the other affected herds, no heifers had returned from the feedlot in Nevada. As a result, no conclusive link can be drawn between the feedlot and the introduction of infection at the dairy. Dairy B had a reputation of selling relatively high-producing cows, and other dairies (such as Dairy A and Dairy C) frequently bought Dairy B cattle at local auction markets. Based on the fact that the infected cow in Herd A originated from this herd, a quarantine was placed on Dairy B in December 2007.

Herd testing in December 2007 detected 63 CFT responders (2.4 percent). Of these, 17 were gamma responders (27.0 percent). All 63 cows were necropsied and 4 cows were confirmed infected by culture, histopathology, or PCR. The four infected cows were CFT responders and γ-IFN positive within 1 month before being euthanized and necropsied. Additional whole-herd testing was performed in March 2008 and between December 2007 and July 2008. Approximately 170 cows were necropsied with no other infected cows found. There were 110 CFT responder cows identified during the 3 herd tests, representing 1.6 percent of cows tested.

Dairy B sold approximately 3,200 cows to 208 operations from 2003 to 2007 and had purchased approximately 113 cattle from 60 operations. Herd B chose to depopulate, and in July 2008, approximately 5,000 cows and calves were sent to slaughter. At slaughter, no suspicious lesions were identified. The source of infection in this herd was never identified but the purchase of cattle, or contact with cattle from other operations, appear to be the most likely sources of introduction.

Herd C: Herd C was a 500-cow dairy located in Fresno County. This herd was composed primarily of Jerseys and Jersey-crosses. Calves were fed pooled, unpasteurized colostrum and then sent offsite, returning at 120 days-of-age. Herd C bought replacement heifers and cows from sale yards and auctions and bought bulls directly from other dairy operations. Most cull cows were shipped directly to slaughter.

The herd was tested because of trace-ins from Herd B, which included 16 animals purchased in August 2007. In March 2008, a complete herd test was conducted and 2.9 percent of the herd were CFT responders. No infected animals were identified. Herd B cows were removed from the herd and necropsied. In April 2008, a cow originating from Herd B that was CFT negative in March was confirmed as infected with *M. bovis*. The herd was quarantined in April 2008 and 1,014 cattle were depopulated in August 2008. No additional infected animals were detected during enhanced slaughter inspection.

Herd C herd became infected by purchasing additions, specifically cull dairy cattle. The spoligotype pattern identified (n=27) from isolates from Herds A-C is similar to a strain identified in a New Mexico herd in 2005 and in numerous Mexican-origin cattle. In addition to California and New Mexico, this isolate has been found in six other States. The strain was also found in 11 cattle and 1 deer in Texas. Three California human isolates also matched this strain. However, the strain was not the same as the strain responsible for the 2002–2003 California TB outbreak. Caution should be used when interpreting similarities in spoligotyping patterns. Many of the isolates identified in California match strains identified in Mexican-origin feeder cattle from neighboring States, which indicates a possible linkage. However, a direct link cannot be made in the absence of epidemiologic information.

Additionally, some strains may not be exact matches when evaluated further using other genotyping methods.

Herd D: Also located in Fresno County, the owner of Herd D is a well-known breeder, embryo supplier, exporter of registered cattle, and seller of breeding bulls throughout the United States and embryos throughout the world. Accredited TB-free until 2001, this herd is connected through frequent animal movements with additional operations managed by family members. It was identified based on a trace-in of a Holstein heifer sold to Herd B in 2003 through a county fair. Herd D reportedly purchased 14 head of cattle in the past few years but has sold thousands of bulls. Most culls (90 percent) were sent directly to slaughter, while the remaining 10 percent were sold through sale yards. Calves are raised at another family member's dairy and fed unpasteurized hospital milk.

A whole-herd test was performed in April 2008 and 2.0 percent of the cattle responded to the CFT. Subsequent testing of CFT responders by CCT identified one reactor and two suspects. The three cows were sent for necropsies and *M. bovis* was recovered from the caudal mediastinal lymph node in the reactor cow. This cow and her dam, granddam, and great-granddam were all born to Herd D. Additional testing identified no other infected cattle. Herd D implemented an APHIS-approved TB management plan.

Spoligotype results in this herd indicated a different strain than that found in Herds A, B, and C. This particular strain was identified in 40 cattle in Mexico, cattle in 6 U.S. States, 1 elk in Oklahoma, and 3 humans in New Jersey. The source of infection in this herd has not been identified. Potential sources include cattle contact through family member operations that had contact with cattle from other operations. Employees are also a potential source of introduction. According to the herd's owner, the wife of a dairy employee who worked in the calving area around the time the infected cow was born died from TB.

Herd E: This herd is primarily Holstein cattle located in San Bernardino County with approximately 1,500 lactating cows. The herd is also associated with two other dairy operations through a calf-raising facility, and animals are transferred among these three operations. Herd E was tested because the owner purchased two 4–H heifers from Herd D in 2005. A whole-herd CFT was conducted in December 2008. Of the 31 cows (2.1 percent) that responded to the CFT, only one cow was γ-IFN positive. The animal was sent to necropsy in January 2009 and confirmed as *M. bovis*-infected based on histopathology, culture, and PCR.

Herd E was quarantined in October 2008 based on the identification of a trace-out from Herd D. Testing of this herd is still being conducted. Spoligotyping of the isolate did not match the California strain found in 2002 or either strain in the 2007–2009 TB outbreak. The strain did match an untraceable California slaughterhouse surveillance isolate discovered in 2004. A human isolate found in San Diego (year unknown) also matches the strain. The source of infection in this herd has not been identified. Potential sources include contact with cattle from family members' operations that had contact with cattle from other operations and calves raised offsite.

Additional herd testing: Herds were identified for testing based on exposure to one or more of the affected herds detailed above. As of July 30, 2009, 251 herds had been tested at least once in association with the outbreak. CFT testing has been performed on 418,619 cattle; 10,994 of which responded to the CFT. Testing by γ-IFN was conducted on samples from 10,840 cattle— 224 of which tested positive.

Table 2.4 Testing performed in California, FY 2007 through July 2009 (251 initial herd tests and 53 retests)

Test	Positive[1]	Negative	Tested
CFT	10,994	407,625	418,619
CCT	50	1232	1282
γ-IFN[2]	224	10,559	10,840

[1] Includes suspects/reactors.
[2] Includes 57 tests with no results.

2.2.3 Summary

The outbreak in California highlights at least three major concerns for TB in the State's dairy industry. First, two herds were directly infected from purchasing cattle from other operations. While not confirmed, the other three herds may have also been infected from purchased cattle. Second, young dairy calves may be exposed to Mexican -origin cattle at feeding facilities. This practice greatly increases the risk of spreading diseases such as TB. Third, the likelihood that employees are spreading the disease to cattle has not been confirmed in any of these herds. This possibility has not been fully investigated primarily because of the time lag between exposure and disease identification, and because human testing is not under the purview of USDA or the California Department of Food and Agriculture.

Three different strains of TB were identified during the California outbreak, suggesting that multiple introductions of TB have occurred without any specific point source identified. Although all of the *M. bovis* strains detected in this event have been detected previously in the United States, it is not known if the disease has been residing in native cattle between detection or was reintroduced. To prevent future outbreaks, sources of TB in dairy cattle in California need to be clarified and eliminated.

2.3 Tuberculosis in Michigan cattle herds, 1998–2009

2.3.1 Background

Michigan TB program: Michigan achieved TB-free status in 1979. The last occurrence of TB in cattle in Michigan prior to that status occurred in 1974. In November 1975, a hunter killed a 9.5-year-old female wild deer in Alcona County in the northeast corner of Michigan's Lower Peninsula. The deer had gross lesions suggestive of TB and *M. bovis* was cultured (Schmitt et al., 1997). Livestock was not kept near the area where the deer was killed. This occurrence of *M. bovis* in wild deer was thought to be a result of spillover from cattle. At the time, very few occurrences of *M. bovis* in wild deer had occurred and it was thought that the disease could not be maintained in that population. As a result, the 1975 case was treated as an isolated incident and no follow-up survey of wildlife in the area was conducted.

In 1993, a TB-positive cow identified at slaughter was traced to an Isabella County herd in which no further infection was found, thereby allowing Michigan to retain its AF status. Further investigation was not warranted at that time and genetic profiling results for this isolate are unavailable.

In November 1994, a wild 4.5-year-old male deer with gross lesions suggestive of TB was killed during hunting season and *M. bovis* was cultured from this deer (Schmitt et al., 1997). The deer was killed in Alpena County; approximately 9 miles from the 1975 case. It was thought that the infected deer killed in 1994 might be associated with TB in livestock, and in spring 1995 all cattle within a 9-mile radius of the kill site were tested. TB was not found. In fall 1995, the Michigan Department of Natural Resources requested hunter participation in a survey to determine if *M. bovis* existed in wild

deer within the 9-mile radius. Of the 354 deer examined, 15 had gross lesions and were culture-positive for *M. bovis.* This prompted increased testing in wild deer in 1996, and regular testing of deer has continued with infected wild deer being found every year.

The area of highest risk included 15 to 20 townships on the shared boundary of Alpena, Alcona, Oscoda, and Montmorency Counties in the northeast Lower Peninsula. This core area is known as Deer Management Unit (DMU) 452. In fall 1997, whole-herd testing of dairy and beef herds within the core area was initiated. In spring 1998, the first TB-positive cattle herd in the area was identified. Additional cattle herds were later identified and Michigan officially lost its AF- status in June 2000. As of 2009, Michigan is divided into three zones (Figure 2.6) with different TB statuses: the AF zone consists of the Upper Peninsula; the MA zone is composed of all or parts of the northernmost 13 counties in the Lower Peninsula; and the MAA zone consists of the remainder of the counties in the Lower Peninsula. As of March 2009, 45 affected dairy and beef herds have been identified since 1998.

The origin of the *M. bovis* outbreak beginning in 1994 will probably never be known. It is generally accepted that the deer found in 1975 was probably not an isolated incident and *M. bovis* was likely present at low levels in wild deer in northeastern Michigan at that time. Prevalence gradually increased due to factors such as increased deer feeding practices that resulted in identification of the 1994 positive deer.

DMU 452 consists largely of forests, swamps, and rocky, sandy, unproductive soils. Because much of the land is good deer habitat, ill-suited for agriculture, and relatively inexpensive, it became an attractive area to establish privately owned hunt clubs. The first hunt club was established in the heart of DMU 452 in the 1880s when public hunting was unrestricted (O'Brien et al., 2006). The club provided protection for deer and deer numbers within the club increased. Other clubs were established, and by 1938 there were approximately 250 clubs in the area (O'Brien et al., 2006). Some clubs grazed cattle on their land from the 1940s to the 1960s and some could afford caretakers and supplemental feed for deer during harsh winters. Widespread feeding did not become commonplace until the 1980s and 1990s, when competition grew among clubs to attract deer to facilitate hunting for club members (O'Brien et al., 2006). During this time, large amounts of feed were transported to the area and the sale of deer feed became a significant part of the local economy (Hickling, 2002). Winter feeding became common, as clubs felt compelled to provide feed to prevent deer from moving to neighboring clubs' feed piles. The normal deer concentration that the habitat of DMU-452 could support was estimated at 31 to 37 deer/sq. mi. (Schmitt et al., 1997). It was estimated that the deer population peaked at 50 to 60 deer/sq. mi. from 1989 to 1992 (Hickling, 2002).

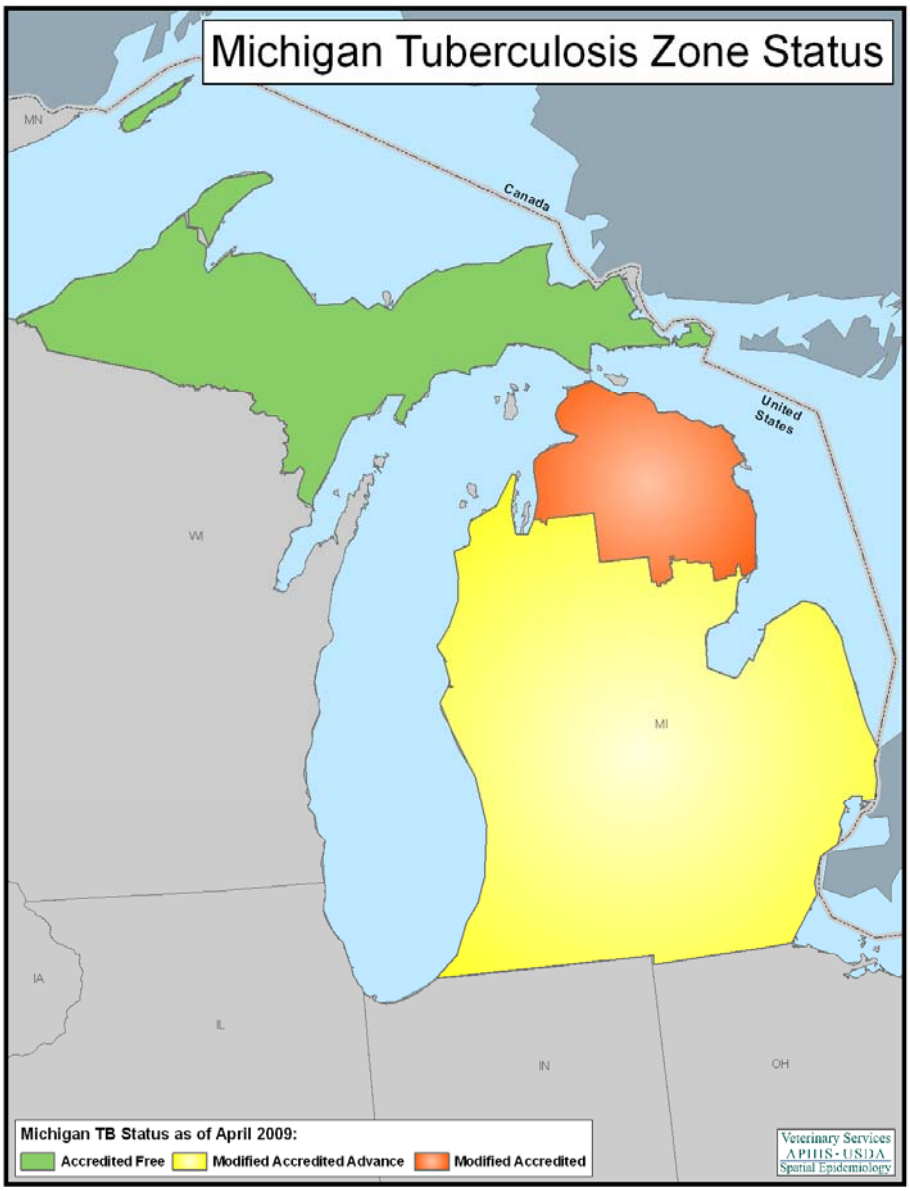
Disease transmission modeling based on annual prevalence results in the TB core area subsequent to 1994 suggests that *M. bovis* arose from cattle-to-deer interaction in the mid 1950s (McCarty and Miller, 1998). These authors reasoned that a more recent introduction within 5 to 10 years prior to 1994 would have required relatively high transmission rates to achieve the prevalence seen in deer in 1995. Given those transmission rates, TB prevalence in deer should have been doubling subsequent to 1995, which was not observed (McCarty and Miller, 1998).

Another study based on extensive historical research of deer numbers, climate, forage availability, the establishment of hunt clubs, supplemental deer feeding, and TB occurrence in cattle in Michigan estimated that *M. bovis* in deer in the core area most likely arose in the 1930s (Miller and Kaneene, 2006). In the 1920s, the highest TB reactor rates in the State were in Alcona and Alpena Counties, the heart of DMU 452 (O'Brien et al., 2006). Prevalence of TB in cattle decreased in the 1930s and 1940s and increased in the 1950s. At one point, Michigan accounted for 30 percent of all TB reactors in the United States (Miller and Kaneene, 2006).

While the 1950s or 1930s are both plausible time periods for the origin of infection in Michigan deer, the exact origin will probably never be known. However, it is commonly accepted that the infection was maintained in deer in the northeastern Lower Peninsula subsequent to the 1975 positive hunter-

killed deer; therefore, the initial introduction of TB into the deer population was most likely prior to 1975.

Figure 2.6 Michigan TB zones as of April 2009



Michigan cattle industry: Michigan was ranked sixth in the United States in number of operations with dairy cows and eighth in number of dairy cows, according to the National Agricultural Statistics Service (NASS) 2007 Census of Agriculture (Table 2.5). The State was ranked 33rd for the number of operations with beef cows and 38th for the number of beef cows. Less than 10 percent of cattle and calves in Michigan are located in the MA zone. The vast majority of cattle and calves in Michigan are in the MAA zone.

While the cattle industry in Michigan is significant and the State is ranked among the top 10 dairy states in the United States, the hunting industry is also very important. Deer are highly valued in

Michigan and are designated as the State game mammal. A 2001 estimate of the value of deer hunting to Michigan's economy was more than $506 million (U.S. Department of the Interior, 2001). In comparison, the value of cattle and calves sales in Michigan was $299 million in 2002. (USDA, 2004).

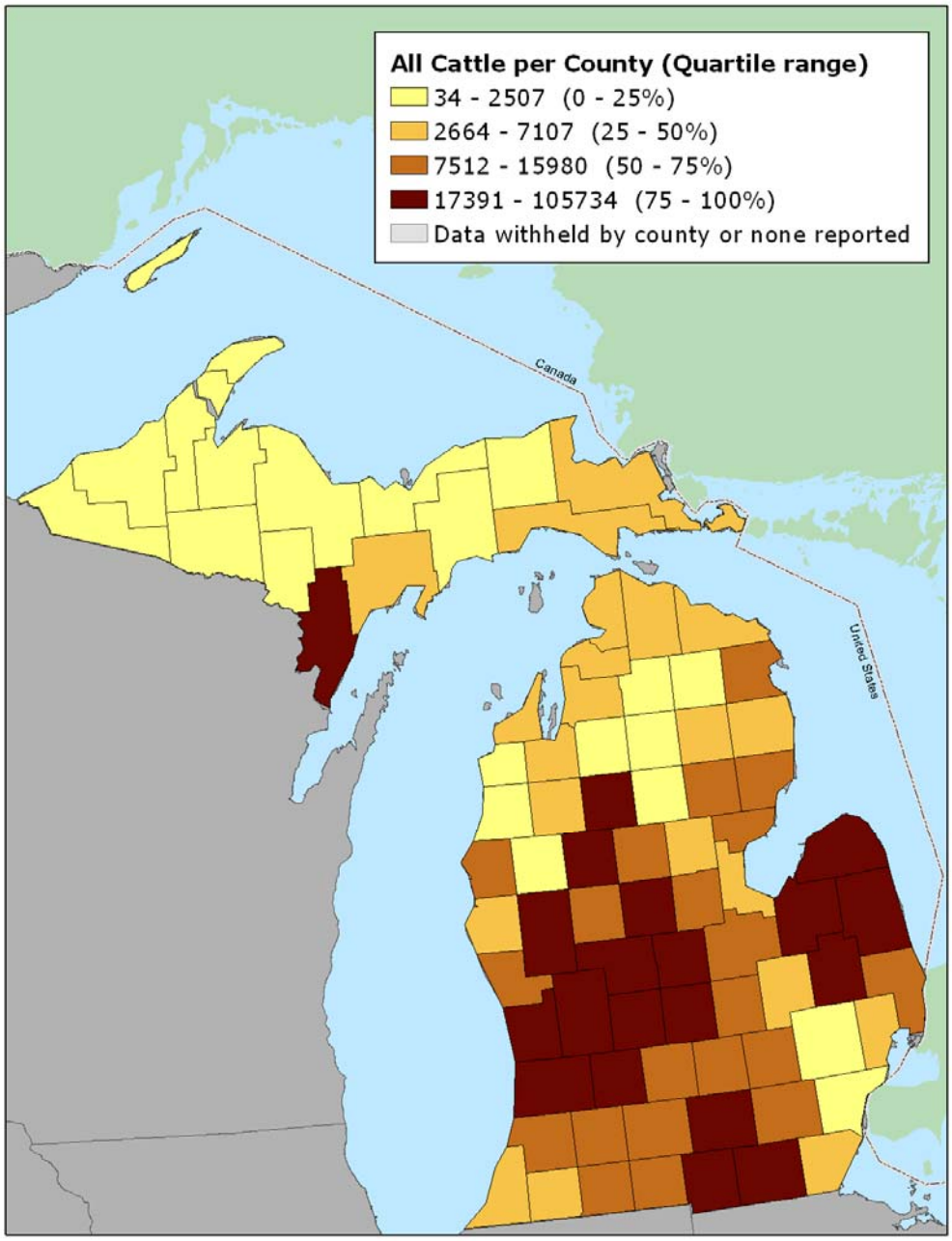
Table 2.5 Michigan TB zones by cattle population, 2007

	TB Zone							
Population	MA Zone[1]	AF Zone	MAA Zone[2]	MI Total	U.S. Total	MI Rank in U.S.	Four-county Area[3]	MA Zone Percent of MI Total
No. farms w/ cattle/ calves	1,191	761	12,502	14,454	963,669	26	419	8.2
No. cattle/calves	69,455	48,961	929,790	1,048,206	96,347,858	30	22,179	6.6
No. farms w/ cows/heifers that calved	922	624	8,593	10,139	818,992	31	323	9.1
No. cows/ heifers that calved	31,746	23,978	397,980	453,733	42,101,375	32	11,099	7.0
No. farms w/ beef cows	762	506	6,580	7,848	764,984	33	240	9.7
No. beef cows	12,745	9,288	83,389	109,500	32,834,801	38	4,334	11.6
No. farms w/ milk cows	188	150	2,309	2,647	69,890	6	93	7.1
No. milk cows	18,442	12,400	308,564	344,233	9,266,574	8	6,765	5.4

[1]Includes all of Iosco and Ogemaw Counties, including those parts inside the MAA zone
[2]Not including those parts of Iosco and Ogemaw Counties inside the MAA zone
[3]Includes all of Alpena, Alcona, Montmorency, and Oscoda Counties. DMU-452 is located in these counties.
Source: NASS 2007 Census of Agriculture

Figure 2.7 presents a graphical depiction of the number of cattle and calves in Michigan by county according to the NASS 2007 Census of Agriculture.

Figure 2.7 Number of cattle and calves in Michigan by county, 2007



Source: NASS 2007 Census of Agriculture

2.3.2 Description of the outbreak

Affected herds in Michigan: Between 1998 and 2008, 45 affected cattle herds were identified on 40 premises in Michigan (Figure 2.8). The first herd was identified in June 1998 and the most recent was diagnosed in December 2008. At least one new TB-positive herd has been diagnosed every year since 1998 (Table 2.6) with all but five identified as a result of testing in the affected areas. Of the 45 affected herds, 68.9 percent were in Alpena and Alcona Counties (Table 2.7). Only two counties (Antrim and Emmet) are outside of the high-risk area bounded by Interstate 75 and Route 55 in the northeastern Upper Peninsula. All counties in which affected cattle herds have been found are in the MA zone in Michigan and infected wild white-tailed deer have been found in all of the counties in

which affected cattle herds were found (Figure 2.9). None of the affected herds have been larger than 500 animals (Table 2.8).

Table 2.6 Number of TB affected cattle herds in Michigan, by year

1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	Total
3	1	7	8	7	6	1	3	4	3	2	45

Table 2.7 Number of TB affected cattle herds in Michigan, by county and by herd type

	County							
Herd Type	Alpena	Alcona	Montmorency	Antrim	Oscoda	Emmet	Presque Isle	Total
Beef	16	11	1	2	3	1	1	35
Dairy	3	1	3	1	0	1	1	10
Total	19 (42.2%)	12 (26.7%)	4 (8.9%)	3 (6.7%)	3 (6.7%)	2 (4.4%)	2 (4.4%)	45 (100.0%)

Table 2.8 Number of TB affected cattle herds in Michigan, by herd size and by herd type

	Herd Size			
Herd Type	Fewer than 50	50–99	100–500	Total
Beef	15	12	8	35
Dairy	2	2	6	10
Total	17	14	14	45

A dairy herd owner who elected not to depopulate after being declared affected in September 2000 found additional infected animals in July 2004, after the herd had been considered TB-free. This herd was infected again in March 2009; however, it was not considered a newly infected herd because it had not been released from quarantine. Deer is the likely source for re-infection of this herd.

Four depopulated beef premises were later found to be TB-positive after the herds were repopulated. One of the beef premises (herd 17) depopulated in September 2001 was diagnosed positive in April 2002, less than a year after repopulation (herd 22). All but two animals in the repopulated herd originated outside of Michigan, with most of the cattle coming from Montana. No additional TB-positive farms were found to be associated with this herd. Re-infection of this premises was attributed to deer as it is located in DMU-452.

Herd 15 was in the heart of the TB core area and had the highest within-herd prevalence. Cattle in this herd were wild and spent most of their time away from barns and people. Part of the herd was tested in 1995, but several animals escaped and testing was not completed. In 2001, this operation was successfully whole-herd tested and 32 percent of the animals tested culture-positive. Herd 15 was probably the source of infection for herd 20 (the one infected animal in herd 20, a bull, came from herd 15) and for herd 12 (all three infected cattle in herd 12 came from herd 15). However, since

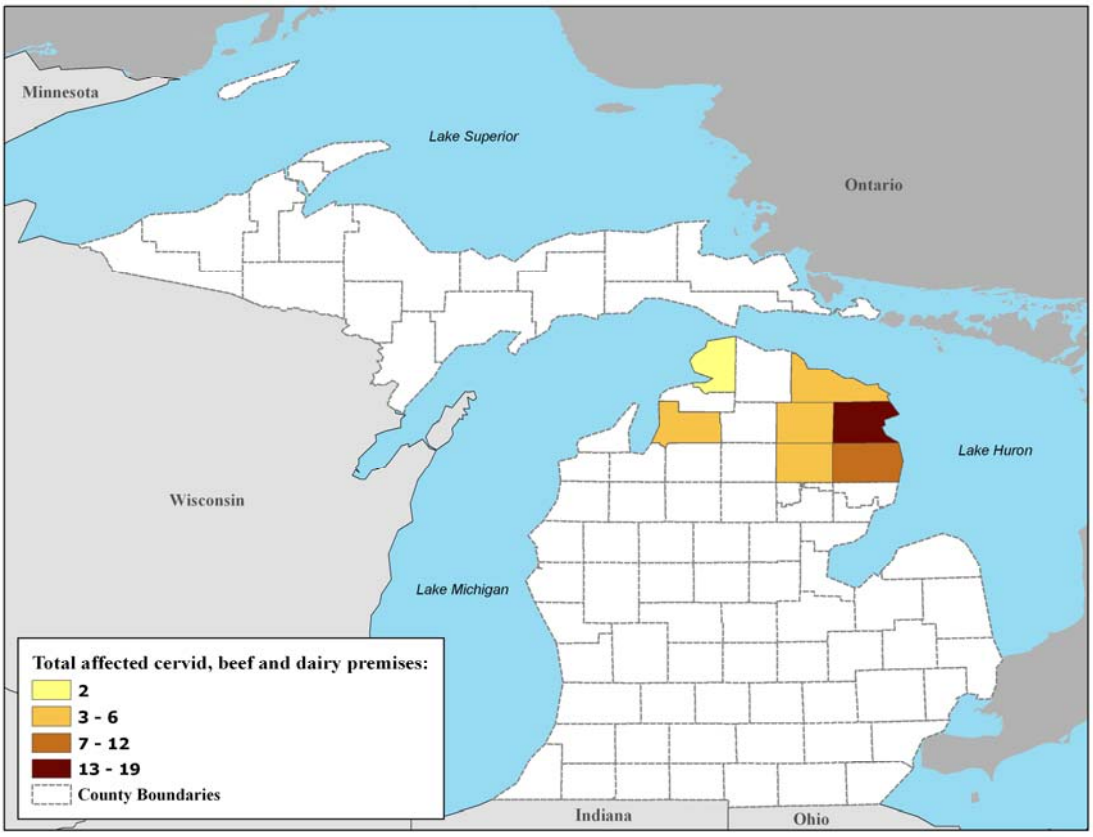
herds 20 and 12 were located in an area in which TB was endemic in deer, the possibility of infection by deer could not be ruled out.

Forty of the 45 affected herds in Michigan have been detected by area testing; movement and slaughter traces have identified very few of the infected herds. In 2000, four herds located within 2 miles of each other were found to be affected. These herds were located along the southern boundary of DMU 452, and in some cases shared fence lines. One of the 4 herds (herd 11) received an animal from one of the neighboring TB-affected herds. Deer were most likely the source of infection, as the area was heavily wooded. However, the possibility of infection due to neighboring cattle, fence-line contact, or cattle that escaped a neighboring herd could not be ruled out. The four herds were all identified as TB-affected within four months of one another (June to September 2000). Approximately 1 year later another neighboring herd (herd 18) was diagnosed TB-positive, likely due to deer. Another relatively tight cluster of positive herds was identified along Highway M 65 in Alpena County within DMU 452 with infection in these herds attributed to deer.

As a general rule, owners of infected beef herds in Michigan elect to depopulate. Beef cow-calf herds rely on calf sales to provide income and quarantines limit their ability to market their calves. Dairy herds depopulate less often, as they rely on milk sales for income and can continue to ship milk while under quarantine. Herds that do not depopulate and are released from quarantine are monitored because Michigan requires annual whole-herd testing for all herds in the MA zone.

An estimate of the period prevalence of affected herds in the MA zone can be obtained using the 2007 Census of Agriculture numbers from Table 2.5. This estimate assumes that the number of herds in each TB zone in Michigan has remained relatively constant since 1998. Given this assumption, the approximate percentage of affected dairy and beef herds in the four-county region of Alpena, Alcona, Montmorency, and Oscoda (all in the MA zone) since the start of the outbreak is 7.5 and 12.9 percent, respectively. The approximate percentage of affected dairy and beef herds in the entire MA zone is 5.3 and 4.6 percent, respectively.

Figure 2.8 Location of TB-affected cattle and cervid herds as of December 31, 2008

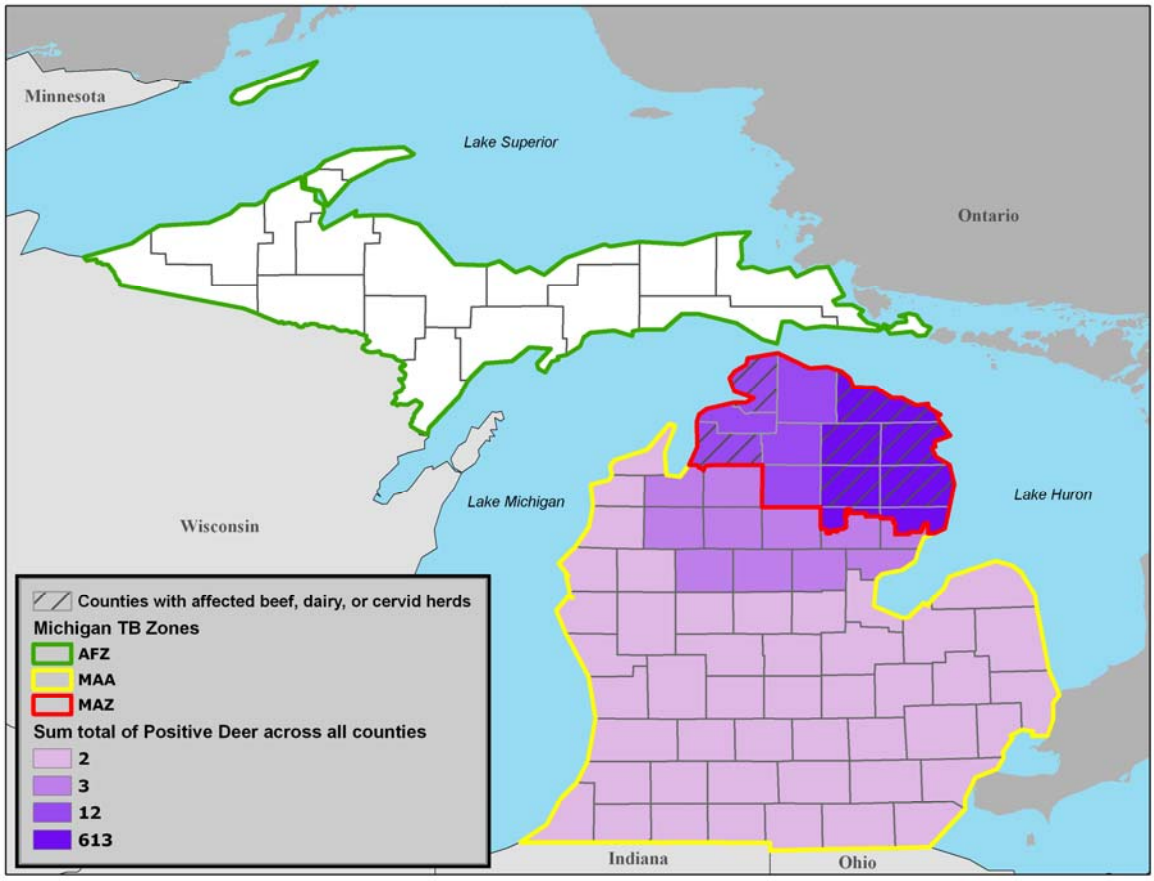


Risks associated with wildlife in Michigan: Deer are the primary reservoir of infection among wildlife species in Michigan. Cattle contact with deer—and especially contact with feed that deer have eaten from—is considered the most important risk factor for *M. bovis* infection. Land in Michigan's northeastern Lower Peninsula is of marginal quality for agricultural use. Pastures with access or close proximity to wooded areas are common, cattle are frequently fed in pastures, and feed is stored in pastures. On many operations, there is ample opportunity for deer to have contact with cattle feed.

Management practices that reduce the risk of introducing bovine TB to a herd include feeding cattle once per day (feeding less often is higher risk because feed may be exposed to wildlife), feeding cattle within buildings or within deer-proof fenced areas, storing feed in enclosed areas that prevent wildlife access, providing water only in troughs or tanks near buildings, and maintaining a minimum of 1 mile distance between cattle pens and wooded areas that provide good deer cover. The Wildlife Risk*A*Syst for Bovine TB (http://web2.msue.msu.edu/bulletins/Bulletin/PDF/FAS113.pdf) is a document designed for Michigan farmers to assess the risk of bovine TB infection on an operation with primary emphasis on deer and cattle contact.

Infected deer may also transmit infection to other deer. An experimental study by Palmer et al. (2001) housed experimentally infected deer (group 1) with uninfected deer (group 2) in the same pens in a Biosecurity Level 3 facility for 4 months. Subsequently, some group 2 deer were housed with a new set of uninfected deer (group 3) for 6 months. At necropsy, all deer from groups 1, 2, and 3 had tuberculous lesions and *M. bovis* was confirmed by culture. Infection may have occurred through inhalation or ingestion by nasal secretions, saliva, or contaminated feed—all of which are possible routes of infection when deer congregate to eat from the same feed piles.

Figure 2.9 Number of free-ranging deer infected with TB in Michigan, 1975–2008



In a similar study, experimentally infected deer and uninfected calves switched pens on a daily basis in a Biosecurity Level 3 facility, never coming in direct contact with each other (Palmer et al., 2004). The infected deer were given excess feed to ensure that leftover feed was available for the calves when pens were switched. In the second phase of the study, excess feed from experimentally infected deer pens was transferred to uninfected calf pens, with no movement of animals between pens. In the first phase of the study, in which calves and deer switched pens, all nine calves had tuberculous lesions or *M. bovis* cultured from tissues. In the second phase of the study, which involved only feed transfer, four of the nine calves had tuberculous lesions or *M. bovis* cultured from tissues. This study showed that M. bovis could be transmitted from deer to cattle through indirect contact.

An observational case-control study examining environmental and farm management factors associated with TB on northeastern Michigan cattle farms indicated that farms with poor deer exclusion practices were more likely to be TB-infected (Kaneene et al., 2002). The study also indicated that a larger amount of open land surrounding the farm was associated with decreased risk of bovine TB infection.

Elk are highly susceptible to bovine TB infection and are the primary reservoir involved in a bovine TB outbreak in Manitoba, Canada. Approximately 1,000 elk are present in the northern Lower Peninsula of Michigan. Elk naturally congregate in large groups (as opposed to white-tailed deer who congregate only in certain situations, such as supplemental feeding grounds) causing elk to be a species of concern with regard to TB. Although infected elk have been found in Michigan, their

numbers are small enough that they are not considered of great importance in sustaining the outbreak in Michigan.

The role that other wildlife species play in cattle infection is uncertain. *M. bovis* has been isolated from wildlife species in Michigan other than deer and elk, including black bear, coyotes, foxes, raccoons, opossums, bobcats, and badgers. All of these animals have been found in the vicinity of endemically infected deer. Infection in these species is thought to be a result of feeding on infected deer carcasses or from feed piles in which infected deer had contact. Gross and histologic lesions have been rare among these species (O'Brien et al., 2006). The extent of TB lesions in an animal provides some indication of its ability to excrete the disease agent, and effective excretion is necessary to act a reservoir host (Bruning-Fann et al., 2001). Since very little disseminated infection has been found in Michigan animals other than ruminants, these other species are considered to be spillover hosts incapable of maintaining the infection. Experimental infection of mice and voles can result in extensive lesions, and voles shed TB significantly in feces. However, there is little understanding of the extent to which these rodents serve as a potential source of infection of other species (O'Brien et al., 2006).

Testing and control measures: Since the TB outbreak, testing requirements for Michigan cattle herds have been more stringent than the requirements set forth in the Tuberculosis Eradication Program's UM&R (USDA, 2005). Michigan required whole-herd testing of all cattle herds in the State by December 31, 2003 and this deadline was met. Testing of dairy herds was the first priority; therefore, all dairy herds in Michigan were tested by June 2001 to satisfy the requirements of the Federal Pasteurized Milk Ordinance. Michigan regulations also require annual whole-herd TB testing for all herds in high-risk areas. In 2001, Alcona, Alpena, Montmorency, and Presque Isle Counties were designated high-risk areas.

In March 2002, the State established three areas to assist with immediate control: an infected zone (Alcona, Alpena, Montmorency, and Presque Isle Counties); a surveillance zone (Cheboygan, Crawford, Iosco, Ogemaw, Oscoda, and Otsego Counties); and a disease-free zone (all other counties). Each zone had different testing and movement requirements. With the exception of terminal operations in which animals leave the operation only for slaughter, all cattle herds in the infected zone were required to undergo annual whole-herd testing and all herds in the surveillance zone were required to undergo whole-herd testing every 2 years. Michigan also required that all herds in the disease-free counties bordering the surveillance zone (Antrim, Arenac, Charlevoix, Emmet, Gladwin, Kalkaska, and Roscommon) complete two whole-herd tests by the end of 2003. Herds in the disease-free zone did not have annual testing requirements other than completing at least one whole-herd test prior to 2003.

In April 2004, USDA approved split-State status for Michigan. Initially, this status was comprised of two zones that were designated either MA or MAA. The MA zone included all of Alcona, Alpena, Antrim, Charlevoix, Cheboygan, Crawford, Emmet, Montmorency, Oscoda, Otsego, and Presque Isle counties, as well as portions of Losco and Ogemaw counties north of the southernmost boundaries of the Huron National Forest and the Au Sable State Forest. This zone retained the annual whole-herd testing and movement restrictions formerly required in the infected zone. The MAA zone included all Michigan counties outside the MA zone. In September 2005, the Upper Peninsula became a separate AF zone, making Michigan a three zone, three-status State. Surveillance testing in the MAA zone has been conducted through random selection of herds within the zone and by whole-herd testing on selected herds (1,800 herds were tested over a 2-year period). Random selection of herds for whole-herd testing has also been practiced in the Upper Peninsula; however, fewer herds were selected. In 2007, 775 herds were tested in the MAA zone compared with 25 herds in the Upper Peninsula. The State also required annual reconciliation of herd inventories by regulatory or accredited veterinarians for all cattle herds in the MA zone to account for all changes in inventory.

In 2008, Michigan began using risk-based surveillance for herds outside the MA zone. The goal during the first year was to test 500 herds and to accumulate 1,500 surveillance points. Herds were assigned different surveillance points based on risk. Animals tested as a result of traces from herds

infected within the past 10 years were assigned six points; herds within a 10-mile circle of infected deer found outside of the MA zone were assigned four surveillance points; herds in counties adjacent to the MA zone were assigned three points; and all other herds were assigned one point. Herds in the border counties and those with one surveillance point were selected randomly. Annual testing is still required for all herds in the MA zone under the risk-based system. In 2009, the risk-based system continued with a few modifications and refinements are proposed over the next few years.

Since 2000, Michigan has had a mandatory official identification program for cattle, whereby all cattle are required to bear official identification ear tags before being moved. Official electronic ear tags became mandatory for cattle in the infected zone in 2002 (later designated as the MA zone). In March 2007, official electronic identification ear tags became mandatory for all cattle in Michigan.

The Michigan Department of Agriculture began surveillance of livestock moving into the Upper Peninsula in 2004 (the Upper and Lower Peninsula are connected by the Mackinac Bridge). Detailed surveillance of all vehicles transporting livestock across the bridge is conducted to ensure that animal identification and movement certificate requirements are met. The check station at the bridge is staffed 24 hours a day, 7 days a week, and regulatory personnel are also present at all sale yards in the State to verify adherence to animal identification and movement requirements.

Plans for a fencing program began in spring 2001, when USDA's Wildlife Services provided funding to construct fencing on eligible farms to prevent deer access to stored feed. To be eligible for funding, farms were required to be located in the MA zone, undergo an evaluation by Wildlife Services, provide documentation of damage by deer to stored feed, lie within close proximity to a bovine TB case, and be willing to use the fence as designed (e.g., keep gates closed when not in use). Through 2006, 54 farms received fencing. The majority of fences built were woven wire, although a few were electrobraid or other types. The average cost per farm was approximately $10,000, with a total expenditure of $529,000. USDA funding for this program was discontinued after 2006.

In August 2008, the Michigan Department of Agriculture began a cost-share program that provided funds for producers in the core TB area to build fences or hoop barns to protect feed from deer. Approximately $384,000 was available to fund up to 75 percent of fencing costs and up to 65 percent of hoop barn costs. Maximum funding was set at $15,000 per producer. Before applying for funding through the program, producers were required to complete a wildlife risk assessment through Wildlife Risk*A*Syst. The assessment evaluated the risk of deer access to livestock and feed storage areas with results in a numerical risk score. Farm owners could apply for funding if their feed storage was considered at risk.

There have been many variations of baiting and feeding bans in areas of Michigan since 1998. Baiting is defined as providing feed materials for wildlife to aid in hunting, and feeding is providing feed for any reason other than baiting. Initially, voluntary restrictions were sought, but in 1999 a mandatory baiting and feeding ban was imposed in the five-county TB area. In 2001, baiting with small amounts of feed was allowed to increase the deer harvest (O'Brien et al., 2006). A complete baiting and feeding ban was imposed in 2002 in a seven-county area from which most of the TB-positive deer had been found. This ban remains in effect. In 2008, all baiting and feeding was banned in the entire Lower Peninsula following the discovery of chronic wasting disease (CWD) in a captive deer in Kent County.

The baiting and feeding bans have had mixed success. Large-scale feeding in the banned areas has been greatly reduced but smaller feeding sites remain (O'Brien et al., 2006). Sales of bait and feed are unregulated and have continued in all areas of Michigan. Enforcement efforts have included aerial surveillance, and although violators are fined, many view the fines as inconsequential. Repeat violators are subject to additional penalties (e.g., seizure of firearms) but these have not been consistently enforced.

Spoligotyping results: TB cases identified in Michigan have predominantly been associated with a single genetic strain of *M. bovis* identified by spoligotype 640033777777600. A few isolates identified

in Michigan have had minor variations of this spoligotype pattern. In many instances, laboratory artifact was responsible for the variation, and those isolates are considered the same strain as the predominant Michigan strain. Other slight differences are likely due to mutations in the genome. Although it is likely the TB infections in Michigan are due to a single strain of *M. bovis* circulating among deer, cattle, and other wildlife, spoligotyping data alone cannot conclusively prove that all isolates originated from the same outbreak strain.

The spoligotype pattern associated with Michigan has also been found in Mexico and is endemic in feral swine on Molokai Island, Hawaii. However, no proven epidemiologic links exist between the Michigan outbreak and Hawaii or Mexico. Additional genotyping tests demonstrate a distinct difference between the Michigan strain and the strain endemic in Hawaii. As spoligotyping can be a useful tool to demonstrate no relationship between herds in an outbreak (such as in California), the converse is not true (e.g., matching strains do not mean a link exists in the absence of epidemiologic evidence and additional genetic fingerprinting). Most available evidence points to the origin of the outbreak in deer in Michigan well before its discovery in 1994.

Human infection: Since 1994, *M. bovis* with the same spoligotype profile as the outbreak strain found in cattle and deer has been isolated from two Michigan residents (Wilkins et al., 2008). One case involved a 29-year-old man who shot a deer just outside of the DMU 452 in 2004. The man had cut one finger with a knife while field dressing the animal. He noticed that the deer had chest lesions which were likely TB and subsequently buried the carcass. A few weeks later, the man's finger became inflamed and he sought medical treatment. A TB skin test was administered with TB-negative results. The man's finger did not respond to antimicrobial treatment and his wound was incised and drained on two different occasions. Approximately 2 months after the injury, *M. bovis* was cultured from the wound. The deer carcass was later recovered and cultured positive for *M. bovis.* Spoligotyping results indicated that the deer and the man were infected with the same strain that matched the strain circulating in Michigan cattle and deer (Wilkens et al., 2008).

Another human case involved a 74-year-old man hospitalized in 2002 with persistent fever and a nonproductive cough. A skin test and sputum smear were both negative for TB. The man remained hospitalized and 5 days later acid-fast-bacteria were identified. The man died on day 16 of hospitalization while culture results were pending. The TB strain was later identified as identical to the strain circulating in cattle and deer in Michigan. The man had grown up on a farm drinking unpasteurized milk, but not in the current TB-endemic area of Michigan. He had moved to Detroit and upon retirement moved near DMU 452. He had previously hunted deer and ate venison, but not within 10 years of his death. However, the man fed deer and handled a deer carcass from DMU 452 in 2000. Pathology results from a lung lobectomy for another ailment in 1999 showed no evidence of TB and it was surmised that infection occurred subsequent to 1999 (Wilkins et al., 2008).

When infected cattle herds in Michigan are identified, the Michigan Department of Agriculture notifies the Michigan Department of Community Health, which in turn notifies the local health department in the vicinity of the affected farm. The local health departments contact owners of affected operations, provide information about TB, and offer TB skin tests. As of mid-2008, 30 people associated with affected operations had been tested—two of which had positive TB skin-test results (Ankney, 2009).

2.3.3 Summary

Michigan is the first State in which *M. bovis* was recognized as persistent in a wildlife reservoir. Bovine TB has proven very difficult to control in wild deer, and by the time this reservoir was confirmed, *M. bovis* was well-established in the wild deer population of the northeastern Lower Peninsula. Fifteen years have elapsed since *M. bovis* was discovered in Michigan deer, which coincides with the beginning of the current *M. bovis* endemic in the State. Cattle contact with infected deer or deer contact with cattle feedstuffs or water sources have been suggested as primary causes of many of Michigan's cattle herd infections. Implementing management practices that prevent deer access to stored cattle feed or areas where cattle are fed are the primary means of preventing TB infection in these cattle herds. The role species other than deer might play in the introduction and spread of bovine TB is not well understood. The primary means of controlling *M. bovis* in Michigan

deer have been population control and the ban on baiting and feeding in endemic areas. Population control alone is not a viable option for TB eradication in the State and it is unlikely that illegal feeding and baiting practices will cease. An effective vaccine for deer may assist in the control efforts.

Michigan has been proactive in developing testing, movement, and animal identification regulations. The State has requirements for electronic identification of cattle State-wide, annual whole-herd testing of all herds in the MA zone, and random testing of a subset of herds in the remainder of the State, including the AF zone. These State-imposed requirements are more stringent than the requirements imposed by the USDA (USDA, 2005). A risk assessment tool has also been developed to gauge the risk of introduction of *M. bovis* by wildlife onto operations. Adherence to recommended practices to prevent deer contact with cattle feed is recommended to prevent further infection; however, there are no accurate estimates as to when *M. bovis* might be eradicated from Michigan.

2.4 Tuberculosis in Minnesota cattle herds, 2005–2008

2.4.1 Background

Minnesota TB status: In July 2005, a Minnesota beef-cattle herd in Roseau County tested positive for *M. bovis* and was officially declared affected with bovine TB. Discovered through routine slaughter surveillance, this was the first infected herd identified in Minnesota since 1971. Subsequent testing revealed infection in several adjacent and epidemiologically-linked cattle herds as well as wild cervids (Table 2.9). As a result, APHIS downgraded the State status from AF to MA. By February 2008, 11 cattle herds in Minnesota were identified as TB-positive. In October 2008, Minnesota was granted split-State status, declaring a MA zone for portions of Beltrami, Roseau, and Marshall Counties. The remainder of the State was designated MAA.

Table 2.9 Number of TB infected cattle and deer in Minnesota as of July 2008, by species and year

	Number of *M. bovis* Isolates		
Year	Cattle	Deer	Total
2005	16	0	16
2006	9	6	15
2007	3	11	14
2008	7	14	21
Total	35	31	66

After the first affected herd was identified, State-wide surveillance was implemented to determine the extent of bovine TB. During this effort, 1,500 herds were tested. The affected herds were clustered in a small geographic area.

After split-State status was granted, Minnesota purchased and depopulated 46 herds (6,200 animals) in the highest risk areas to minimize further propagation of bovine TB. One additional herd (herd 12) was identified as affected through the buyout. Information on this herd was not available at the time of this assessment; however, additional herds have not been found as of August 2009. Owners who did not accept the terms of the buyout underwent a herd risk assessment (similar to Michigan's Wildlife Risk*A*Syst) to evaluate the potential for deer-to-cattle interactions on their operations. Based on the assessment, recommendations were made to minimize the potential for those interactions and

producers were obligated to follow the recommendations given by the State. Additionally, Minnesota provides funding to help owners construct fences to minimize deer contact with cattle or their feed.

Minnesota cattle industry: Minnesota ranks fourth in the United States in the number of dairy farms and cattle-on-feed operations (Table 2.10). The State has more than 2.25 million cattle and calves on 28,034 farms (Figure 2.10) that includes 881,842 adult breeding cows on 21,094 farms. There were approximately 7,000 cattle-on-feed operations in 2002 with an inventory of more than 500,000 cattle.

Table 2.10 Number of cattle and calves and number of farms in the United States and Minnesota

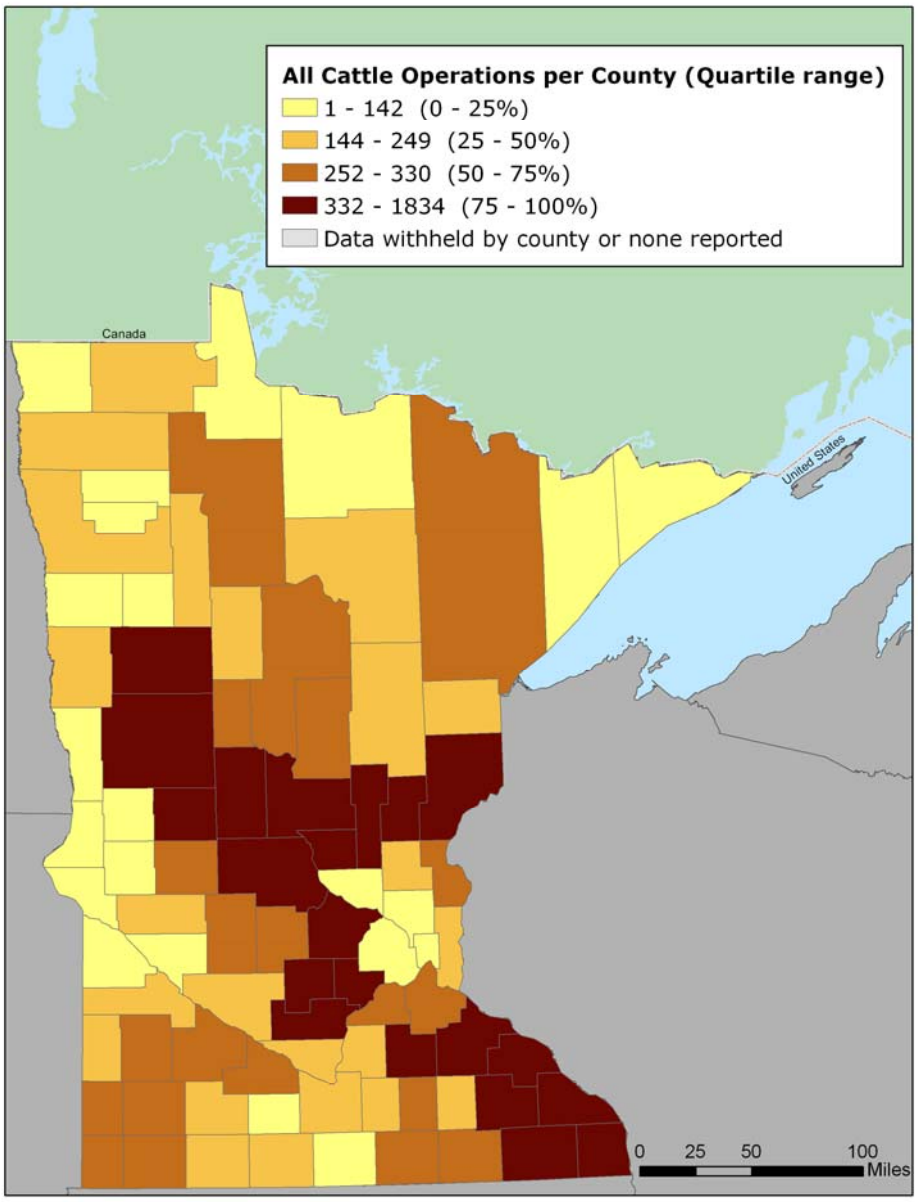
	State				County		
	U.S.	MN	% U.S.	Rank	Three County*	% MN	Rank
Cattle and Calves, Farms							
Cattle and cows	1,018,359	28,034	2.8	12	988	3.5	21
With cows	864,823	21,094	2.4	15	848	4.0	18
Beef cows	796,436	15,565	2.0	17	791	5.1	11
Dairy cows	91,989	6,474	7.0	4	82	1.3	42
With other	875,850	25,087	2.9	10	890	3.5	21
Cattle on feed	80,743	6,991	8.7	4	88	1.3	65
Cattle and Calves, Animals							
Cattle and cows	95,497,994	2,265,997	2.4	13	66,389	2.9	27
With cows	42,502,230	881,842	2.1	17	32,015	3.6	18
Beef cows	33,398,271	403,594	1.2	27	26,452	6.6	6
Dairy cows	9,103,959	478,248	5.3	5	5,563	1.2	47
With other	52,995,784	1,384,155	2.6	11	34,374	2.5	35
Cattle on feed	14,905,545	518,036	3.5	8	3,131	0.6	62

* Beltrami, Roseau, and Marshall Counties
Source: NASS 2002 Census of Agriculture

Beltrami, Roseau, and Marshall Counties are in the northern part of the State and contain more than 66,000 cattle and calves on 988 farms (Table 2.10). The three counties do not account for a significant portion of Minnesota's dairies or feedlots, but they are among the top 12 counties in Minnesota for cow-calf production.

The counties currently affected are an important part of cow-calf production in Minnesota (6.6 percent of beef cows) and likely play a central role in the northwest Minnesota cow-calf industry (a larger area than the three affected counties). The expansion of the three-county outbreak would threaten significant dairy and cattle-on-feed populations, placing 7 to 9 percent of U.S. dairies and cattle-on-feed operations at risk.

Figure 2.10 Number of Minnesota operations with cattle and calves, 2007



Source: NASS 2007 Census of Agriculture

2.4.2 Description of the outbreak

The index herd was discovered when TB-suspicious lesions were found on a 5-year-old cull cow at slaughter. The herd consisted of nearly 600 head of purebred Tarentaise and Angus cattle. Of the 63 caudal fold responders from the initial whole-herd test, 6 animals cultured positive, 5 of which were Tarentaise cows 3 to 10 years-of-age.

Four of the 11 infected herds represented secondary infections and were found by area testing which began in fall 2005. These herds averaged 275 head and an average of 3 infected animals were found in each herd. The remaining six affected herds were recently discovered; two in fall 2006 and four in winter 2007–2008. Five of the six herds had at least one previously negative whole-herd test. These herds were smaller (97 head on average) than the index or secondary herds and typically had only one infected animal.

The index herd stands out because of its size and, unlike the other herds, the majority of animals added to the herd were purchased out-of-State (90 percent of trace-ins). Two of the six infected animals were from Iowa.

In the other herds, almost all additions were from in-State (97 percent of trace-ins). The animals brought into Michigan from out-of-State were not culture-positive and the majority were born on the farm. Additionally, 5 of the 6 infected cattle in the index herd were Tarentaise whereas only 1 of 17 infected cattle from the other 10 herds were Tarentaise (Table 2.11).

Table 2.11 Infected cattle from the index herd

	Sex	Breed[1]	Age (in years) when Necropsied	Approx. DOB	Animal Origin
1	F	TR	6	1999	Home raised
2	F	TR	10	1995	Out-of-State; purchased 11/1999[2]
3	F	TR	3	2002	Home raised
4	F	AN	5	1999	Home raised
5	F	TR	8	1997	Out-of-State; purchased 1/2000
6	F	TR	5	2000	Home raised

[1]AN = Angus; TR = Tarentaise
[2]Likely moved January 2000

The emerging epidemiological information indicates that the index herd received a large influx of purebred Tarentaise cows and Angus bulls, mostly through out-of-State purchases. One of these purchases is likely responsible for the introduction of *M. bovis* into the herd. Of the 96 traces into this herd between 1998 and 2005, more than 75 percent were from two States. Two Tarentaise cows purchased out-of-State were later identified as infected. They were 3- and 5-years- old and presumably already infected when added to the index herd. Over the next 5 years, infection spread throughout this operation, ultimately giving rise to 63 CFT responders, 13 CCT reactors, and 6 additional infected cows from 4 of 8 pastures (subgroups) that make up this operation. Evidence suggests that infection also spread to adjacent herds, spilled over to deer, and infected other cattle herd operations that purchased cattle predominantly from northwest Minnesota.

Another possible scenario is that bovine TB existed in Minnesota before the index herd was found. Slaughter surveillance was the primary surveillance program used to detect bovine TB before the index case was discovered. The sensitivity of slaughter inspection is reported to be just 50 percent (Ducrot et al., 1997). In 1999 and 2000, suspicious granuloma submissions were at the lowest point in the past 25 years (Kaneene et al., 2006). The median time from infection to detection of bovine TB by visual inspection of carcasses at slaughter is estimated at 300 weeks (5.75 years) (van Roermund et al., 2003; Fischer et al., 2005). The 5 years to detection is consistent with expectations found in the literature and may indicate bovine TB was present in Minnesota prior to the identification of the index herd.

The discovery of six affected herds since October 2006 may be a result of secondary spread from the index herd or new infections from other sources. The TB outbreak may not have been contained, the delay in detecting the six affected herds may have been a result of poor testing sensitivity, low levels of surveillance, or continuous active transmission of *M. bovis* in northwest Minnesota.

Moreover, the six herds differed from the other affected herds in several ways. They were smaller than the other herds and had fewer infected animals. Five of the six herds had only one infected

animal, while the sixth herd had two infected animals. In comparison, the index herd had six infected animals and the secondary herds each had three infected animals (with the exception of herd 3 that had only one infected animal).

One reason fewer positive animals were found in the secondary herds than the index herd may be the duration between a herd's exposure to TB and detection. The index herd was discovered approximately 5 years after exposure. The duration between exposure and detection for the secondary herds was approximately 2.5 years—half the time of the index herd.

In herds 6, 7, and 8, infected animals were less than 2-years-old and the average time for detection was only 1.5 years. In herds 9, 10, and 11, infected animals were more than 3-years-old, and the herd could have been infected at any time since the index herd in 2000 (approximately 3 years on average). However, if the herds became infected after the most recent negative whole-herd test, the time elapsed to detection would be a little more than 1 year. Therefore, the number of infected animals found in these herds suggests that they were recently infected.

Table 2.12 Earliest and latest dates TB was possibly transmitted to each affected herd

	Potential date of Exposure			
Affected Herd	Earliest	Latest	Link to index herd	Possible source
1	Jan 2000[1]			Cows purchased at 3- and 5-years-old
1	Jan 2000[2]	Jan 2005		Index animals in herd
2	Summer 2002	Fall 2005	Yes - Adjacent premises	
3	Spring 2004	Fall 2005	Yes - Adjacent premises	Fence-line contact; deer exposure
4	Spring 2002[3]	Fall 2005	Yes - Traced out	Three calves purchased; probably infected first year of life in Herd 1
5	Spring 2000	Fall 2005	No	Purchased young stock from herds 1, 2, or 3 via market.[4]
5	May 2005	Fall 2005	No	Infected cow in herd
6	Feb 2005	Sep 2006	Yes - Adjacent premises [5]	Deer exposure; fence-line contact
7	Feb 2005	Sep 2006	No[5]	Deer exposure
8	Spring 2006	Sep 2007	No	Deer exposure
9	Spring 2003	Dec 2007	No	
10	Spring 2000	Jan 2008	Yes	Area spread via deer or direct contact with herd 2 or 3 cattle
11	Spring 2000	Dec 2007	No	

[1]Index animals brought from Iowa
[2]Two were calves and a yearling at time index animals entered the herd in January 2000
[3]Born on herd 1 in spring 2002, most likely infected there before being sold in February 2003
[4]This herd had more traces into it than all other herds combined (159 from MN)
[5]Infected animals were born spring 2005 and index herd quarantined in July 2005. Given this short window, it is just as likely (if not more) that the animal was exposed to deer as a young animal—especially for herd 7 which, unlike herd 6, was not adjacent to index herd.

An epidemiological link between the recently discovered herds and the index herd would provide evidence that transmission of TB occurred before fall 2005 and that the prior negative whole-herd tests were false-negatives due to poor sensitivity of the CFT.

Affected herds 2, 3, and 4 were epidemiologically linked to the index herd. Two were adjacent to the index herd and one purchased three yearlings from the index herd in February 2003. Herd 5 did not have a clear epidemiological link to the index herd. Only two of the herds (6 and 10) had an epidemiological link to the index herd, but in both cases the link was weak.

The lack of an epidemiological link between the recently discovered herds and the index herd casts doubt as to whether the newly discovered herds were infected during the same time as the secondary herds. Furthermore, an epidemiological link to deer exposure exists for several of these herds. The infected animals in herds 6, 7, and 8 were all young animals, which are most likely to interact with deer on the premises. Herds 6, 9 (summer pasture), and 10 were within 2 miles of an infected deer.

Five of the six herds had a negative whole-herd test before an infected animal was discovered. Herds 9 and 10 had two negative annual whole-herd tests before an infected animal was discovered in winter 2007–2008. As noted, these herds were much smaller on average than the index and secondary herds. The 2 largest recently discovered herds had approximately 200 adult cows. The other 4 herds had fewer than 40 adult cows.

The prior negative whole-herd tests may indicate that the herds were truly negative. However, applying a screening test of low sensitivity for a low prevalence disease in small herds increases the risk of obtaining false negative results. The probability of correctly identifying an affected herd (herd sensitivity) is dependent on the test sensitivity within herd prevalence and the cutoff value for the number of positive tests needed to classify a herd as positive.

A model was constructed in @Risk to assess the risk of false-negative herds and to determine what role, if any, lower herd sensitivity may have played in this incident. The model was constructed to simulate the expected number of false-negative herds given the situation in 2005. The specific inputs for the model are presented in Table 2.13.

Table 2.13 Inputs for @Risk model used to simulate expected number of false negative herds

Input Parameter	Value	Comment
N (population at risk)	150	Number of herds tested the first year after finding index herd
Herd size	Random draw from herd size distribution	Based on number of animals tested for whole-herd test conducted in MA zone
Herd prevalence	Random draw from a pert distribution (0, 4, 5) over N	Pert distribution is minimum, most likely, max number of infected herds
Within-herd prevalence	3%	Based on actual within-herd prevalence in first five infected herds

Given a sensitivity of 0.82 and a specificity of 0.96 for the CFT, 2.5 is the mean number of false-negative herds (for 5,000 iterations). Therefore, the six herds discovered since the initial case finding represent twice the number of false-negative herds expected. The 95 percent confidence interval was 0 to 6 herds, which indicates a 5 percent likelihood of missing 6 herds by whole-herd testing. Therefore, these herds likely represent recent TB transmission; not missed infection.

Three specific aspects of the Minnesota TB epidemiology were assessed to determine if the recently discovered herds were infected for a longer period of time and missed, or whether active TB

transmission continues. A summary of the findings and their interpretation are presented in the Table 2.14.

Table 2.14 Findings and interpretation used to determine the current status of bovine TB in the Modified Accredited Zone

	Findings	Interpretation
Epidemiology analysis	Few animals infected Young animals infected	Rapid detection of recent infection. Recent infection. (deer exposure > index herd)
Epidemiology link to index herd.	No link for several herds	Recent infection. (deer exposure > index herd)
Sensitivity of detection	Few false negative herds expected	Recent infection > missed infection

Conclusions indicate that active transmission of bovine TB continued for 2 to 3 years after the initial TB case was discovered in Minnesota because of cattle exposure to infected deer.

Risk associated with wildlife in Minnesota: After the identification of the index herd, the Minnesota Department of Natural Resources conducted surveillance of hunter-harvested white-tailed deer within a 15-mile radius of the first four *M. bovis*-infected premises; 1 of 474 deer tested was positive for *M. bovis.* The infected deer was harvested 1.2 miles south of the index herd and less than 3.1 miles from the other initially infected cattle herds. This prompted targeted culling and surveillance of 90 deer on the infected farms during spring 2006—1 additional TB positive deer was found.

The infected deer appeared to be associated with *M. bovis*-infected cattle herds in the region based on proximity and the fact that the deer and cattle had the same strain of bovine TB. As a result, the Minnesota Department of Natural Resources instituted more rigorous sampling protocols to establish prevalence of *M. bovis* in the deer population. Before the fall hunt, approximately 15,000 deer over 18 months-of-age inhabited the 1,728-square-mile surveillance zone. It was determined that samples from 1,000 of these deer would provide a 95 percent confidence interval of detecting *M. bovis* if prevalence in the deer population is less than 1 percent.

As of July 2008, the Minnesota Department of Natural Resources planned to conduct hunter-harvested deer surveillance during the fall hunting season. If *M. bovis*-infected deer are identified, targeted culling will be conducted in the spring around areas where infected deer have been found. Culling methods include sharpshooters, baiting, landowner shooting permits, and the use of aerial shootings. As of April 2008, the Minnesota Department of Natural Resources tested 4,043 deer in the surveillance zone and identified 18 TB-positive deer.

The infected deer were 1.5 to 7.5-years-of-age, which is consistent with sampling conducted in Michigan in which adult deer, specifically males, were more likely to be infected with *M. bovis*. The majority of deer tested within the surveillance zone (62 percent) have been adult deer. To date, no *M. bovis*-positive fawns have been found. Deer appear to be spatially associated with known affected cattle herds. The average distance between affected cattle herds and *M. bovis*-positive deer in the TB management area is 4.9 miles (StDev=3.6 km (2.2 mi); Min=0.3 km (0.2 mi)). Furthermore, 61 percent (11 out of 18) of infected deer and 63 percent (5 out of 8) of suspect deer are within 3.1 miles of affected cattle herds. All positive and suspect deer were within 6.8 miles of *M bovis*-affected herds.

Based on fall hunter-harvested sampling, 0.43 percent of white-tailed deer in the MA zone tested positive for bovine TB from 2005 to 2007, which is similar to the overall apparent prevalence for Michigan white-tailed deer. However, apparent prevalence in fall hunter-harvested deer appears to be higher in the management zone (0.71 percent) and in the established core zone (1.97 percent) compared to the remainder of the proposed MA zone. These apparent prevalence estimates are

lower than those reported in Michigan's core zone, which ranged from 1.2 to 4.9 percent (Michigan Department of Natural Resources, 2008).

Spoligotyping results: Bovine TB cases identified in Minnesota have been associated with a single genetic strain of *M. bovis* identified by spoligotype 664073377777600. This relationship has been confirmed through additional DNA fingerprinting techniques. Although it is likely that TB infections in Minnesota are due to a single strain of *M. bovis* circulating among deer and cattle, how the isolate was introduced or how it continues to spread cannot be determined by spoligotyping data alone. The strain identified in Minnesota most closely resembles a strain identified in feeder animals of Mexican origin identified in the southwestern United States, which may help support the theory that this strain was originally introduced through cattle movement. However, a limitation of spoligotyping is that it may group strains that are unrelated. Therefore, in the absence of epidemiologic evidence and results from other genotyping tests, a conclusion cannot be made about this relationship.

2.4.3 Summary

The initial source of infection of bovine TB in Minnesota cannot be confirmed but is thought to be from the introduction of infected cattle. Regardless of the source of introduction, the active transmission of bovine TB continues in cattle and deer within the designated MA zone. Cattle, white-tailed deer, and other fomites are all potentially responsible for the spread of *M. bovis* in the State. Because of the significance of the cattle industry in Northern Minnesota, controlling bovine TB in both cattle and free-ranging white-tailed deer continues to be a priority in this area.

2.5 Tuberculosis in New Mexico cattle herds, 2002–2009

2.5.1 Background

New Mexico TB status: Since the mid-1980s, concerns about bovine TB in New Mexico cattle have fluctuated. The State was granted AF TB status in 2000; however, two TB-positive beef steers were traced from west Texas feedlots to eastern New Mexico herds in 2002. Additional infection was not found in the herds-of-origin for these two steers. As a result, the source herds remained classified as unaffected. Two TB-infected cull cows subsequently discovered at slaughter were traced back to two dairies. Testing of the source herds confirmed the presence of TB in other cattle within each herd, and the two herds were classified as affected. Subsequently, the entire State was reclassified as MA.

In 2005, New Mexico had split-State status with the majority of the State classified AF, and a small MAA zone in parts of Curry and Roosevelt Counties in the Clovis-Portales area of eastern New Mexico.

On September 11, 2008, New Mexico's bovine TB status was downgraded to MAA status for the entire State. This status downgrade was based on the discovery of an affected dairy found by traceback from the slaughter of a culture-positive cow in April 2007. A second affected operation was discovered by testing of an adult dairy cow at a sale in Texas as the cow was about to re-enter New Mexico. The cow had been illegally transported from the New Mexico operation to Texas and the operation was declared affected based on epidemiologic evidence. New Mexico subsequently applied for a change in split-State status (a regulatory option accepted by USDA in March 2009). This change designated a MAA zone that encompasses the entire area of Curry and Roosevelt counties with the balance of New Mexico returned to AF status.

New Mexico cattle industry: New Mexico has approximately 326,000 dairy cows (Figure 2.12), 100,000 replacement dairy heifers, 1.5 million beef cattle (Figure 2.11), and thousands of head of rodeo stock. Many cattle of Mexican origin are pastured in New Mexico before being shipped to feedlots in other States. There are 172 dairies with an average herd size of 2,100 cows in New Mexico, and the State ranks first in the nation in dairy herd size. There are 54 calf-raising facilities in New Mexico, the largest of which manages more than 10,000 heifers and 18,000 steers. The State has 11 public livestock auction yards.

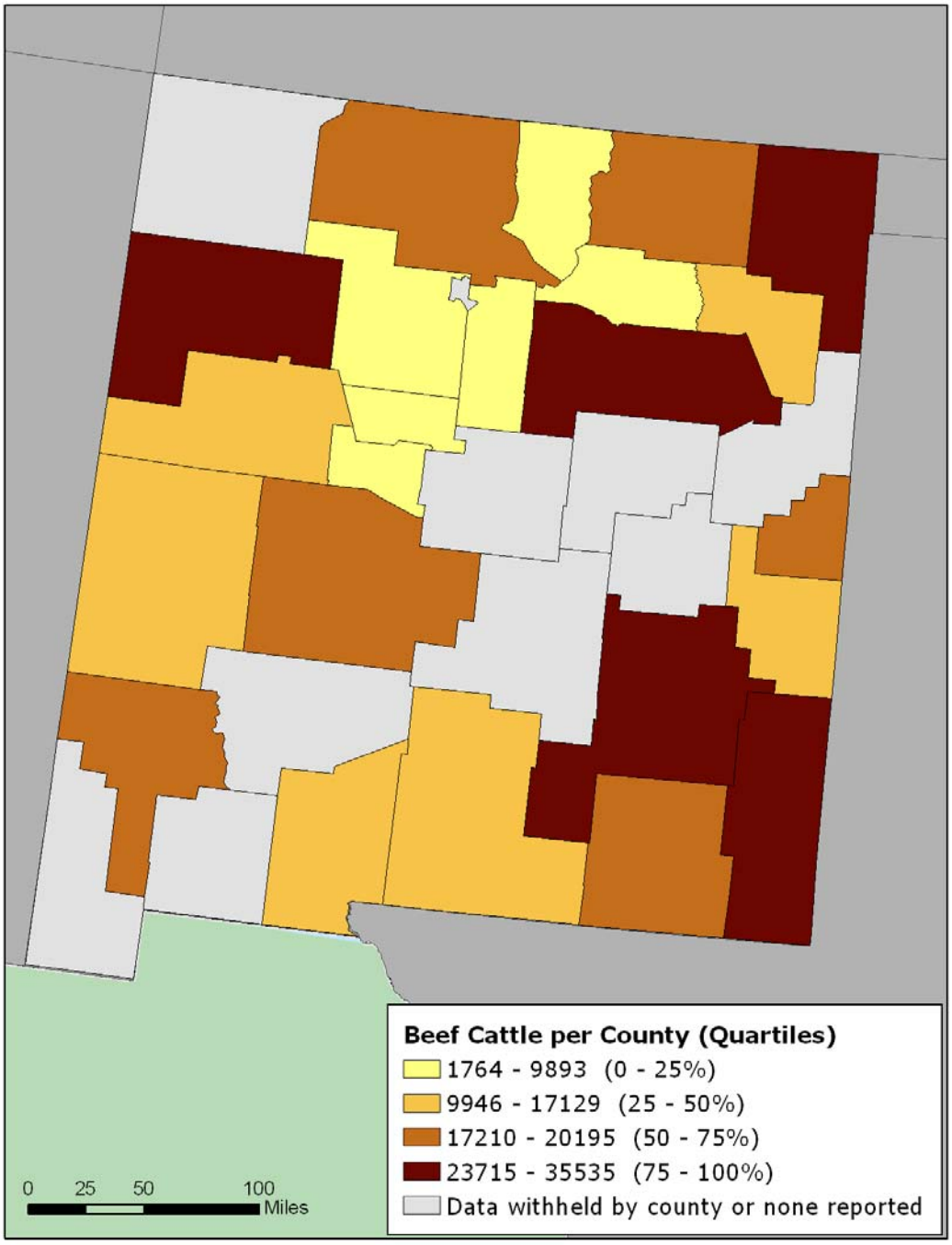
According to the 2007 Census of Agriculture, New Mexico ranks 37th in the United States for number of farms with milk cows (272) and 9th in milk cow inventory (326,400) (Table 2.15).

Table 2.15 Number of cattle and calves and number of farms in the United States and New Mexico, 2007

		State			County		
	U.S.	NM	% U.S.	Rank	Two County	% NM	Rank*
Cattle and Calves, Farms							
Cattle and cows	963,999	9,508	1.0	34	580	6.1	16,7
With cows	818,992	8,380	1.0	32	446	5.3	16,10
Beef cows	764,984	8,208	1.1	31	390	4.8	21,11
Dairy cows	69,890	272	0.4	37	71	26.1	3, 2
With other	788,633	6,933	0.9	34	491	7.1	13,5
Cattle on feed	50,009	34	0.1	39	11	32.4	2,3
Cattle and Calves, Animals							
Cattle and cows	96,347,858	1,525,976	1.6	21	341,889	22.4	1, 4
With cows	42,101,375	856,573	2.0	19	159,831	18.7	2,3
Beef cows	32,834,801	530,173	1.6	21	34,809	6.6	9,12
Dairy cows	9,266,574	326,400	3.5	9	125,022	38.3	2,3
With other	54,246,483	669,403	1.2	23	182,058	27.2	1,4
Cattle on feed	16,098,910	154,556	0.1	17	2,471	0.2	1,6

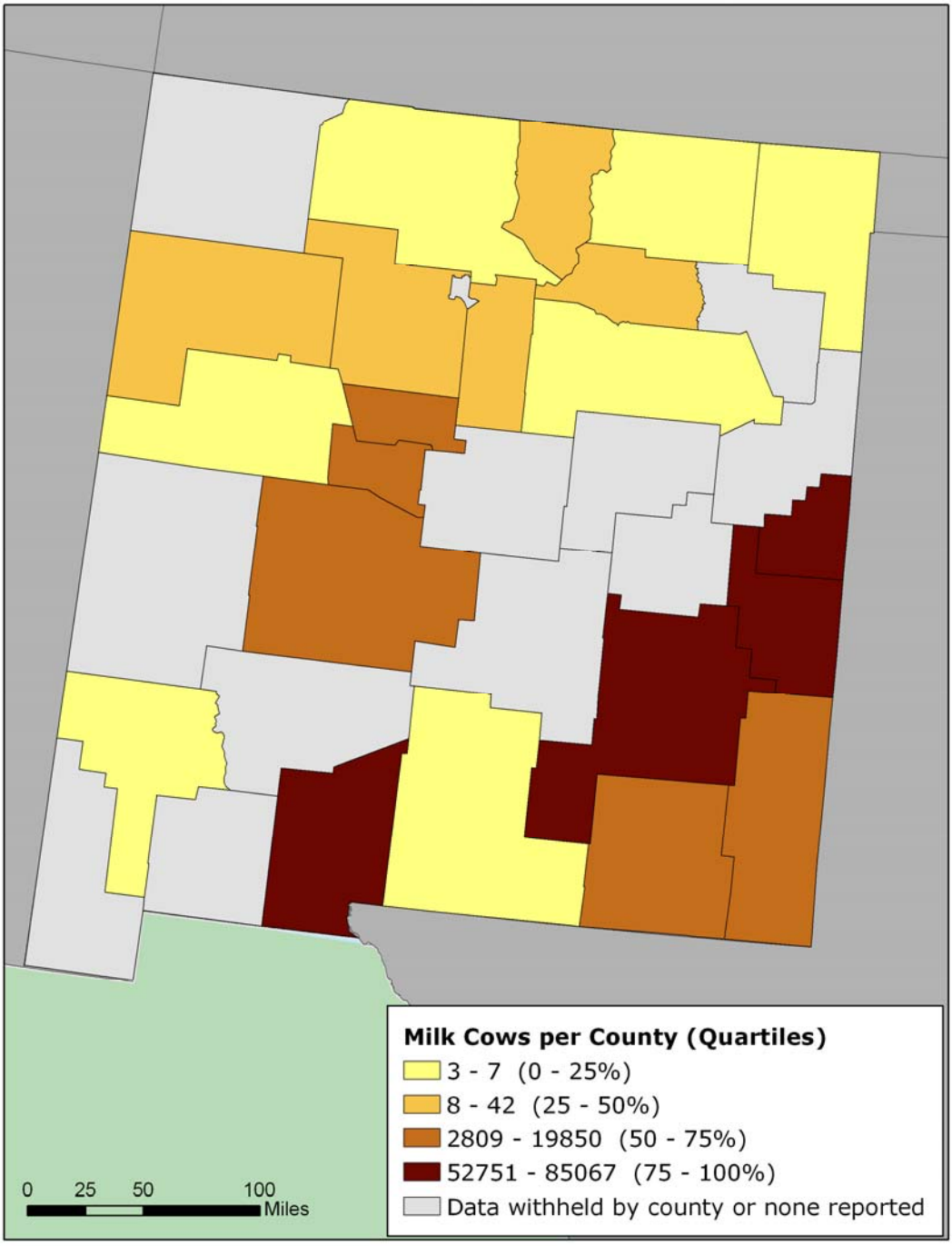
*Rank of Curry, Roosevelt counties among all 29 NM counties.
Source: NASS 2007 Census of Agriculture.

Figure 2.11 Number of beef cattle in New Mexico by county, 2007



Source: NASS 2007 Census of Agriculture

Figure 2.12 Number of milk cows in New Mexico by county, 2007



Source: NASS 2007 Census of Agriculture

Curry and Roosevelt counties are currently in the MAA zone and account for 342,000 cattle and calves on 580 farms (i.e., approximately 160,000 adult breeding cows on 446 farms in the two counties). Roosevelt and Curry counties also rank second and third in the number of dairies in the State, respectively. More than one-third of New Mexico's dairy cows and one-fourth of farms with milk cows are in these two counties.

The affected counties are central areas of dairy production in New Mexico (comprising 38.3 percent of the State's dairy cows) and have a significant calf raising industry (27.2 percent of New Mexico calves on farms without cows). Dairy producers in these two counties likely have strong connections to farms in Chaves County, the largest dairy county in New Mexico in terms of cattle and operations. The extent and nature of the counties' relationship to the large number of farms with only calves and the overlapping beef industry in the eastern portions of New Mexico are not well described. It is possible that the affected population encompasses a greater area than Curry and Roosevelt counties. Surveillance efforts to detect new bovine TB cases should be extended to other adjacent counties (Chaves, Quay, and San Miguel) with significant susceptible populations.

2.5.2 Description of New Mexico outbreak

Affected herds and cases: For purposes of this section, cases and affected herds are numbered sequentially from 1-11. "Case" is used to refer to infected individual animals originating from herds that remained classified as unaffected while "herd" is used when the herd is declared affected. Since 2002, New Mexico has identified four affected herds. Seven additional infected animals (cases) have been found, but these animals had no known connection to a specific herd or additional infected animals were not identified in the source herd.

There have been three temporal clusters of infection in New Mexico: 2002, 2004, and 2007–2008. Most of the infected beef cattle were considered cases because additional infected animals in the herd-of-origin were not found. Cases 1, 2, and 5 were beef cattle identified at slaughter and traced from west Texas feedlots. Additional infected animals were not found in the herds-of-origin, and therefore not classified as affected herds. More recently, an affected mixed herd (herd 11) was identified, though it was discovered by a traceback of an infected adult dairy cow. The low number of affected beef herds may reflect the transient nature of beef feeding facilities more than the absence of transmission of *M. bovis* among beef cattle. In contrast, dairy herds are larger and relatively stable compared to beef feeding channels, which improves the likelihood that infection can spread and be detected. Tracebacks to dairy herds have usually resulted in the identification of additional cases in those herds and a designation of "affected herd" (herds 3, 4, and 10).

The beef and dairy industries in New Mexico appear to be interconnected. Beef operations may purchase dairy calves and there have been multiple tracebacks to such operations from feedlots. The most recently discovered affected herd was a feedlot that also purchased and re-sold cull dairy cows. These few herds showed adequate connections between beef and dairy producers to transmit TB. Further, there is an overlap of the top beef and top dairy production areas in New Mexico. These connections must be managed with improved biosecurity practices to avoid continual TB spread between the two industries.

Mexican-origin cattle played a predominant role in the source of *M. bovis* in New Mexico. Four TB-positive animals were linked to Mexican-origin cattle based on epidemiological investigations and two others were linked by genotyping results. The herds from which cases 1 and 2 originated import Mexican steers and the herd-of-origin for case 5 and affected herd 3 had direct exposure to Mexican-origin roping steers. *M. bovis* isolates from case 9 and herd 11 were most closely related to strains of Mexican-origin.

Genotyping results show that the three temporal clusters of bovine TB cases were unrelated. For example, *M. bovis* isolates from cases 6 through 8 were considered significantly different from *M. bovis* isolates from the infected dairy herds in the 2002 cluster (herds 3 and 4). *M. bovis* isolates from the 2007–2008 cluster are different from isolates from the first two temporal clusters. Herd 10 had an *M. bovis* isolate most closely related to isolates from two other affected dairies in Texas and Arizona from 2004 and 2005, respectively.

The number of cattle tested by CFT and the numbers of responders, by year, are presented in Table 2.16. In total, 692,773 CFTs have been conducted since 2002.

Three-fourths of the CFTs (75.6 percent) from 2003 to 2008 have been conducted in dairy operations. More than 3 percent of test-eligible cattle on these premises were classified as suspects—more than twice the national average of 1.3 percent.

Table 2.16 Number and percent CFT responders in New Mexico 2003-2008, by premises type

Premises Type	Number Responders	Total Animals Tested	Percent Responders
Dairy	16,723	523,403	3.2
Other premises	962	39,703	2.4
Beef	406	65,814	0.6
Livestock market	168	39,458	0.4
Farm or ranch	25	7,860	0.3
Interstate movement	44	16,523	0.3
All	18,328	692,761	2.9

Spoligotyping results: M. Bovis isolates identified in New Mexico from 2000 to 2008 have been associated with four different strains through spoligotyping. These strains have also been identified in Mexican-origin animals and herds in Arizona, California, Colorado, Idaho, Kansas, Missouri, Nebraska, New Jersey, New York, North Dakota, Oklahoma, South Dakota, Texas, and Washington. One of these strains is also similar to the strain seen in Hawaii and Michigan. A limitation of spoligotyping is that it may group strains that are unrelated. Therefore, in the absence of epidemiologic evidence and results from other genotyping tests, a conclusion cannot be made about the relationship between these four strains and outbreaks in the other States. However, based on the epidemiologic evidence in the herds in New Mexico, there does appear to be a relationship between strains in cattle of Mexican-origin and cattle in the southeastern United States.

2.5.3 Summary

In New Mexico, isolates identified by spoligotype 264073777777600 came from cases 6 through 9 and herd 10. *M. bovis* strains identified by this spoligotype have been isolated from cattle in California, Colorado, Kansas, North Dakota, Nebraska, New Jersey, New York, Oklahoma, Texas, and Washington as well as Mexico between 1997 and 2008.

The New Mexico cattle industry has experienced a series of *M. bovis* cases and affected herds since 2002. Characteristics that appear to be typical of *M. bovis* occurrence in New Mexico are:

- The herd-of-origin is usually affected with TB when TB-positive dairy cattle are found, but when positive beef cattle are found the herd-of-origin (typically a feedlot) is rarely found to be affected with TB
- Mexican-origin cattle are often implicated as the most probable source
- Genetic fingerprinting suggests a relationship between infection in cattle of Mexican-origin and cattle in New Mexico and other parts of the Southwest
- The numerous cases and affected herds represent separate introductions of *M. bovis* (not uncontrolled spread of a single outbreak)

Multiple risks exist for the introduction and spread of bovine TB in New Mexico; importation of Mexican-origin steers, dairy replacement heifer management, the biosecurity practices of calf raisers, and the large influx of purchased additions all pose potential risk for exposure to *M. bovis*. This brief

review of the bovine TB cases in New Mexico suggests that both beef and dairy herds face intermittent exposure to *M. bovis*.

2.6 Summary of outbreaks

Analyses of the four outbreaks in California, Michigan, Minnesota, and New Mexico identified several risk factors for the introduction and spread of bovine TB. In California and New Mexico, molecular fingerprinting techniques revealed several strains of *M. bovis*, indicating multiple sources of introduction. Risk factors for California and New Mexico included the importation and commingling of Mexican-origin steers, the management and biosecurity practices used by calf raisers for dairy replacement heifers, and the large influx of purchased additions. In States with similar practices and risk factors, both beef and dairy herds may face intermittent exposure to *M. bovis*.

Conversely, Michigan and Minnesota each had just one strain of *M. bovis*, indicating a point source of introduction and local area spread. The same strains were identified in the wildlife of each respective State, making cattle contact with infected white-tailed deer (especially contact with feed contaminated by deer) an important risk factor for the introduction and spread of bovine TB in Michigan, Minnesota, or any other area in which infected wildlife reside.

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3. Legal and Illegal Importation of Cattle

3.1 Introduction

In 2008, the U.S. cattle population was just over 96 million (NASS, 2009) (Figure 3.1). The top five cattle-producing States were Texas (13.6 million head), Nebraska (6.5 million head), Kansas (6.7 million head), California (5.5 million head), and Oklahoma (5.4 million head). These five States represent 39 percent of the total U.S. cattle population. Of these States, Texas and California share a border with Mexico and none share a border with Canada. Cattle of Mexican origin have been associated with *M. bovis* identification through slaughter and are believed to be a risk factor for U.S. cattle.

3.2 U.S. cattle imports and regulations

Of bovines legally imported into the United States in 2008, less than 0.01 percent were from Australia, 70 percent were from Canada, and 30 percent were from Mexico (U.S. Department of Commerce, 2009). Live bovine imports from Canada include cattle and bison, although bison account for a very small proportion. The last time the United States imported cattle from a country other than Australia, Canada, or Mexico was in 2003 from New Zealand; however, import permits may have been issued for other countries. These permits indicate an interest in exporting to the United States but were not reflected in the actual import numbers. Recent trends show that the number of cattle imported from Canada is increasing while the number imported from Mexico is decreasing (Table 3.1). A notable deviation was caused by the Canadian border closure during the U.S. Bovine Spongiform Encephalopathy (BSE) outbreak in 2004.

Table 3.1 U.S. live bovine imports by country and by number of head

	Number of Head							
Country	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**
Canada	1,308,670	1,688,814	513,344	135	562,647	1,045,125	1,425,998	1,610,973
Mexico	1,130,168	816,460	1,239,531	1,370,476	1,256,404	1,256,973	1,090,094	702,661
Australia	12	4	0	0	0	0	0	29
New Zealand	0	0	12	0	0	0	0	0
Norway	350	0	0	0	0	0	0	0
Guatemala	0	1	0	0	0	0	0	0
Total	2,439,200	2,505,279	1,752,887	1,370,611	1,819,051	2,302,098	2,516,092	2,313,663

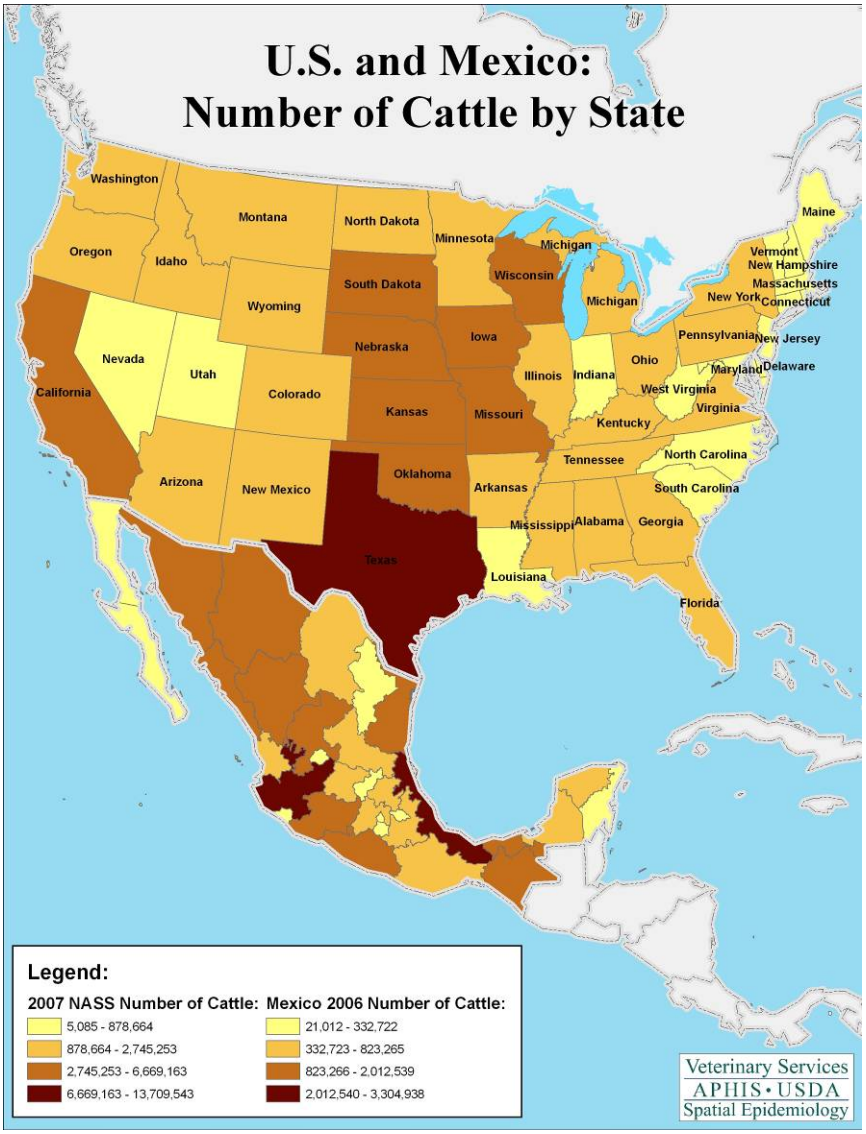
Source: World Trade Atlas. U.S. Dept. of Commerce Bureau of Census. Accessed 1/29/ 2009.

Part 93 of 9 *CFR* states that any imported cattle not going directly to slaughter must have a health certificate attesting that the cattle have tested negative for bovine TB. The importation of Holstein steers, spayed heifers, Holstein cross steers, and Holstein cross spayed heifers from Mexico is prohibited. No cattle from a herd with evidence of TB or suspected TB will be allowed entry into the United States until the herd achieves accredited status.

All cattle entering from TB zones within Mexico must be branded with an “M” unless the animals are headed directly for slaughter. The regulations in 9 *CFR,* part 93 allows the importation of intact cattle from Mexico if the cattle are test-negative at the port of entry. Tests are administered by the port

veterinarian. In addition to border testing, some States, such as Colorado (Colorado Department of Agriculture, 2009) and Texas (Texas Animal Health Commission, 2008), require annual TB testing for any Mexican-origin rodeo and exhibition cattle used for timed events, rodeos, or team events. Colorado regulations also mandate that only steers and spayed heifers from Mexico be allowed into the State. Nebraska allows cattle from Mexico to enter if they adhere to CFR TB import regulations and have additional testing and identification (Nebraska administrative code, 2009). Additionally, any cattle imported from Canada must originate from an AF or MAA Province.[3] Breeding cattle and bison entering from the Province of Manitoba are prohibited.[4]

Figure 3.1 Number of cattle in the United States (2007) and Mexico (2006), by State



[3] VS Memo 591.64
[4] VS Notice 02-17

3.3 U.S. cattle imports from Mexico

3.3.1 Cattle imports from Mexico by State of destination

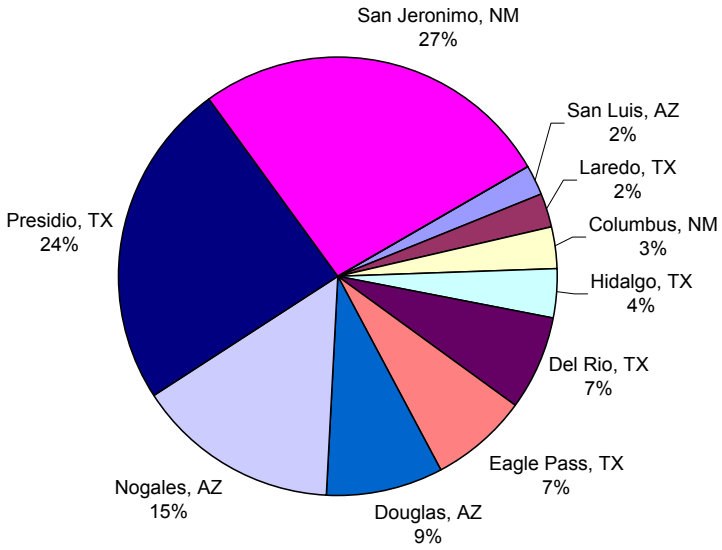
In 2008, total cattle imports from Mexico were approximately 800,000 head.[5] The top five destination States were Texas (450,547), New Mexico (216,764), California (64,905), Arizona (38,966), and Colorado (10,352). The five States represent 98.6 percent of all Mexican cattle imports; however, they are not necessarily the final destinations of these cattle because once cattle enter the United States, they move on certificate of veterinary inspection (i.e., interstate health certificate). A 2004 study suggests that most cattle imported from Mexico into Texas and New Mexico tend to stay in those States (Skaggs et al., 2004). This study also refers to cattle going as far as Mississippi, Idaho, Oregon, Washington, and Iowa.

3.3.2 Cattle imports from Mexico by type and port

According to the VS Import Tracking System, 98.5 percent of cattle imported from Mexico in 2008 were feeder cattle and 1.5 percent were imported for competition. Purpose categories include breeding, commercial, confiscated, exhibition, other, and slaughter. Feeder cattle include backgrounding animals and cattle destined for feedlots. Feedlot cattle usually go to slaughter in the United States; however, cattle on pasture may return to Mexico for further finishing or slaughter.

Most cattle imports from Mexico enter through the land border ports in Texas, New Mexico, and Arizona (Figure 3.2). The largest numbers of cattle enter through San Jeronimo, New Mexico (27 percent), Presidio, Texas (24 percent), Nogales, Arizona (15 percent), and Douglas, Arizona (9 percent). These four ports account for 75 percent of all Mexico cattle imports.[3] The current requirements for importing Mexican cattle for bovine TB are provided in VS Notice 09-07.

Figure 3.2 Percentage of Mexican cattle imports, by port of entry FY 2008



Source: Veterinary Services Import Tracking System

3.3.3 Rodeo cattle from Mexico

Competition cattle or rodeo cattle represent a very small percentage of cattle imported from Mexico. However, rodeo cattle move frequently throughout the United States and, therefore, are at highest

[5] Veterinary Services Import Tracking System. Accessed 1/29/2009.

risk for exposure to infected animals or exposing susceptible animals to disease. Additionally, imported rodeo cattle are moved to pasture where they may commingle with domestic cattle populations during the off season.

In Texas, 17 *M. bovis*-positive cattle were found associated with a feedyard. Many of these animals were from Mexico and all were used for roping and rodeo cattle (Texas Animal Health Commission, 2009a). Of the total Mexican-origin cattle TB investigations, 11 percent were related to rodeo and roping purposes (Texas Animal Health Commission, 2009b).

Corriente cattle represent the majority of imported rodeo or competition cattle. Corriente cattle are also imported into the United States for breeding purposes (DH Corriente Cattle, 2009; Jandal Corriente Cattle, 2009; Nota Bene Company, 2009). Offspring are used for breeding, rodeos, and competitions. Some in industry believe that Mexico rodeo steers pose less risk to than domestic steers because Mexico has additional TB testing requirements that are not required in the United States (Welch, 2009). The industry practice of feeder cattle being diverted to rodeo cattle is addressed in a later chapter of this assessment.

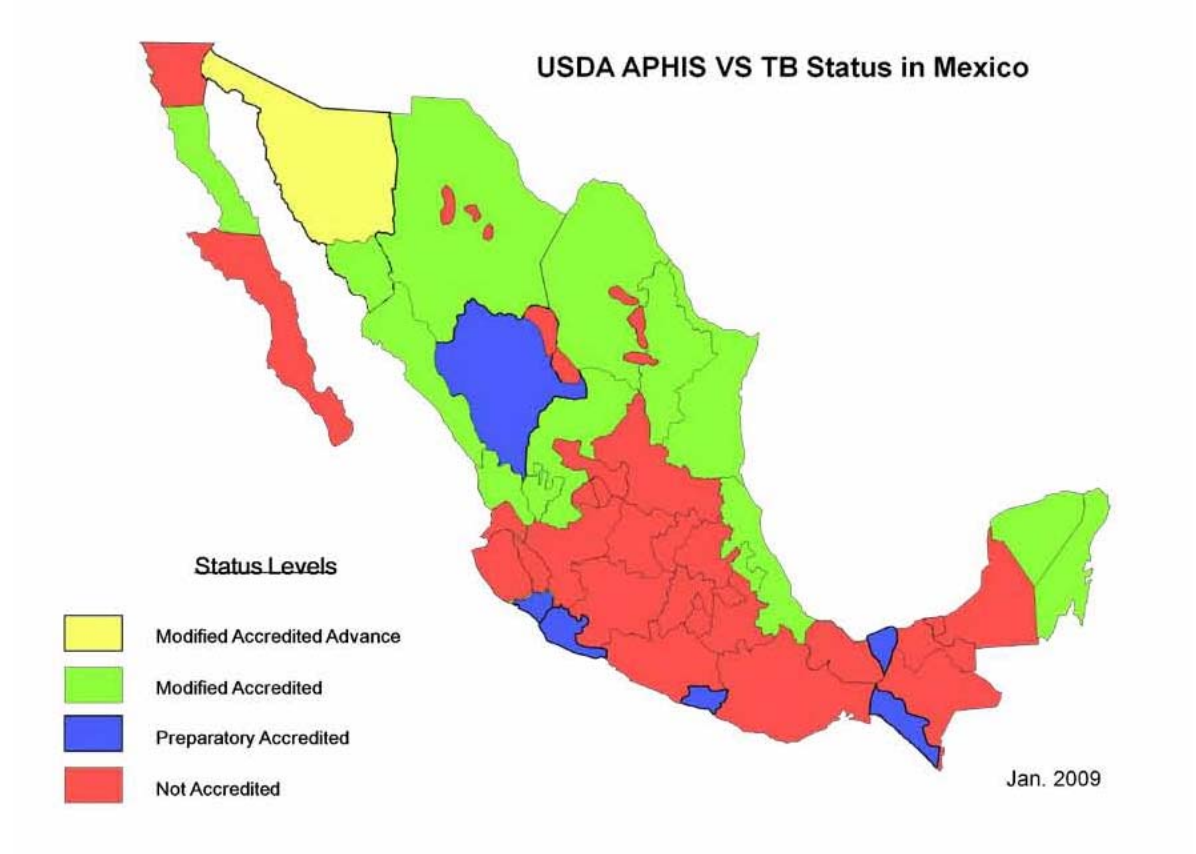
3.3.4 Mexican TB status

Mexico has had a bovine TB eradication program since 1972. APHIS, together with U.S. States and industry, has been working with Mexico for over a decade to establish equivalency between the TB programs in the United States and Mexico and to harmonize international TB requirements. In 1993, the Binational Committee (BNC) for TB was formed under the auspices of the USAHA. The committee addressed bovine TB issues on both sides of the border (Willer, 2005). The BNC meets twice yearly, with the first meetings held in August and October 1993.

APHIS recognizes equivalent TB status of some Mexican States and zones consistent with the current U.S. bovine TB classification scheme: MAA, MA, AP, and nonaccredited.[6]

[6] VS Memo 09-07

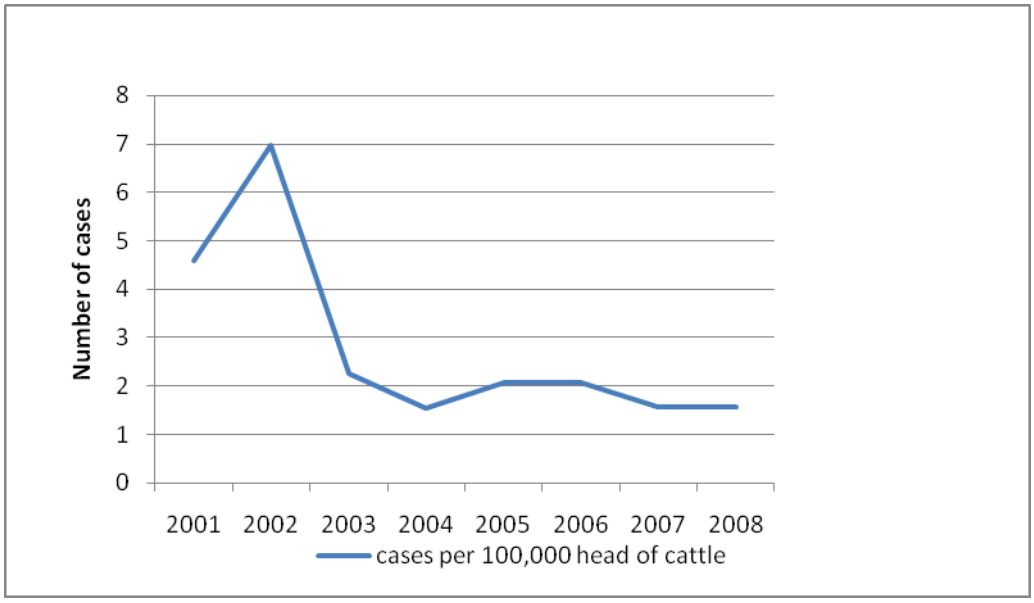
Figure 3.3 Mexico bovine TB status levels



Source: SAGARPA (Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food- Mexico)

Slaughter cases may be identified as Mexican-origin by an official Mexican animal ID present on their ears at the time of slaughter, an "M" brand, or through epidemiologic investigation. Sometimes the investigation can determine the specific Mexican state of origin. Since 2003, the number of Mexican-origin cases identified at slaughter has decreased. This is consistent with the decrease in cattle entering the United States. Annually 1-2 cases per 100,000 head of cattle imported are identified (Figure 3.4).

Figure 3.4 The number of Mexican-origin slaughter cases identified per 100,000 head of cattle imported from Mexico, 2001-2008



3.3.5 Mexico cattle imports by State of origin

In FY 2007, almost 85 percent of cattle imported from Mexico came from five Mexican States:[7] Chihuahua (31 percent), Sonora (23 percent), Tamaulipas (17 percent), Coahuila (9 percent), and Nuevo Leon (5 percent). Four of the States border the United States. Northern Sonora has MAA status, Southern Sonora has MA status, Tamaulipas has MA status, and Chihuahua, Coahuila, and the nonbordering State of Nuevo Leon have MA status and a few nonaccredited zones.

Cattle entering from Chihuahua and Durango move through the San Jeronimo port in New Mexico. While the majority of these cattle remain in Texas and New Mexico some cattle are shipped as far as Mississippi, Idaho, and Oregon (Skaggs et al., 2004).

3.3.6 Illegal entry of Mexican cattle

Although data systems that collect information on illegal entry of Mexican-origin cattle are scarce, anecdotal references about illegal entry do exist. Reports of illegal entry go as far back as 1893 (NY Times, 1893). Another historical article mentions the arrest of a cattle smuggler who was charged by U.S. Customs in 1953 (ISA Cattle Company, 2009). An article in 2006 mentions one U.S. ranching family near the border turning back 468 trespassing cows from Mexico over a 2.5-year period (Associated Press, 2009). An article in 1999 reported on ranchers concerned about stray Mexican cattle causing disease and damage to crops and property (LA Times, 2009). The same article referred to stray Mexican cattle damaging onion fields, costing thousands of dollars. Smuggled cattle have also been identified through an ongoing cattle fever tick control and eradication program in some U.S. border States (Pelzel, 2005, Wagner, 2002).

[7] Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria.

3.4 U.S. cattle imports from Canada

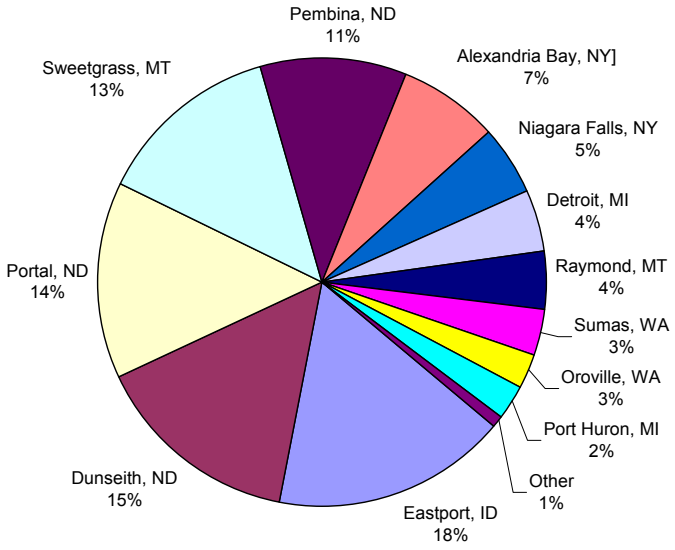
3.4.1 Canadian cattle imports by State of destination

In 2008, cattle imports from Canada totaled 1,586,499.[3] The top five destination States were Nebraska (424,446), Washington (350,921), Pennsylvania (178,450), Utah (146,259), and Minnesota (120,017). These States represent 77 percent of all Canadian cattle imports.

3.4.2 Canadian cattle imports by type and port

Of the cattle imported from Canada in 2008, 57 percent were destined for slaughter, 39 percent were feeder cattle, and 3 percent were for breeding. Most cattle imports from Canada enter through the land border ports of Idaho, North Dakota, and Montana. Five ports in these States account for 70 percent of all Canadian cattle imports[3]: East Port, Idaho (17 percent), Dunseith, North Dakota (15 percent), Portal, North Dakota (14 percent), Sweetgrass, Montana (13 percent) and Pembina, North Dakota (11 percent) (Figure 3.5).

Figure 3.5 Percentage of Canadian cattle imports, by U.S. port of entry, FY 2008



Source: USDA-APHIS-VS Import Tracking System

3.4.3 Canadian cattle exports to the U.S. by province of origin

In 2008, cattle exports to the United States originated in nine Canadian provinces. The top four exporting provinces were Alberta (30 percent), Manitoba (22 percent), Saskatchewan (22 percent), and Ontario (13 percent) (World Trade Atlas, 2009). All four provinces are along the United States-Canada border and cover approximately 75 percent of the border.

3.4.4 Canadian cattle populations with TB

Canada's animal health officials consider all of their provinces as TB-free. The Riding Mountain Eradication Area within Canada's Riding Mountain National Park (Manitoba) continues to undergo increased TB testing because of the higher risk in this area. APHIS has classified Manitoba as MAA for import purposes (NCIE, 2009).

VS collects slaughter data on animals with lesions consistent with bovine TB. In 2008, only three culture-positive animals were traced back to Canada (British Columbia). Two of these animals were

linked by the same owner. In total, there have only been five cases of bovine TB tracebacks to Canada in the past 7 years.

3.4.5 Illegal Canadian cattle imports to the United States

Illegal movement of cattle also occurs at the Canadian border. On August 25, 2004, two men were caught smuggling six Canadian cattle across the border into New York (High Plains Midwest Ag Journal, 2009). In June 2003, a rancher with properties in Alberta, Canada and Montana tried to smuggle 24 rodeo bulls from Canada through the Port of Del Bonita, Montana. In January 2004, the same rancher brought in an additional six bulls by the same route (Great Falls Tribune, 2004). The rancher told officials at the port that he was only bringing in horses (CBC News, 2004). These incidents occurred during the United States ban on importing live Canadian cattle because of BSE.

3.5 Summary

Approximately twice as many cattle are imported into the United States from Canada than from Mexico. Canadian cattle are considered to present a low risk of TB introduction to U.S. cattle due to the low prevalence of TB in Canada.

Bovine TB in the United States has been linked to Mexico for several years through epidemiologic investigation and genotyping of *M. bovis* isolates. There are many instances in which cattle legally imported from Mexico were later found to be positive for bovine TB at slaughter, likely due to the poor sensitivity of the original screening test. After importation Mexican rodeo cattle off the rodeo circuit are often pastured with domestic cattle, creating the opportunity for exposure and transmission of bovine TB. Many feeder cattle imported from Mexico pose the same problem due to management practices in the United States which present opportunities for commingling.

Cattle imported legally are subjected to tests and regional restrictions established by Mexico, and the United States continues to work with Mexico to improve its bovine TB status. The United States and Mexico recently outlined a 5-year strategic plan (Strategic Plan for Reducing the Risk of Importing Tuberculosis-Infected Cattle from Mexico, 2008–12, USDA–APHIS–VS) to help reduce the risk of bovine TB introduction into the United States from Mexico. From 2001 through February 2009, 236 out of 329 slaughter cases were traced to Mexico. The historical trend of positive bovine TB slaughter cases traced back to Mexico seems to be decreasing proportionally with the decrease of cattle imports from Mexico.

Illegal cattle entries from Mexico pose a risk to the U.S. cattle industry but this is difficult to quantify due to a lack of available data. Illegal cattle are not tested and may originate from any region in Mexico that may or may not have cattle with bovine TB. The Mexican States bordering the United States are recognized as MA or MAA status. The higher-risk zones in these bordering States are not located near the border. The likelihood of illegal cattle movements from higher risk zones is low due to geographic proximity. These entries occur by stray cattle crossing the border in remote areas of Arizona, New Mexico, and Texas where fencing is limited or damaged, or through intentional entry by smugglers. These areas may be remote; however there is almost always a U.S. cattle ranch in the vicinity. If illegal Mexican cattle commingle with cattle from these ranches, the U.S. cattle may be exposed to bovine TB and may go undetected until U.S. ranchers take their cattle to slaughter.

The importation of bovine TB, particularly from Mexican-origin animals, continues to be of concern. Many *M. bovis*-positive animals identified through slaughter channels are traced to Mexico and little information is available on where native U.S. cattle exposure to Mexican-origin animals may occur. Surveillance could target these areas if additional information is gained as to where mixing of U.S. and Mexican cattle occurs.

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4. U.S. Cattle Industry Practices

4.1 Introduction

Multiple management practices associated with transmission of bovine TB among cattle herds have been identified in the scientific literature (Table 4.1). Successful transmission of disease once exposure occurs is highly dependent on the animal's immune status, the herd's stocking density replacement strategies, and the farm's housing, fencing, and feed storage facilities (Phillips, 2002). All actions at the farm level aimed at excluding disease from the herd are referred to as biosecurity, while biocontainment refers to efforts aimed at controlling the spread within and between groups once the agent is present on the operation (Maunsell, 2008). Management choices resulting in suboptimal biosecurity practices may place the operation at higher risk of disease introduction.

Purchasing animals as herd additions may place the herd at increased risk of disease introduction. Purchased animals were identified as the most likely source of bovine TB in all but 1 of 31 affected herds in Northeast England (Gopal, 2006). Herds in this region were repopulated using sources throughout the United Kingdom (UK) after the foot-and-mouth disease outbreak in 2001. The risk of disease introduction was attributed to a larger number of animal sources, greater geographic distribution of animal sources, and more circuitous routes taken by newly arrived animals (i.e., more potential for exposure during commingling). In 11 of 31 affected herds, reactors were estimated to be 1 year-of-age or younger when they moved from their most likely source holding; therefore, test ineligible animals (those less than 12 months-of-age) should not be overlooked as a source of infection.

In an analysis of nine outbreaks of *M. bovis* among cattle and cervid herds in Canada, risk factors for disease spread among herds were identified: sourcing animals from affected herds, contact with infected animals in neighboring herds, and increasing herd size (Munroe, 1999). Herds that were investigated because they had received animals from a reactor or affected herd[8] (trace-outs) had the highest risk of a positive disease outcome when compared with herds tested within a given radius of an affected herd (area testing). Herds investigated because of pasture or fence-line contact with neighboring affected herds (traceback) or at the request of the owner (because the owner suspected contact with infected animals) also demonstrated higher risk when compared with area testing. The authors concluded that the movement of animals or direct contact with neighboring affected herds greatly increased the risk of spreading bovine TB. Larger herds were thought to be associated with management practices that increase the risk of transmission of bovine TB. The greater potential for movement of animals from a larger number of sources is realized in larger herds due to a need for large numbers of replacement animals and breeding stock.

Dispersal of infected cattle through sales of replacements from areas in which affected herds are associated with a wildlife reservoir may be one explanation for the spread of bovine TB from Southeast England into other parts of the country. In Great Britain, cattle movement patterns were consistently shown to better predict bovine TB distributions than other variables (Gilbert, 2005). The predictive power of this variable exceeded that of distance to previous disease cases, more so than the total number of movements onto a farm and number of movements from an infected area.

[8]An affected herd was one with at least one culture-positive animal. A reactor herd had at least one animal that was positive or suspicious on mid-cervical, comparative cervical, or gross or histopathological test for *M. bovis*.

Table 4.1 Management practices listed in the literature related to increased risk of bovine TB

Risk factors evaluated and identified as significant	Reference
Purchased animals identified as the most likely source in 30/31 affected herds: • Wide range in the number of source operations that supplied cattle to the herd • Imported animals • Source animals were 1-year-old or younger • Herds restocked after depopulation	Gopal et al., 2006
Movements from areas in which bovine TB is reported outperform other predictors of disease occurrence (long distance jump-spread) • Proportion of movements from affected areas arriving at a location outside the endemic core are most closely associated with disease presence	Gilbert et al., 2005
Outside of wildlife endemic areas, the majority of TB incidents were attributed to purchased cattle and contiguous spread • The herd incidence, duration of incident, and number of reactors in confirmed incidents increase significantly with herd size	Goodchild et al., 2001
Increasing herd size (beef and dairy): • 16-35 (odd ratio (OR) 2.9) • 36-80 (OR 5.8) • >80 animals (OR 9.3) Reason for investigation as part of the outbreak: • Herds that purchased animals from a reactor/positive herd (OR 57.8) • Herds that had been a source of animals for a reactor/positive herd (OR 14.9) • Pasture or fence-line contact with a reactor/positive herd (OR 31.8)	Munroe et al., 1999
Cattle purchase (OR 5.8) The presence of mixed (dairy and beef) operations (OR 4.9) compared with dairy or beef only operations	Marango et al., 1998

4.2 The role of management practices in current U.S. bovine TB outbreaks

Recent outbreaks of bovine TB in California and New Mexico have not been attributed to a wildlife reservoir. Dispersal of cattle from regions of the United States where bovine TB is maintained in a wildlife reservoir may be a plausible hypothesis for the introduction of bovine TB into new geographic areas. However, molecular analyses show no connection between the TB strains in California or New Mexico and wildlife reservoirs have been identified. Dairy and beef herds have been identified as affected (Table 4.2).

Table 4.2 Number of herds declared affected, by herd type FY 1998–through April 2009

Herd Type	Number of Herds
Beef	53
Dairy	24
Mixed	2
Cervid	5

Source: USDA–APHIS–VS (unpublished).

Exposure of U.S. cattle to cattle of Mexican origin has also been suggested as one source of disease introduction. In the recent outbreak in New Mexico, the exposure of a dairy cow that had been initially purchased at an auction market and was housed in a feed yard with beef cattle ultimately became infected, suggesting that this exposure resulted in TB transmission. This reconditioned cow was transported illegally to Texas and sold at a replacement sale where she tested positive for bovine TB before being transported back to New Mexico. A reconditioned cow is a milk cow that was culled from a herd and sent to a cull-cow sale then purchased and diverted from slaughter channels. The purchaser is often a dealer who returns the cow to an acceptable body condition and health status then sells her to another producer to be milked.

The time devoted to planning and sourcing cattle is a small fraction of the time spent planning a dairy expansion (Faust, 2001). Areas where dairies are expanding may be at increased risk as they attempt to source replacements from other areas of the United States producing surplus heifers or from dispersal sales in areas where the industry is contracting.

In the United States, the movement of purchased cattle from farm to farm occurs over large geographic areas. For example, as reported in the NAHMS Beef 2007–08 Study, 19.2 percent of the 24.1 percent of beef cow-calf operations that brought cattle or calves onto the operation reported that arriving shipments traveled 100 or more miles (USDA, 2009). A higher percentage of beef cow-calf operations in the West region[9] (26.4 percent) than in the East region (9.7 percent) of the United States reported that arriving shipments traveled 100 miles or more. Similar movement data are not available for the dairy industry.

The commingling of cattle from different sources creates opportunities for bovine TB exposure and transmission. Purchased lots of cattle may originate from several herds. Animals are grouped in truck-load sized lots (50,000 lb). To reach a trucking threshold, cattle purchased from areas with relatively small herd sizes are most likely commingled with animals from other sources (Thomson, 2006). These cattle have a higher probability of being exposed to disease because of the increased number of source herds.

4.3 Management practices related to TB risk in the beef cow-calf industry

The NAHMS Beef 2007–08 study, conducted in 24 States representing 79.6 percent of U.S. cow-calf operations and 87.8 percent of U.S. beef cows, provides information on management practices that

[9] Beef 2007-08 study States/Regions:
West: California, Colorado, Idaho, Montana, New Mexico, Oregon, Wyoming.
Central: Iowa, Kansas, Missouri, Nebraska, North Dakota, South Dakota
South Central: Oklahoma, Texas
East: Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, Tennessee, Virginia

could be used to estimate the percentage of U.S. beef cow-calf operations engaging in practices that may increase the risk of exposing their herds to TB (Wells, 2000). The following describes the results of the NAHMS Beef study as it relates to bovine TB.

4.3.1 TB awareness in the beef cow-calf industry

In the NAHMS Beef 2007–08 study, producers were asked about their familiarity with a number of cattle diseases. Producers on one-half of the operations (50.1 percent) knew some basics or were fairly knowledgeable about bovine TB. On nearly one-third of operations (31.4 percent), producers recognized the name of the disease but not much else (Table 4.3).

Table 4.3 Percentage of cow-calf operations by level of producer familiarity with bovine TB

Percent Operations								
Level of Familiarity								
Fairly Knowledgeable		Know Some Basics		Recognized Name, Not Much Else		Had Not Heard of Before		
Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Total
22.8	(1.2)	27.3	(1.3)	31.4	(1.3)	18.5	(1.1)	100.0

Approximately 1 of 20 operations that brought cattle onto the operation during the previous 3 years required testing for TB (5.4 percent) (Table 4.4). The percentage of operations required testing of new cattle for bovine TB was similar across herd sizes. These numbers reflect voluntary testing, not federally- or State-required testing.

Table 4.4 Of cow-calf operations that introduced new cattle during the previous 3 years, percentage that required TB testing for new cattle

Percent Operations									
Herd Size (Number of Beef Cows)									
1-49		50-99		100-199		200 or More		All Operations	
Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
5.3	(2.6)	4.5	(2.6)	7.6	(2.9)	5.3	(1.9)	5.4	(1.7)

Of operations that brought cattle onto the operation during the previous 3 years, a higher percentage in the Southeast region (7.3 percent) required testing for TB compared with operations in the West (0.7 percent) and Central (3.0 percent) regions.

4.3.2 Introduction of cattle onto beef cow-calf operations

Producers may choose to replace a portion of their breeding herd or purchase and feed other classes of cattle to take advantage of long- or short-run economic market conditions. For these reasons and others, cattle movement occurs from farm to farm, either by direct sales or through livestock auction markets within the beef cow-calf industry.

The percentage of operations that brought on any class of cattle during the 12 months preceding the NAHMS Beef 2007-08 study interview increased as herd size increased, with just over 1 of 4 of operations with 1 to 49 cows (27.6 percent) bringing cattle onto the operation and nearly 7 of 10 of operations with 200 or more cows (69.9 percent) bringing cattle onto the operation (Table 4.5). More than one third of operations (34.5 percent) brought on any class of cattle.

Table 4.5 Percentage of cow-calf operations that brought any beef or dairy cattle or calves onto the operation during the previous 12 months, by cattle class and by herd size

Percent Operations Herd Size (Number of Beef Cows)										
	1-49		50-99		100-199		200 or more		All operations	
Cattle Class	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
Preweaned beef calves w/dam	2.9	(0.6)	3.2	(1.0)	7.7	(1.7)	4.2	(1.0)	3.4	(0.5)
Beef heifers weaned, not bred	5.6	(0.9)	6.8	(1.4)	8.3	(1.6)	10.9	(1.5)	6.2	(0.7)
Bred beef heifers	2.4	(0.6)	4.8	(1.3)	8.1	(1.5)	7.9	(1.5)	3.5	(0.5)
Beef cows (pregnant)	7.4	(1.0)	12.6	(2.0)	13.9	(2.1)	17.5	(2.4)	9.2	(0.8)
Beef cows (not pregnant)	2.0	(0.5)	2.7	(1.0)	5.5	(1.5)	6.0	(1.3)	2.6	(0.4)
Weaned beef bulls	14.9	(1.3)	25.7	(2.5)	33.6	(2.7)	43.1	(2.6)	19.5	(1.0)
Weaned steers (all types)	2.9	(0.6)	2.8	(1.0)	4.3	(1.1)	4.9	(1.0)	3.1	(0.5)
Preweaned dairy calves	0.2	(0.1)	2.2	(1.2)	0.0	(--)	0.2	(0.2)	0.5	(0.2)
Weaned dairy heifers/cows	0.2	(0.1)	1.6	(0.9)	0.4	(0.3)	0.3	(0.2)	0.4	(0.2)
Weaned dairy bulls	0.6	(0.3)	0.0	(--)	0.0	(--)	0.5	(0.2)	0.4	(0.2)
Any	27.6	(1.6)	43.0	(2.8)	58.0	(2.9)	69.9	(2.3)	34.5	(1.3)

Overall, the percentage of cattle and calves brought on operations was approximately one-half (47.8 percent) of the beef cows in inventory. This estimate is greatly influenced by the 3.1 percent of operations that brought on weaned steers in numbers that greatly exceeded their beef cow inventory. Nearly one-half of cattle brought on (49.9 percent) were weaned steers, followed by weaned but not bred beef heifers (15.5 percent), and pregnant beef cows (14.4 percent). Weaned steers accounted

for a higher percentage of cattle and calves brought on operations with 1 to 49 cows (75.4 percent) compared with operations with 200 or more cows (28.7 percent).

For operations that brought any cattle or calves onto the operation during the previous 12 months, about one of three (34.8 percent) brought cattle onto the operation from a sale barn or auction (Table 4.6). Most operations (70.3 percent) brought cattle directly from another beef operation(s).

Table 4.6 For cow-calf operations that brought any cattle or calves onto the operation during the previous 12 months, percentage of operations by source of cattle and calves and by herd size

	Percent Operations Herd Size (Number of Beef Cows)									
	1-49		50-99		100-199		200 or more		All operations	
Source	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
Sale barn/auction	30.2	(3.2)	39.8	(4.4)	43.0	(3.7)	40.7	(3.4)	34.8	(2.1)
Directly from another beef operation	75.0	(3.0)	62.5	(4.3)	62.4	(3.6)	68.7	(3.1)	70.3	(2.0)
Directly from a dairy operation	3.7	(1.3)	8.4	(3.2)	0.6	(0.4)	0.4	(0.3)	3.8	(1.0)
Other	3.8	(1.4)	5.8	(2.2)	2.2	(1.0)	1.2	(0.8)	3.7	(0.9)

4.3.3 Beef cattle contact with other animals

Disease agents can be brought onto an operation by animals newly introduced to the herd, through contact with animals that are not part of the operation, or by inanimate objects such as equipment. Nearly all operations (96.3 percent) reported that at least some beef cattle had fence-line (nose-to-nose) contact or commingled with one or more of the animals listed in Table 4.7. More than two-thirds of operations reported beef cattle contact with wild cervids and dogs (72.6 and 69.7 percent, respectively). Beef cattle on approximately one-half of the operations had contact with cats and horses (55.4 and 44.5 percent, respectively).

Table 4.7 Percentage of cow-calf operations by whether or not any beef cattle on the operation had fence-line contact (nose-to-nose) or commingled with the following animals during the previous 12 months.

	Percent Operations						
	Contact						
	Yes		Don't Know		No		
Animal Type	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Total
Wild cervids (e.g., elk, deer)	72.6	(1.3)	12.0	(0.9)	15.4	(1.1)	100.0
Captive cervids (e.g., elk, deer)	3.2	(0.5)	2.5	(0.4)	94.3	(0.6)	100.0
Captive bison	0.9	(0.3)	0.9	(0.2)	98.2	(0.3)	100.0
Mexican-origin cattle	1.0	(0.3)	2.1	(0.4)	96.9	(0.5)	100.0
Dairy cattle	3.1	(0.5)	1.5	(0.3)	95.4	(0.6)	100.0
Pigs	12.1	(0.9)	4.8	(0.6)	83.1	(1.1)	100.0
Sheep	5.2	(0.6)	1.3	(0.3)	93.5	(0.7)	100.0
Goats	9.6	(0.9)	1.6	(0.3)	88.8	(0.9)	100.0
Horses or other equids (e.g., ponies, donkeys, mules, burros, etc.)	44.5	(1.4)	2.8	(0.5)	52.7	(1.4)	100.0
Camelids (e.g., llamas, alpacas, etc.)	2.8	(0.5)	1.4	(0.3)	95.8	(0.6)	100.0
Chickens, other poultry, or their litter	15.3	(1.0)	2.3	(0.4)	82.4	(1.1)	100.0
Dogs	69.7	(1.3)	9.0	(0.8)	21.3	(1.2)	100.0
Cats	55.4	(1.4)	12.3	(0.9)	32.3	(1.3)	100.0
Any of the above	96.3	(0.6)	0.1	(0.1)	3.6	(0.6)	100.0

Nearly all beef cows (97.8 percent) were on operations in which at least some beef cattle had fence-line contact or commingled with 1 or more of the animals listed below, and approximately 9 of 10 cows (88.0 percent) were on operations in which contact occurred with wild cervids (Table 4.8). Dogs were the next most common animal that beef cattle had exposure to, followed by cats and horses. Fewer than 1 of 20 cows were on operations in which any beef cattle had exposure to camelids, dairy cattle, cattle of Mexican origin, captive bison, or captive cervids.

Table 4.8 For cow-calf operations in which beef cattle had fence-line contact (nose-to-nose) or commingled with the following animals during the previous 12 months, percentage of beef cattle on these operations.

Animal Type	Percent Beef Cows	Standard Error
Wild cervids (e.g., elk, deer)	88.0	(0.9)
Captive cervids (e.g., elk, deer)	3.9	(0.6)
Captive bison	2.1	(0.5)
Cattle of Mexican origin	1.8	(0.4)
Dairy cattle	3.8	(0.5)
Pigs	15.4	(1.0)
Sheep	6.9	(0.8)
Goats	7.8	(0.7)
Horses or other equids (e.g., ponies, donkeys, mules, burros, etc.)	58.2	(1.3)
Camelids (e.g., llamas and alpacas)	2.9	(0.5)
Chickens, other poultry, or their litter	13.5	(0.9)
Dogs	79.9	(1.1)
Cats	63.9	(1.4)
Any of the above	97.8	(0.3)

For operations that were aware of fence-line contact with the specific animals, the percentage of operations in which beef cattle had fence-line contact or commingled with wild cervids was higher on operations with 200 or more cows than on operations with 1 to 49 cows. A higher percentage of operations with 200 or more cows reported beef cattle had contact with horses than did all other operation sizes.

Beef cattle can be exposed to disease agents through feedstuffs contaminated by other animals. More than 8 of 10 operations (81.2 percent) were aware that 1 or more of the animals listed in Table 4.9 had access to cattle feed or minerals. Wild cervids most commonly had access to cattle feed or minerals, followed by dogs, cats, and horses or other equids.

Table 4.9 Percentage of cow-calf operations in which the following animals had access to the operations' cattle feed or minerals during the previous 12 months

	Percent Operations						
	Yes		Don't Know		No		
Animal type	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Total
Wild cervids (e.g., elk, deer)	63.3	(1.4)	6.2	(0.7)	30.5	(1.3)	100.0
Captive cervids (e.g., elk, deer)	2.5	(0.4)	1.2	(0.3)	96.3	(0.5)	100.0
Captive bison	0.3	(0.1)	0.5	(0.2)	99.2	(0.2)	100.0
Mexican-origin cattle	0.3	(0.1)	0.8	(0.2)	98.9	(0.2)	100.0
Dairy cattle	1.6	(0.4)	0.5	(0.1)	97.9	(0.4)	100.0
Pigs	7.8	(0.8)	2.2	(0.4)	90.0	(0.9)	100.0
Sheep	3.2	(0.5)	0.6	(0.2)	96.2	(0.5)	100.0
Goats	5.4	(0.7)	0.9	(0.3)	93.7	(0.7)	100.0
Horses or other equids (e.g., ponies, donkeys, mules, burros)	27.5	(1.3)	1.1	(0.3)	71.4	(1.3)	100.0
Camelids (e.g., llamas and alpacas)	1.9	(0.4)	0.8	(0.2)	97.3	(0.4)	100.0
Chickens, other poultry, or their litter	7.9	(0.8)	1.5	(0.3)	90.6	(0.8)	100.0
Dogs	44.1	(1.4)	8.7	(0.8)	47.2	(1.4)	100.0
Cats	39.0	(1.4)	10.7	(0.9)	50.3	(1.4)	100.0
Any of the above	81.2	(1.2)	0.2	(0.1)	18.6	(1.2)	100.0

Approximately 9 of 10 cows (88.8 percent) were on operations in which 1 or more of the animals listed in Table 4.10 had access to cattle feed or minerals. Nearly 8 of 10 cows (79.5 percent) resided on operations in which wild cervids were known to have access to cattle feed or minerals.

Table 4.10 For cow-calf operations in which the following animals had access to the operations' cattle feed or minerals during the previous 12 months, percentage of beef cows on these operations

Animals	Percent Beef Cows	Standard Error
Wild cervids (e.g., elk, deer)	79.5	(1.0)
Captive cervids (e.g., elk, deer)	3.0	(0.5)
Captive bison	1.1	(0.4)
Mexican-origin cattle	0.8	(0.2)
Dairy cattle	1.7	(0.3)
Pigs	10.4	(1.0)
Sheep	3.8	(0.6)
Goats	4.9	(0.6)
Horses or other equids (e.g., ponies, donkeys, mules, or burros)	36.0	(1.3)
Camelids (e.g., llamas and alpacas)	1.9	(0.4)
Chickens, other poultry, or their litter	7.4	(0.7)
Dogs	52.2	(1.4)
Cats	45.4	(1.4)
Any of the above	88.8	(0.8)

4.4 Management practices related to TB risk in the dairy industry

Results from the NAHMS Dairy 2007 Study

The NAHMS Dairy 2007 study was conducted in 17 of the Nation's major dairy States.[10] States in the western United States have shown the largest growth in the number of milk cows since 1992. Arizona, California, Colorado, Idaho, Kansas, Nevada, New Mexico, Oregon, and Utah have all increased dairy cow numbers since 1992. States in the southeast, including Alabama, Arkansas, Louisiana, and Mississippi, had the largest percentage decline in dairy cows, but these States represented less than 5 percent of the overall dairy population. In 2007, California had the largest number of dairy cows (1.79 million) followed by Wisconsin (1.245 million), and New York (628,000).

[10] Dairy 2007 States/Regions
West Region: California, Idaho, Texas, New Mexico and Washington
East Region: Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia and Wisconsin

4.4.1 The introduction of cattle onto dairy operations

Herd replacements or additions can serve as reservoirs of economically important infectious diseases for the adult herd (Maunsell, 2008). Since dairy heifers in North America are typically raised in continuous flow systems (versus an all in-all out approach), ample opportunity exists for the introduction of infectious disease agents and transmission within and among age groups (Maunsell, 2008).

From 1996 to 2007, approximately 4 of 10 operations brought cattle onto the operation (Table 2.14). Cattle are brought onto operations for several reasons: as a source of replacements for culled cows or heifers (whether purchased or born on the operation and raised offsite), to replace mortality losses, and to increase herd size.

Almost 4 of 10 operations (38.9 percent) brought at least 1 new addition onto the operation during 2006 (Table 4.11). Approximately one of eight operations brought on bred dairy heifers, lactating dairy cows, or dairy bulls (12.2, 13.8, and 12.5 percent, respectively). A lower percentage of large operations brought on preweaned calves compared to small operations (1.0 and 3.8 percent, respectively), but a higher percentage of large operations brought on dairy heifers, bred dairy heifers, dairy bulls, and "any beef or dairy cattle" compared with medium or small operations.

Table 4.11 Percentage of dairy operations that brought the following classes of cattle onto the operation during 2006, by herd size

	Percent Operations							
	Herd Size (Number of Dairy Cows)							
	Small (Fewer than 100)		**Medium** (100-499)		**Large** (500 or More)		**All Operations**	
Cattle Class	**Pct.**	**Std. Error**	**Pct.**	**Std. Error**	**Pct.**	**Std. Error**	**Pct.**	**Std. Error**
Preweaned calves (dairy or beef)	3.8	(0.8)	2.5	(0.6)	1.0	(0.3)	3.4	(0.6)
Dairy heifers (weaned but not bred)	5.3	(0.8)	7.6	(1.2)	16.3	(2.6)	6.4	(0.7)
Bred dairy heifers	8.9	(1.0)	18.1	(1.8)	34.7	(2.6)	12.2	(0.9)
Lactating dairy cows	13.2	(1.3)	16.0	(1.7)	13.0	(1.9)	13.8	(1.0)
Dry dairy cows	4.1	(0.8)	4.3	(0.9)	5.5	(1.5)	4.3	(0.6)
Beef heifers and cows	0.9	(0.3)	2.5	(0.7)	1.1	(0.6)	1.3	(0.3)
Dairy bulls (weaned)	11.4	(1.1)	14.1	(1.6)	22.5	(2.4)	12.5	(0.9)
Beef bulls (weaned)	1.5	(0.4)	2.2	(0.6)	1.5	(0.5)	1.7	(0.3)
Steers (weaned)	2.0	(0.5)	1.3	(0.5)	0.7	(0.6)	1.8	(0.4)
Any cattle	35.6	(1.7)	44.3	(2.3)	61.6	(2.8)	38.9	(1.4)

On the majority of operations (89.8 percent) cow replacements born and raised on the operation entered the milking string during 2006 (Table 4.12). Replacements accounted for more than one-third of cow inventory (38.4 percent). Almost all operations (97.0 percent) had some replacements enter

the milking string during 2006. Just over 14 percent of operations sourced replacements from cattle born off the operation.

Table 4.12 Percentage of dairy operations and percentage cow inventory, by source of cow replacements that entered the milking string in 2006

Replacement Source	Percent Operations	Standard Error	Percent Dairy Cows*	Standard Error
Born and raised on operation	89.8	(0.8)	27.8	(0.8)
Born on operation raised off operation	6.8	(0.6)	8.0	(0.7)
Born off operation	14.1	(1.0)	2.6	(0.2)
Any replacements	97.0	(0.5)	38.4	(0.8)

*Number of replacements that entered the milking string during 2006, as a percentage of the January 1, 2007, cow inventory.

4.4.2 Heifers raised off the operation

Raising heifers at a separate site (calf ranches) has many potential advantages. Calf-ranch personnel are usually dedicated to working only with calves, which can result in increased attention to the feeding and health of calves and also decreased exposure to adult cow disease. If calves are not commingled with older animals or animals from other operations, their exposure to disease agents is reduced.

Fewer than 1 of 10 operations (9.3 percent) raised any heifers off the operation (Table 4.13). The percentage of operations that raised heifers offsite increased as herd size increased for all heifer classes. Less than 5 percent of small operations raised any heifers offsite, compared with 15.5 percent of medium operations and 46.0 percent of large operations. Almost one-third of large operations (35.3 percent) raised preweaned calves offsite, compared with 7.1 percent of medium operations and 1.7 percent of small operations. Similar herd-size differences in the percentages of operations that raised heifers offsite were observed among all heifer classes.

Table 4.13 Percentage of dairy operations that raised any heifers off-site, by heifer class and by herd size

	Percent Operations							
	Herd Size (Number of Dairy Cows)							
	Small (Fewer than 100)		Medium (100-499)		Large (500 or More)		All Operations	
Heifer Class	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
Preweaned	1.7	(0.5)	7.1	(1.2)	35.3	(2.9)	4.6	(0.5)
Weaned	4.3	(0.7)	14.6	(1.6)	44.2	(2.9)	8.6	(0.7)
Bred	4.1	(0.7)	11.5	(1.5)	22.5	(2.3)	6.7	(0.6)
Any of the above	4.7	(0.7)	15.5	(1.7)	46.0	(2.9)	9.3	(0.7)

Approximately 8 of 10 operations that sent heifers offsite to be raised (81.1 percent) retained ownership of the heifers sent. A total of 9.4 percent of operations sold heifers sent offsite and repurchased the same animals while 9.5 percent of operations sold the animals sent and replaced them with different animals.

Ideally, heifer-raising facilities would house only animals from a single operation. When producers were asked to choose the description that best described their primary offsite rearing facility, more than one-fourth (27.7 percent) sent heifers to a single rearing facility where heifers did not have contact with cattle from other operations, but the majority (51.3 percent) sent heifers to a single rearing facility where heifers had contact with cattle from other operations (Table 4.14).

Table 4.14 Percentage of operations that sent heifers offsite to be raised by primary off-site rearing facility

Off-Site Rearing Facility	Percent Operations	Std. Error
Heifers sent to a single rearing facility and did not have contact with cattle from other operations	27.7	(3.3)
Heifers sent to multiple rearing facilities and did not have contact with cattle from other operations	8.5	(2.1)
Heifers sent to a single rearing facility and had contact (commingled) with cattle from other operations	51.3	(4.0)
Heifers sent to multiple rearing facilities and had contact (commingled) with cattle from other operations	12.5	(3.0)
Total	100.0	

For operations that brought beef or dairy cattle onto the operation during 2006, approximately 15 percent within each herd-size category required TB testing for incoming animals (Table 4.15).

Table 4.15 For the 38.9 percent of dairy operations that brought beef or dairy cattle onto the operation during 2006, percentage of operations that tested individual animals brought onto the operation for TB, by herd size

Percent Operations Herd Size (Number of Dairy Cows)							
Small (Fewer than 100)		Medium (100-499)		Large (500 or More)		All Operations	
Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
12.0	(1.8)	17.8	(2.7)	15.8	(2.3)	13.8	(1.4)

4.4.3 Liquid diets

Although TB of the udder has been shown to be uncommon in the United Kingdom (Goodchild, 2001), infected cows can transmit the disease to multiple calves in a pooled milk feeding system (Monies, 1999). In an outbreak of TB on a dairy farm in Cornwall, calves were fed raw milk. The herd was under TB restriction following a herd test that identified eight infected cattle, including five calves less than 3 months-of-age. Twelve of 22 in-contact calves had tuberculous lung lesions at slaughter. One cow culled from the herd was shown to have tuberculous abscesses throughout one-fourth of the udder. A typical udder infected with *M. bovis* may excrete tubercle bacteria to the extent of 5×10^2 to 5×10^5 per ml of milk (Zanini, 1998). Properly pasteurizing and handling waste (nonsaleable) milk or

saleable milk reduces pathogen loads without affecting milk quality. However, managing a pasteurization system that consistently provides high-quality nutrition to the calf with decreased pathogens is an intensive process and requires daily monitoring of equipment and the feeding system.

A variety of liquid diets are commonly offered to preweaned calves (Table 4.16). A higher percentage of large operations (28.7 percent) fed pasteurized waste milk compared with medium and small operations (3.0 and 1.0 percent, respectively). Small operations were more likely to feed unpasteurized whole (saleable) milk (32.2 percent) than medium and large operations (17.4 and 12.1 percent, respectively). Similar percentages of operations fed unpasteurized waste milk and unpasteurized whole (saleable) milk (30.6 and 28.0 percent, respectively).

Table 4.16 Percentage of dairy operations by type of liquid diet fed to heifers at any time prior to weaning in 2006, by herd size

	Percent Operations							
	Herd Size (Number of Dairy Cows)							
	Small (Fewer than 100)		Medium (100-499)		Large (500 or More)		All Operations	
Liquid Diet	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
Unpasteurized waste (nonsaleable) milk	32.2	(1.7)	25.7	(2.0)	27.6	(2.8)	30.6	(1.3)
Pasteurized waste (nonsaleable) milk	1.0	(0.3)	3.0	(0.9)	28.7	(2.7)	2.8	(0.3)
Unpasteurized whole (saleable) milk	32.2	(1.7)	17.4	(1.7)	12.1	(1.9)	28.0	(1.3)
Pasteurized whole (saleable) milk	1.3	(0.4)	1.6	(0.8)	2.0	(0.7)	1.4	(0.3)
Other	2.6	(0.6)	3.5	(0.9)	4.9	(1.8)	2.9	(0.5)

Pooling colostrum may increase calves' exposure to pathogens. Approximately one of five operations (21.0 percent) pooled colostrum (Table 4.17). As herd size increased, the percentage of operations that pooled colostrum also increased, ranging from 16.0 percent of small operations to 56.9 percent of large operations.

Table 4.17 For dairy operations that normally hand-fed colostrum, percentage of operations that pooled colostrum from more than one cow, by herd size

Percent Operations Herd Size (Number of Dairy Cows)							
Small (Fewer than 100)		Medium (100-499)		Large (500 or More)		All Operations	
Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
16.0	(1.7)	26.0	(2.4)	56.9	(3.1)	21.0	(1.3)

4.4.4 Dairy cattle contact with other animals

More than 40 percent of operations reported that cats, dogs, and deer or other members of the deer family had contact with cattle, their feed, and/or water supply (Table 4.18). Cattle on operations in the East region were more likely to have contact with sheep, beef cattle, cats, and deer compared with cattle on operations in the West region. Almost 4 of 5 operations in the West region (79.2 percent) and 9 of 10 operations in the East region (95.2 percent) reported that at least 1 of the listed animals had physical contact with cattle and/or their feed, minerals, or water.

Table 4.18 Percentage of dairy operations in which the following animals had physical contact with cattle or contact with their feed, minerals, or water supply, by region

	Percent Operations					
	Region					
	West		East		All Operations	
Animal Type	Percent	Std. Error	Percent	Std. Error	Percent	Std. Error
Chickens or other poultry	9.2	(2.1)	8.3	(0.8)	8.3	(0.8)
Horses or other equids	10.2	(2.2)	13.6	(1.1)	13.3	(1.0)
Pigs	2.0	(0.6)	2.0	(0.5)	2.0	(0.4)
Sheep	0.1	(0.1)	1.0	(0.3)	0.9	(0.3)
Goats	4.8	(1.6)	2.3	(0.4)	2.5	(0.4)
Beef cattle	5.1	(1.5)	11.8	(1.0)	11.3	(1.0)
Exotic species (e.g., llamas, alpacas, and emus)	1.0	(0.6)	0.7	(0.2)	0.7	(0.2)
Dogs	63.4	(2.7)	69.4	(1.4)	68.9	(1.3)
Cats	62.1	(2.8)	87.1	(1.0)	85.2	(0.9)
Deer or other members of the deer family (e.g., elk and moose)	20.9	(2.9)	51.6	(1.5)	49.3	(1.4)
Any animal	79.2	(2.0)	95.2	(0.6)	94.0	(0.6)

Cattle that have direct contact with deer could pose a risk of transmitting diseases such as bovine TB. For operations in which deer or members of the deer family had contact with cattle, their feed, or water, the majority (90.8 percent) reported that cattle could possibly or sometimes have face-to-face contact with deer. There were no differences by region in the percentage of operations that reported face-to-face contact with deer (Table 4.19).

Table 4.19 For operations in which deer had physical contact with cattle and/or their feed, minerals, or water supply, percentage of operations by frequency that members of the deer family had face-to-face contact with cattle, and by region

	Percent Operations					
	Region					
	West		East		All Operations	
Frequency	Percent	Std. Error	Percent	Std. Error	Percent	Std. Error
Never	4.8	(2.1)	9.4	(1.2)	9.2	(1.2)
Possibly	56.3	(8.0)	64.3	(2.1)	64.1	(2.0)
Sometimes	38.9	(7.9)	26.3	(1.9)	26.7	(1.9)
Total	100.0		100.0		100.0	

4.5 Summary

TB can be introduced to cattle herds in a variety of ways. Both cow-calf operations and dairy operations implemented management practices that increase or decrease the risk of TB infection. Practices which may increase the risk of introduction include:

- The introduction of new animals with unknown TB status onto the premises
- Exposure to wildlife or other domestic animals
- Exposure of feed or water to wildlife or other domestic animals
- Offsite heifer rearing or other practices where commingling occurs
- Feeding unpasteurized milk to calves

Approximately one-third of cow-calf and dairy producers introduce new cattle to their operations, thereby increasing the risk of introducing bovine TB and other significant cattle diseases to cattle herds. Additionally, cattle on these operations are frequently exposed to free-ranging deer (72.6 and 49.3 percent for cow-calf and dairy operations, respectively).

A subset of dairy operations (9.3 percent) raised heifers offsite, and 63.8 percent of these operations increase the risk of disease transmission by commingling calves with cattle from other operations.To decrease this risk, producers should minimize the number and source of incoming cattle, be aware of the status of source herds, conduct disease testing prior to exposing the home herd, decrease animal and feed contact with members of the deer family, and limit exposure of cattle to animals from other operations and unknown disease status.

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5. U.S. Captive Cervid Industry

5.1 Introduction

The 1992 *Assessment of risk factors associated with the introduction and spread of M. bovis in the United States* (USDA, 1992) included a discussion of the potential risks for the spread of *M. bovis* that could be associated with captive wildlife operations, including captive cervids. The purpose of this chapter is to provide an update of the 1992 risk assessment as it pertains to the captive cervid industry. Several options for consideration were identified in the risk assessment as suggestions to improve future risk assessments and improve the likelihood of detection of *M. bovis* in captive cervids through surveillance. These options and their current status are:

- *Acquire farm-level data for a more accurate assessment*
  Farm-level data have been difficult to acquire and this consideration has not been successfully pursued on a national level.
- *Establish procedures for tracking the movement of captive Cervidae*
  Tracking movements of captive cervids is currently limited to information obtained in health certificates prior to interstate movement and to the tuberculin testing reporting requirements for obtaining accredited herd status.
- *Consider slaughter indemnity for Cervidae*
  Slaughter indemnity is not currently available for Cervidae
- *Focus efforts to develop a reliable screening test for bovine TB in Cervidae*
  There has been progress made in developing a reliable serologic test for cervids; however, the tests are not yet available for general use (O'Brien et al., 2009).

5.2 Captive cervid distribution

The total number of captive cervids maintained on premises decreased in the United States between 2002 and 2007 (NASS, 2009) (Table 5.1). Between 2002 and 2007, the inventory of captive deer declined from 286,863 to 269,537 and the number of captive elk declined from 97,901 to 68,251. The total number of captive deer farms increased from 4,901 in 2002 to 5,654 in 2007, while the total number of captive elk farms decreased from 2,371 in 2002 to 1,917 in 2007. Comparisons for previous years are not available, as captive cervid inventory was not a part of the Census of Agriculture before 2002.

Table 5.1 Inventories of captive deer and elk, 2007

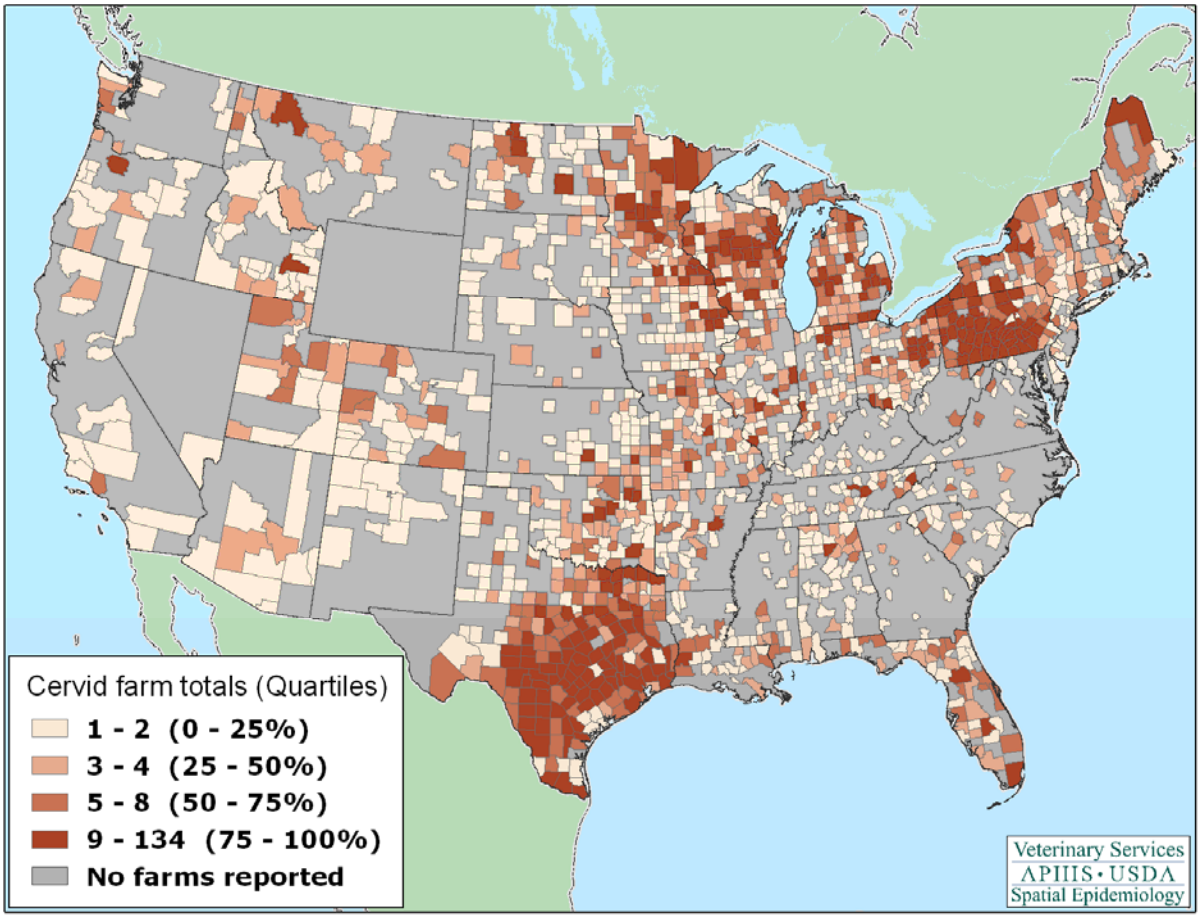
	2002 U.S. Inventory		2007 U.S. Inventory	
	Number of Premises	Number of Animals	Number of Premises	Number of Animals
Captive deer	4,901	286,863	5,654	269,537
Captive elk	2,371	97,901	1,917	68,251

Source: 2007 Census of Agriculture.

Notable increases in the number of captive deer farms occurred in Alabama (49 to 77), Missouri (137 to 204), Ohio (224 to 303), and Pennsylvania (525 to 810), while considerable decreases were seen in the number of captive deer farms in Michigan (403 to 304) and Wisconsin (512 to 250).

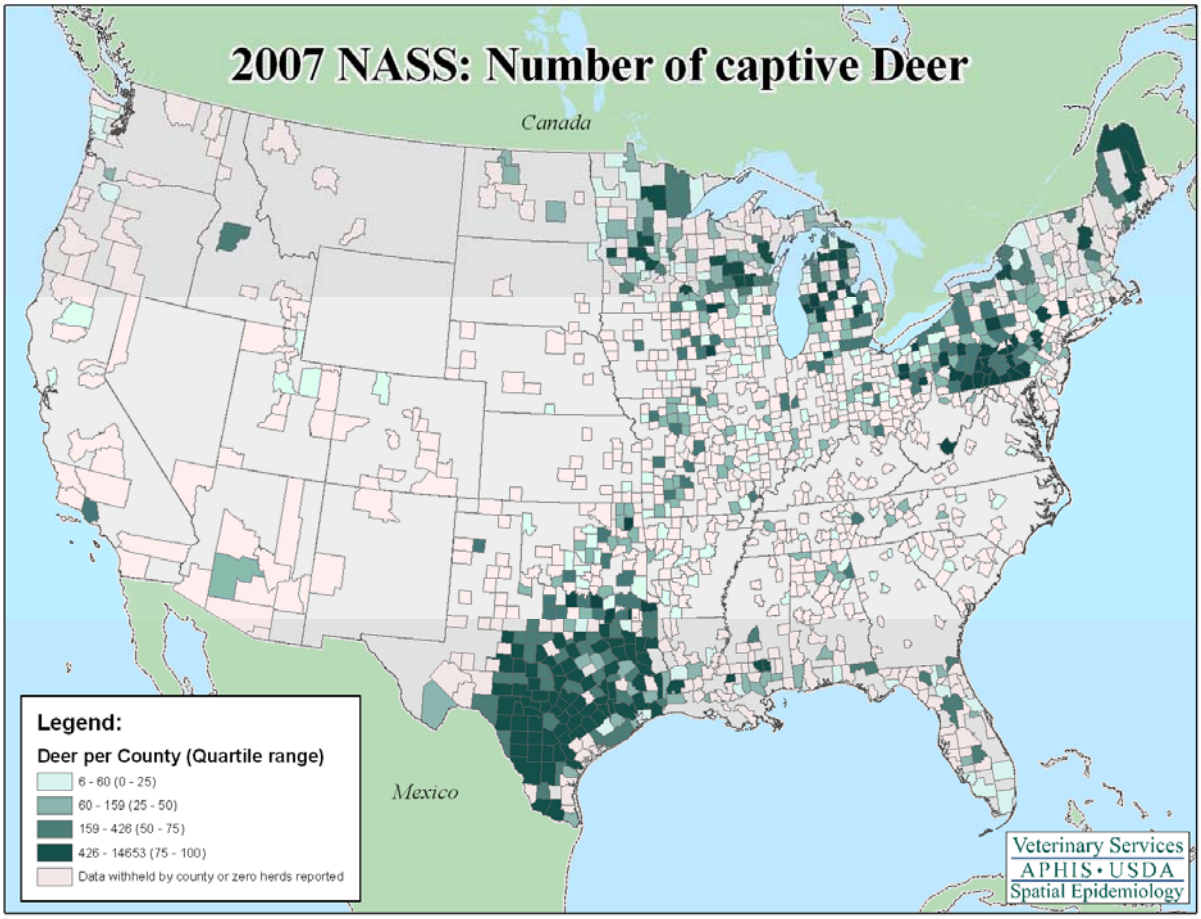
Figures 5.1 through 5.3 illustrate the location of captive cervids according the NASS 2007 Census of Agriculture. Figure 5.4 illustrates the location of cattle in the United States.

Figure 5.1 Number of captive cervid farms by county, 2007



Source: NASS 2007 Census of Agriculture

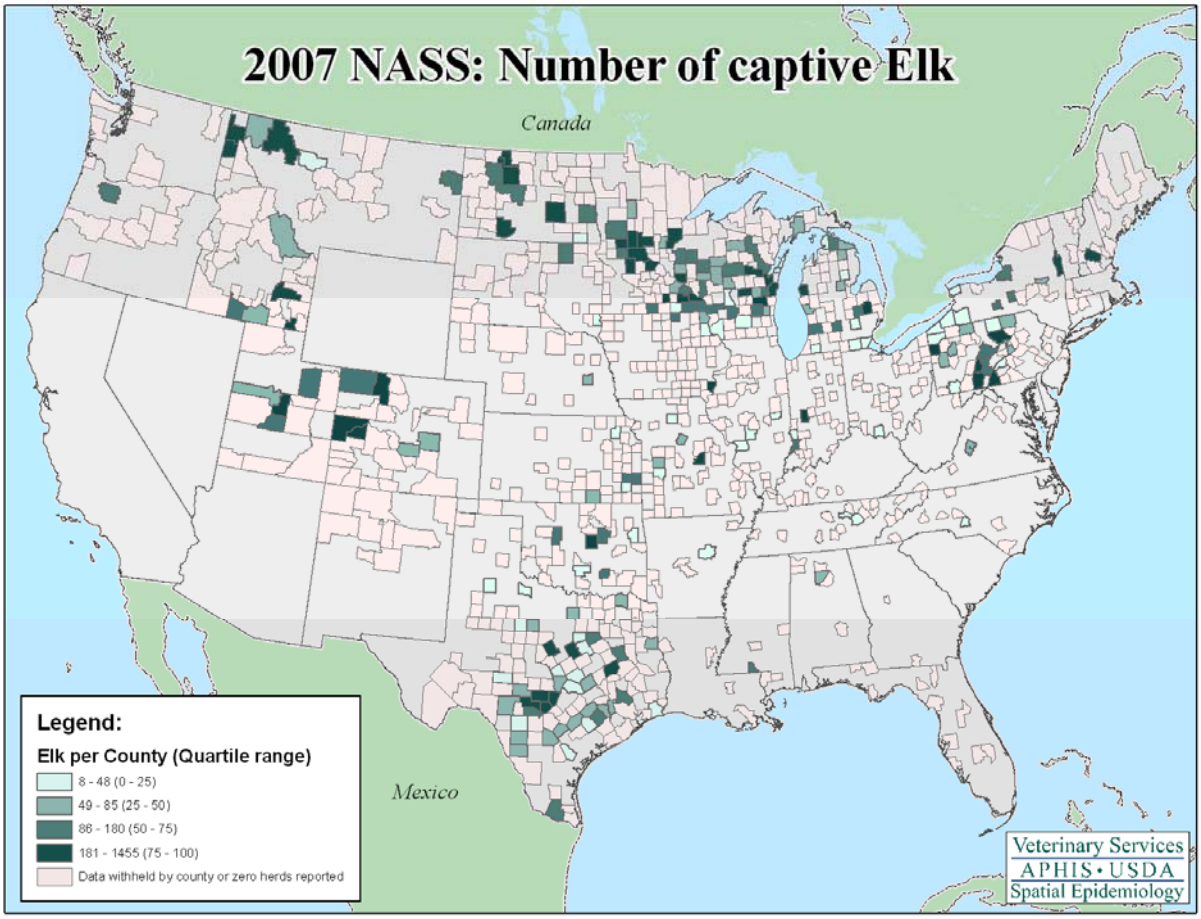
Figure 5.2 Number of captive deer by county, 2007



Source: NASS 2007 Census of Agriculture

The number of captive elk farms declined markedly in Colorado (120 to 69), Iowa (125 to 71), Minnesota (404 to 199), Montana (94 to 39), Nebraska (40 to 20), South Dakota (52 to 30), and Wisconsin (373 to 202). Texas reported a notable increase in the number of captive elk farms (247 to 354).

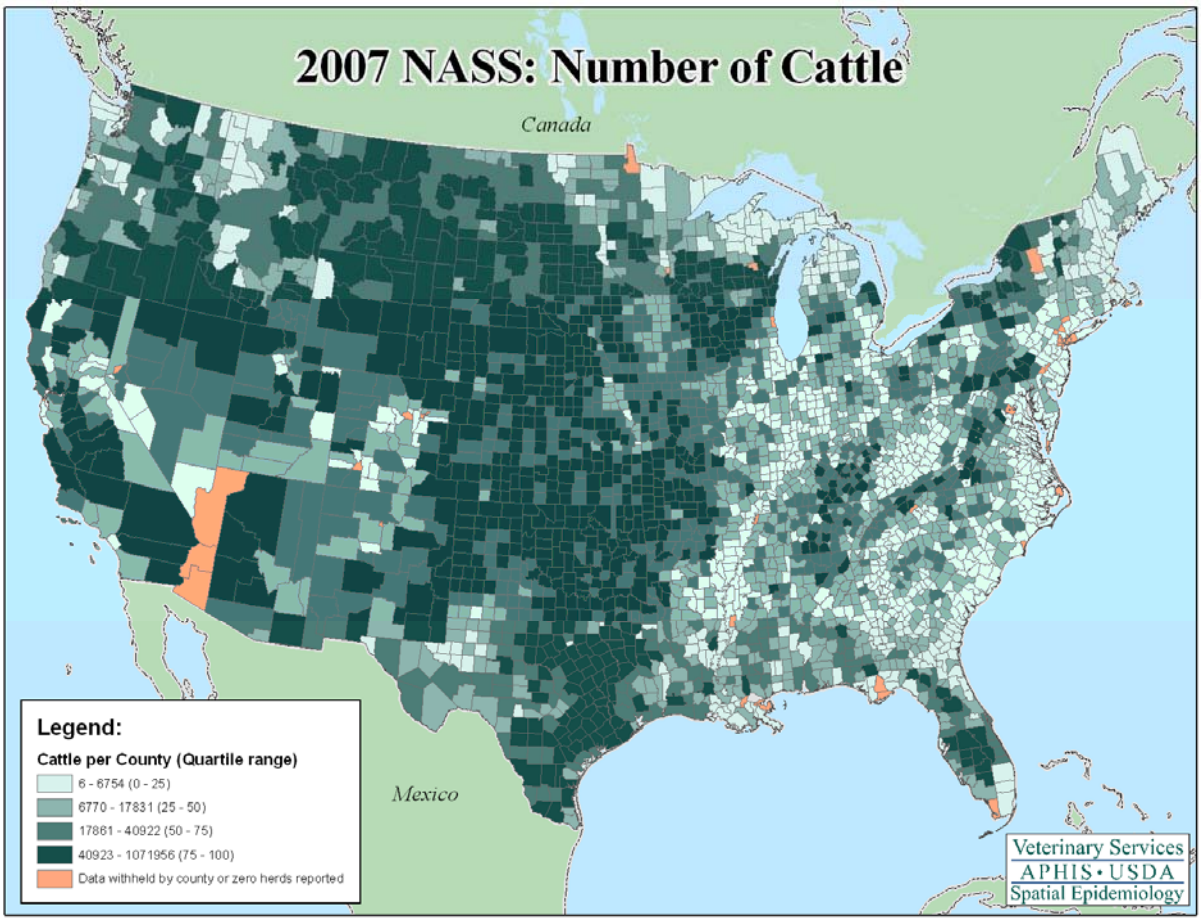
Figure 5.3 Number of captive elk per county, 2007



Source: NASS 2007 Census of Agriculture

For comparison, a map of cattle distribution is provided below:

Figure 5.4 Number of cattle per county, 2007



Source: NASS 2007 Census of Agriculture

Increased regulations, CWD, and the loss of antler-velvet export markets have contributed to the overall decline in the number of captive cervid farms in the United States.

It should be noted that another source has estimated the total number of captive cervid farms at 7,828 (Texas A&M University, 2007). The survey conducted by Texas A&M University concluded that the captive cervid industry is growing and has an overall economic impact of $2.3 billion annually. The difference in conclusions between this study and the Census of Agriculture may be due to growth in particular segments of the industry, such as hunting preserves and hobby farms that would not necessarily be captured in the Census of Agriculture data. Differences may also exist in the collection methods.

5.3 Regulations and regulatory authorities

Captive cervids are regulated by APHIS under *9 CFR,* part 77, subpart C and the TB UM&R, 1999. The SCT and the CCT are currently the only recognized official tests for captive cervids. Both of these tests have practical challenges because they require capturing the animals twice; once to administer the test and again 72 hours later (+/- 6 hr) to read the test results. Bacteriologic culture of

M. bovis is considered the gold standard for confirming an infected animal, but PCR in combination with strong epidemiologic evidence may lead to an animal being declared infected. Specific rules and testing requirements for herd status levels and interstate movements are detailed in the documents listed above and are beyond the scope of this document.

5.4 Bovine TB surveillance in captive cervids

SCT and CCT test submissions data from the TB Eradication Program were obtained for FY 2005 to FY 2008 (NVSL, 2009) (Table 5.2). Skin tests are typically conducted prior to movements, as part of the herd status requirements, prior to sale, or for travel to shows or exhibitions. CCT tests, the only approved antemortem test approved for followup testing to the SCT in captive cervids, were submitted less frequently.

Table 5.2 TB tuberculin tests for captive cervids

TB Eradication Program	FY2005	FY2006	FY2007	FY2008
SCT tests	27,795	27,291	13,037	20,609
SCT responders	3	1	4	1
CCT tests	680	330	435	566
CCT reactors	10	2	1	4

In addition to testing requirements for accreditation and interstate movements, some passive surveillance of captive cervids occurs through the slaughter channels. These submissions are not required, as there is no official slaughter surveillance program for cervids. Instead, they are submitted through routine slaughter inspection that does not include animals that were sent to slaughter after being TB-tested and receiving a suspect or reactor classification. The total number of samples submitted through slaughter surveillance is not available.

Slaughter surveillance has identified three positive cultures of *M. bovis* since FY 2001 (NVSL, 2009). An adult elk with mediastinal lesions typical of TB was found at slaughter in Colorado in 2002. Samples from the elk confirmed *M. bovis* infection. The second case was recently identified in FY 2009 in Nebraska. The 2-year-old female elk had gross lesions at slaughter and was confirmed positive by both histopathology and isolation of *M. bovis.* An affected herd was also identified in Indiana. This herd was traced to two additional cervid herds identified as affected. A cattle herd in Indiana was also linked to these cervid herds through identification of the same genotyping results.

An epidemiologic survey of TB in captive cervids was conducted in Michigan and reported in 2002 (Kaneene et al., 2002). This study used a combination of skin tests and slaughter on 96 privately owned cervid ranches in northeastern lower Michigan. This area was selected because endemic TB in the wild white-tailed deer population was identified in 1995. The cervid farms in this area are surrounded by a reservoir of TB in free-ranging deer. Some cervid farms were thought to have inadvertently fenced in wild deer at the initiation of their operations. This may have introduced *M. bovis*-infected animals into the herds. The study detected only 1 farm with TB-infected cervids and the reported apparent prevalence was 1.1 percent (21 cases out of 1,867 cervids tested). Three additional Michigan herds have since been identified. The authors concluded that the single positive herd was likely a result of fencing in wild white-tailed deer at herd initiation in 1992.

Several States have recently implemented a TB surveillance protocol that uses the mandatory sampling requirement of their State's CWD programs. Many CWD programs require that all captive cervids over 12 to 16 months-of-age that die or are slaughtered must be tested for CWD. The lymph

nodes collected for this CWD surveillance are then also tested for TB. This has proven to be a cost-effective approach for TB surveillance in captive cervids.

5.4.1 Cattle interaction

TB is most commonly transmitted between animals by the respiratory route, generally through the inhalation of aerosols coughed or exhaled by an infected animal. However, physical contact with feed, water, or minerals that has been contaminated by saliva or other discharges can also be a source of infection. Face-to-face contact is another possible route between cattle and captive cervids.

A cross-reference of 2002 Census of Agriculture data indicated that 21.5 percent of operations that owned captive cervids also owned beef cattle. The same data source also estimated that 2.6 percent of captive cervid operations had dairy cattle on their premises (USDA–NASS, 2009). In contrast, the 1992 TB risk assessment quoted that an estimated 50 percent of North American Elk Breeders Association members owned both cattle and elk (USDA, 1992).

In the recent NAHMS Dairy 2007 Study, nearly one-half of all dairy operations (49.3 percent) reported that deer or members of the deer family (including elk and moose) had physical contact with dairy cattle or their feed, minerals, or water supply (Table 5.3). The survey did not distinguish between captive and free-ranging deer. Dairy operations surveyed in the eastern United States more frequently reported physical contact with deer or members of the deer family (or contact with their feed, minerals, or water supply) than States in the western United States.

Table 5.3 Percentage of dairy operations in which dairy cattle had physical contact with deer or other members of the cervid family, by region

Percent Operations					
Region					
West		East		All Operations	
Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
20.9	(2.9)	51.6	(1.5)	49.3	(1.4)

Source: USDA, 2007.

On operations in which deer or members of the cervid family had contact with dairy cattle or their feed, minerals, or water, 90.8 percent reported that cattle could possibly or sometimes have face-to-face contact with deer. Less than one-half of all dairy operations (48.5 percent) limited cattle contact with other livestock, elk, and deer.

A similar national survey was conducted by USDA-APHIS-VS for the beef cow-calf industry in 2008 (USDA, 2009). In contrast to the previous dairy study, participants were asked to distinguish between wild cervid contacts and captive cervid contacts. Only 3.2 percent of cow-calf operations reported that their beef cattle had fence-line contact or commingled with captive cervids within the previous 12 months. Contact with wild cervids was reported on 72.6 percent of operations (Table 5.4).

Table 5.4 Percentage of beef operations in which beef cattle had physical contact with captive or wild cervids

	Percent Operations Contact					
	Yes		Don't Know		No	
	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
Captive cervids	3.2	(0.5)	2.5	(0.4)	94.3	(0.6)
Wild cervids	72.6	(1.3)	12.0	(0.9)	15.4	(1.1)

Source: USDA, 2009.

On an individual-animal basis, only 3.9 percent of beef cattle had fence-line contact or commingled with captive cervids within the previous 12 months. Beef cattle were much more likely to come in contact with wild cervids (88.0 percent) (Table 5.5).

Table 5.5 Percentage of beef cows on operations in which cows had fence-line contact or commingled with captive or wild cervids

	Percent Beef Cows	Std. Error
Captive cervids	3.9	(0.6)
Wild cervids	88.0	(0.9)

Source: USDA, 2009.

5.5 Historical occurrences of M. bovis in captive cervids in the United States

Bovine TB in captive Cervidae has been sporadically reported in the United States as early as 1923. However, most of the early cases did not report the specific mycobacterial organisms involved.

A detailed report of a captive fallow deer herd in Michigan was written in 1965 (Towar et al., 1965). The premises held a variety of species including spider monkeys, llamas, wild sheep, reindeer, donkeys, and bears. The article described advanced lesions in the lungs and lymph nodes of the deer and suggested an aerosol or oral route of infection. Laboratory results confirmed that the causative agent was *M. bovis.*

A report of bovine TB in a captive elk herd in Colorado was published in 1992 (Rhyan et al., 1992). The herd was depopulated after TB lesions were found in 8 of 10 SCT responders. More than one-half of the depopulated adults had gross lesions suggestive of TB and laboratory results confirmed *M. bovis* infection in the herd. The gross lesions varied and were described as resembling bovine TB in cattle or as lesions that more closely resembled ovine abscesses or caseous lymphadenitis.

Ten captive cervid herds have been identified as having one or more isolations of *M. bovis* since the implementation of cervid regulations in 1999 (NVSL, 2009). Four of the herds were found in Michigan, one in Kansas, one in New York, one in Nebraska and three in Indiana.

The 2007 Michigan herd was a trophy-hunting white-tailed deer operation. Tissues were submitted from a 3-year-old female for routine CWD and TB surveillance. Histologic lesions compatible with TB were found. The deer was PCR-positive for *M. tuberculosis* complex, but no fresh tissue was available for culture of *M. bovis.* The 100-acre ranch had been fenced in 1997 and all wild deer were

removed prior to stocking with purchased white-tailed deer. The last purchased deer entered the property in 1999 and no live deer left the property. However, evidence of fence-line contact with free-ranging wild deer was found. Extensive incursions by raccoons into the grain feeding and storage areas were also evident.

The 2008 New York herd had a 14-year-old female fallow deer that tested positive on a SCT for sale and movement. Necropsy revealed extensive gross lesions, and histologic and PCR testing were compatible with TB. The premises also contained red deer, horses, donkeys, cattle, pigs, and alpacas. Multiple whole-herd tests were conducted on the deer from the formation of the herd in 1993 until 2008. Only one deer, a yearling that tested negative for TB, was known to have entered the herd in the 10 years prior to discovery of the positive case.

No detailed epidemiology reports were available for the other three positive herds at the time of this report.

5.6 Mitigation and future needs

A rapid and reliable live-animal test would be a valuable tool for surveillance and control of TB in captive and wild cervids. There are at least seven serologic tests for TB in development by private companies. Some of these have the potential for use in the cervid industry. However, recent reports indicate that the sensitivities of currently available tests range from 46 to 68 percent, with highly variable positive predictive values (O'Brien et al., 2009). A gamma interferon test has also been evaluated for use in cervids, but overall performance has not been favorable and the test is not yet approved for official use (Waters et al., 2008). Further evaluation and improvement of these tests are underway.

Fencing that prevents ingress or egress of wildlife is an important component in reducing the risk of TB infection to and from other livestock or wildlife. Reports of potential TB transmission through fence-line contact from wild to captive cervids have caused concern (USDA, 1992; Rhyan et al., 1995). However, a recently published study suggests that this risk may be minimal (Vercauteren et al., 2007). The study evaluated fence-line contact between captive and wild white-tailed deer in Michigan using animal-activated cameras. Only two naso-oral contacts were documented after 77,165 hours of camera monitoring on six high-fenced farms, even though an average of 5.69 wild deer visitations occurred every 1,000 hours. No contacts between wild elk and farmed deer or elk were recorded. This single study does not eliminate the possibility that fence-line contact is a significant risk for TB transmission in captive cervids, but it does offer evidence that the risk may be low.

The hope of a vaccine that would prevent or reduce the spread of TB is also promising. Recent reports of vaccination of white-tailed deer with *M. bovis* bacillus Calmette-Guerin (BCG) indicate that both oral and parental vaccination is effective (Palmer et al., 2007; Nol et al., 2008). Gross lesions were reduced significantly in vaccinates versus controls following a challenge dose of *M. bovis* and the vaccinated deer were significantly less likely to be culture-positive. The authors of one study concluded that BCG vaccination "shows great potential in controlling disease caused by *M. bovis* infection" (Nol et al., 2008).

5.7 Summary

Based on the small number of positive cases identified in captive cervids in recent history, and the relatively small percentage of cattle that reportedly have physical contact with these animals, it appears that captive cervids pose a relatively low risk for TB transmission to cattle. However, the recent case in Indiana demonstrates the need for additional surveillance in this industry to truly understand the risk. Increased slaughter surveillance could be helpful in more accurately predicting the risk of TB transmission. A reliable serum test and the potential of vaccination of captive cervids would also provide powerful tools in identifying TB and preventing its spread.

5.8 References

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6. Free-ranging Wildlife

6.1 Introduction

Bovine TB has one of the broadest host ranges of all known pathogens, affecting many groups of mammals, including humans (O'Reilly and Daborn, 1995). In some parts of the world, bovine TB has spilled over from cattle into wildlife, where it has persisted as a reservoir of infection and frustrated efforts to eradicate the disease from sympatric livestock populations. Major free-ranging hosts of endemic bovine TB include the European badger (*Meles meles*) in the UK and Ireland, brushtail possum (*Trichosurus vulpecula*) in New Zealand, Cape buffalo (*Syncerus caffer*) and greater kudu (*Tragelaphus strepsiceros*) in southern Africa, bison (*Bison bison*) in Canada's Wood Buffalo National Park, and white-tailed deer (*Odocoileus virginianus*) in Michigan. Many other species, including humans, are susceptible to bovine TB but do not maintain infection in the population (O'Reilly and Daborn, 1995) without a change in population dynamics that enhances disease spread (Cousins and Florisson, 2005). The host status of a species with regard to *M. bovis* may differ between regions or change over time depending on population density or management regime (Cousins and Florisson, 2005). This, in turn, is the basis for efforts to manage or eradicate TB in wildlife and domestic animals.

6.2 Diagnostic methods for bovine TB in wildlife

6.2.1 Current methods

Cousins and Florisson (2005) summarized diagnostic tests that have been applied to the detection of TB in a variety of livestock and nonlivestock species. Even though culture of *M. bovis* remains the gold standard, this technique is impractical for bovine TB diagnosis in live animals. Instead, intradermal tuberculin testing continues to be the tool of choice for most bovine TB control programs. Cell-mediated immunity tests, including the interferon-gamma assay and the lymphocyte transformation assay, have been developed for cattle and wildlife. Additionally, various antibody-based tests, including ELISA and fluorescence polarization assay, have been applied to wildlife with limited success. Because of the lack of well-validated data for the diagnosis of bovine TB in animals other than cattle, Cousins and Florisson (2005) concluded that many of the current tests would fail to meet the validation criteria required by the World Organization for Animal Health (OIE).

6.2.2 New methods

Poor performance of the gamma-interferon assay was a critical limitation for a bovine TB test-and-cull program begun in 2004 for white-tailed deer in Michigan. As an alternative, O'Brien et al. (2009) compared the performance of several blood assays to culture results for *M. bovis* in white-tailed deer. Blood-test sensitivities were moderate (46 to 68 percent), whereas specificities and negative predictive values were all quite high (higher than 92 percent). The authors concluded that such tests could be useful in a well-equipped climate-controlled laboratory with skilled laboratory personnel, but they would be of limited value in field operations performed in adverse weather by staff unskilled in laboratory techniques.

DNA fingerprinting of *M. bovis* isolates has proved a valuable epidemiological tool for identifying sources of bovine TB infection, especially where wildlife sources are suspected (O'Reilly and Daborn, 1995). A novel PCR-based DNA fingerprinting technique for *M. bovis* strains known as DVR spoligotyping has recently been developed. This technique involves the use of a large number of sets of primers and the amplification of target oligonucleotides in order to detect polymorphisms in the spacer regions between the DR (direct repeat) sequences. Advantages of PCR-based DNA fingerprinting methods over standard genomic hybridization methods include the speed with which results can be obtained and the fact that very little DNA sample material is required for analysis, since PCR techniques involve the *in vitro* amplification of DNA. However, DNA fingerprinting techniques do not determine the direction of transmission from wildlife to domestic animals or from domestic animals to wildlife.

6.3 Wildlife and livestock interactions and epidemiology

An infected wildlife population can be defined either as a maintenance or spillover host, depending on the dynamics of the infection (Morris and Pfeiffer, 1995). In a maintenance host, once an infection is established it can persist in the population without any outside source and may also be transmitted to other species. In a spillover host, infection cannot persist indefinitely unless there is re-infection from another species or a change in the population that enhances interspecies transmission (Corner, 2006). Identifying whether a species or population has the status of a maintenance or spillover host is critical to assessing whether disease control measures within the host are necessary, or in predicting whether infection will persist once the source of infection is removed or the behavior of the population is altered. The host status of a species may differ geographically or change over time depending on habitat, population, and management. Maintenance and spillover hosts can both act as reservoirs of disease for other species (Cousins and Florisson, 2005).

Corner (2006) identified several key pieces of information needed to determine the status of an infected wildlife species as a potential reservoir for bovine TB. It is important to determine the nature of the infection in individual animals, the dynamics of infection in the population, the geographic distribution of the wildlife species, and the nature of its interactions with domestic animals and other wildlife. Bovine TB exhibits a range of epidemiologic patterns in different wildlife populations, and the presence of infection in a population does not necessarily mean it will spread to other species.

The mode of transmission and route of infection within and between species is one of the main factors in the epidemiology of TB (de Lisle et al., 2001). Where lesions are located can reveal how an animal acquired the disease. For example, a predominance of lesions in the lungs and thorax is evidence of infection by aerosol transmission. Animals with predominantly mesenteric lesions were probably infected orally. Tuberculous lesions in retropharyngeal lymph nodes, as frequently seen in deer, may indicate either aerosol or oral transmission because these nodes receive lymphatic drainage from nasal and oral cavities. Some animals have infected superficial body lymph nodes that could indicate infection through bite wounds or systemic spread from another primary source of infection (de Lisle et al., 2001).

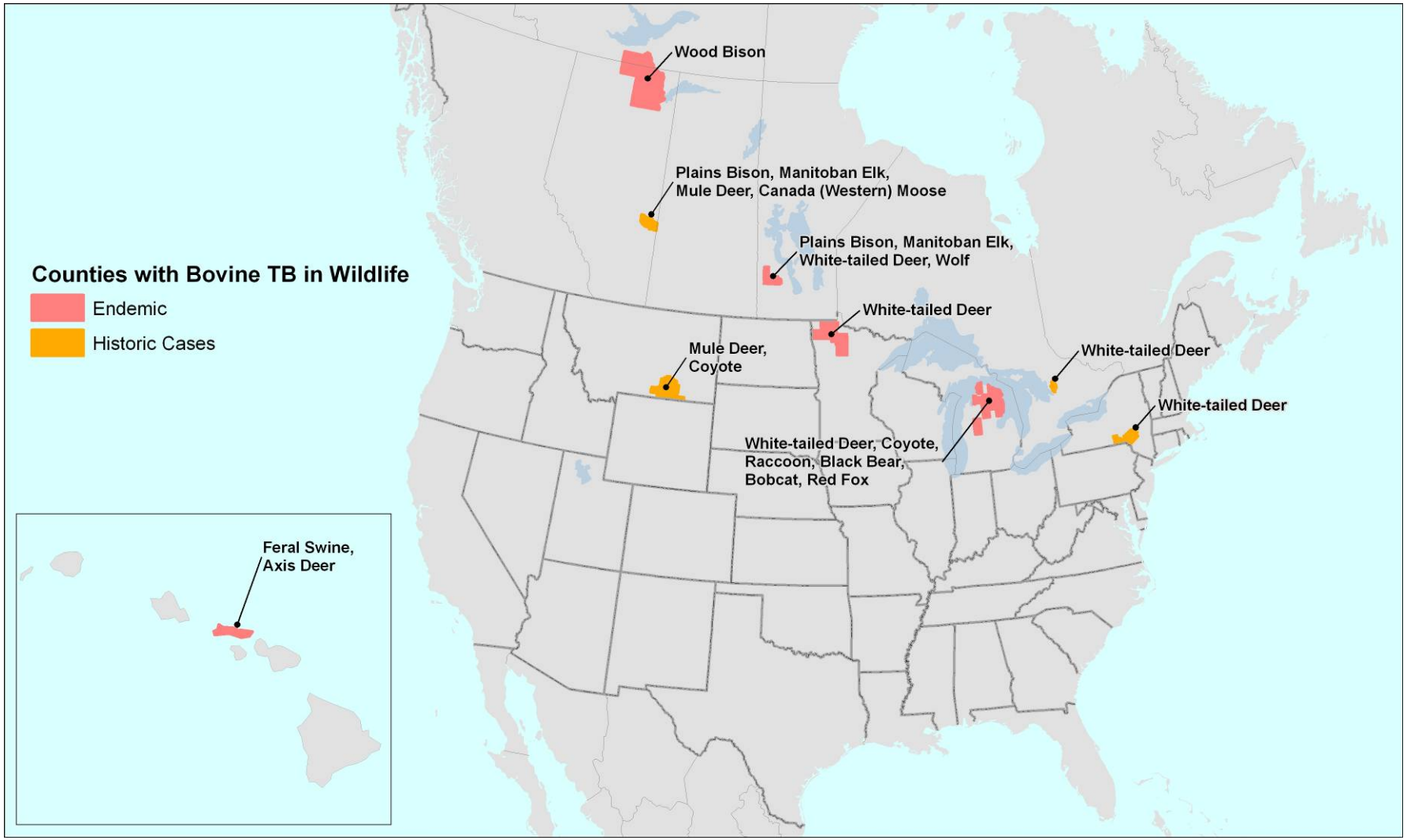
6.4 Bovine tuberculosis of wildlife in North America

6.4.1 Cervidae

Bovine TB has been identified in nine geographically distinct wildlife populations in North America and is endemic in four cervid populations (Table 6.1, Figure 6.1). Before the1990s, bovine TB had only rarely been reported among free-ranging Cervidae in North America. In Canada, Hadwen (1942) confirmed *M. bovis* infection in elk (*Cervus canadensis*), moose (*Alces alces*), and mule deer (*O. hemionus*) that ranged with *M. bovis*-infected bison in the Wainwright Buffalo Park in east-central Alberta. Belli (1962) reported bovine TB in a white-tailed deer in Ontario. In the United States, Levine (1934) and Friend et al. (1963) each reported two cases of bovine TB in white-tailed deer in New York, and Ferris et al. (1961) reported two cases in white-tailed deer in Illinois. In Michigan, a TB-infected white-tailed deer was documented in 1975 (Schmitt et al., 1997) and in Montana, a free-ranging mule deer living near a bovine TB-infected elk ranch was diagnosed with *M. bovis* (Rhyan et al., 1995).

Traditionally, it was assumed that *M. bovis* would not persist in free-ranging deer or elk unless they had continued contact with infected bison, cattle herds, or captive deer (Bleem et al., 1993, Essey and Vantiem, 1995). This paradigm shifted in the mid-1990s after *M. bovis* was diagnosed in a hunter-killed white-tailed deer in Michigan's northeast Lower Peninsula. Hunter-harvest surveillance by the Department of Natural Resources identified an endemic focus of bovine TB in free-ranging white-tailed deer within a five-county area of the Lower Peninsula (Schmitt et al., 1997; O'Brien et al., 2001, 2002; Palmer et al., 2002). In response, the State declared a special deer management unit that encompassed the core area of infected deer. This focus of infection represented the first known reservoir of *M. bovis* in free-ranging wildlife in the United States and the first known epizootic of TB in white -tailed deer in the world (Palmer, 2007).

Figure 6.1 Reported locations of bovine TB in North American wildlife



Sources: See Table 6.1

Table 6.1 Reported cases of *M. bovis* infection in free-ranging wildlife in North America

		Prevalence[1] (%)	Year	Species	Citation
United States					
Hawaii [2]	Endemic	<5.0	1970, 1971, 1972, 1981, 1998	axis deer	Sawa et al., 1974, Essey et al., 1981, Whipple and Palmer 2000
		20.0 [2]–3.8	1980, 1994, 1999–present	feral swine	Essey et al., 1981, 1983, Whipple and Palmer, 2000;
Michigan [3]	Endemic	4.9–0.2	1975, 1994–present	white-tailed deer	Schmitt et al., 1997, Thoen, 2006; Ankney, 2008, O'Brien, 2006
		52–4.8	1996-1999; 2003–05	coyote	Bruning-Fann, 1998, 2001; VerCauteren, 2008; O'Brien, 2006
		4.6-2.4	1996-2003	raccoon	Bruning-Fann et al., 1998, 2001,O'Brien, 2006
		3.3-2.4	1996-2003	black bear	Bruning-Fann et al., 1998, 2001; O'Brien, 2006
		12.5-7.0	1996-2003	bobcat	Bruning-Fann et al., 1998, 2001; O'Brien, 2006
		16.6-10.0	1996-2003	red fox	Bruning-Fann et al., 1998, 2001; O'Brien, 2006
		2.4	1996-2003	opossum	O'Brien, 2006
		0.3	2000, 2001, 2003	Rocky Mountain elk	O'Brien, 2006
		NA	2000	feral cat	Kaneene, 2002
Minnesota [4]	Likely Endemic	1.6–0.5	2005–present	white-tailed deer	USDA, 2008; Carstensen 2008
Montana	Reported	4.9	1994	mule deer	Rhyan et al., 1995
		4.3	1994	coyote	Rhyan et al., 1995
New York	Reported	NA	1933, 1937, 1961	white-tailed deer	Levine, 1934; LeDune 1937; Friend, 1963; Wagner, 1993
Canada					
Alberta [5]	Endemic	5.5	1939–40	Manitoban elk	Hawden, 1942; Tessaro, 1986
		5.6	1939–40	Canadian moose	Hawden, 1942; Tessaro, 1986
		0.8	1939–40	mule deer	Hawden, 1942; Tessaro, 1986
		49.0–42.0	1925–present	wood bison	Tessaro et al., 1990; Joly et al., 2004
		53.7	1923–37	plains bison	Hawden, 1942; Tessaro 1986
Manitoba [6]	Endemic	NA	1937	plains bison	Tabulenas, 1983
		NA	1978	wolf (pups)	Carbyn, 1982; Lutze-Wallace, 2005
		3.6–0.4	1992, 1998–2005	Manitoban elk	Thoen et al., 2006; Lees et al., 2003
		<0.5	1998–2005	white-tailed deer	Thoen et al., 2006; Lees et al., 2003
Ontario	Reported	0.2	1958	white-tailed deer	Belli, 1962

[1] Prevalence reported by specific study.
[2] Foci of infection occurs only on the island of Molokai and is considered endemic. Initial surveys indicated prevalence as high as 20% in feral swine, but this was reduced to 3.8% after culling.
[3] Currently considered endemic in Michigan with a foci of infection occurring in northeastern Michigan.
[4] A foci of infection occurs in northwest Minnesota with an estimated prevalence in white-tailed deer of 1.6 percent in the core area and an overall prevalence of 0.5 percent.
[5] Endemic infection occurs in bison populations of Wood Bison National Park.
[6] Endemic foci of infection occurs in and around Riding Mountain National Park.

A similar situation exists in Manitoba, Canada, where a herd of 2,500 to 4,000 elk (*C. c. manitobensis*) was implicated in an outbreak of bovine TB among 11 cattle herds surrounding Riding Mountain National Park (Lees et al., 2003). Commingling of elk and cattle that feed on the same hay bales was considered the most likely mode of transmission between species. Strain typing of *M. bovis* isolates from wildlife and cattle indicates that it is a unique strain seemingly unrelated to other strains previously identified in Canada. Management tactics employed to help reduce the transmission of *M. bovis* between wildlife and cattle have included reducing the elk population through extended hunting seasons and predator conservation, barrier fencing to protect hay-storage yards from wild elk and deer, legislation to prevent baiting and unnatural cervid herding behavior, and habitat improvement in the Park through prescribed burning (Nishi et al., 2006). In Michigan, a small population of elk resides within the bovine TB-endemic area for white-tailed deer. Although a few of the more than 1,400 elk tested have been *M. bovis*-positive, it appears these are spillover cases possibly affected by ingesting contaminated feed (O'Brien et al., 2006). In the absence of strong congregating factors, elk and deer generally maintain spatial separation with little potential for direct interaction and transmission of TB (Miller, 2002).

It is believed that *M. bovis* was transmitted from cattle to deer in Michigan sometime during the early to mid-1900s when TB was widespread among cattle in the State. Epidemiologic models estimate that spillover from cattle to deer occurred around 1955 when Michigan's deer population was beginning to increase well beyond normal carrying capacity (Palmer, 2007). In the region the outbreak occurred, deer densities increased two- to threefold during the three decades preceding the outbreak and functional deer densities resulting from winter yarding of deer undoubtedly were much higher (Peterson, 2003). Bovine TB likely smoldered within the deer population during this period and finally became evident when prevalence grew to detectable levels. Deer population increases were due to a combination of limited doe harvest and extensive supplemental feeding by hunt clubs (Schmitt et al., 1997; O'Brien et al., 2002).

Michigan adopted two principal management strategies to deal with bovine TB in the affected five-county area (O'Brien et al., 2006). First, it increased hunter harvests to reduce deer population density, and second, it placed restrictions on supplemental feeding and baiting of deer. Voluntary restrictions were initially sought from the public and hunt clubs, followed by a regulatory ban on feeding and baiting within the TB area and the remainder of the State. Additionally, Michigan adopted a moratorium on establishing new captive cervid facilities. Hunter-harvest has reduced the five-county deer population by approximately 50 percent and, concurrent with these management actions, bovine TB prevalence has declined significantly in adult and yearling deer (O'Brien et al., 2006).

Research indicates that supplemental feeding and baiting have been major factors in the propagation and persistence of bovine TB in Michigan's white-tailed deer population (O'Brien et al., 2006). Deer groups with the highest prevalence of *M. bovis* were clustered on private land where feeding and baiting were common practices. *M. bovis* can survive for months on foodstuffs commonly used for deer (O'Brien et al., 2006) and transmission between deer by shared feed has been documented (Palmer et al., 2004b). Shared feed also appears to be the primary route of transmission from deer to cattle (Palmer et al., 2004a; O'Brien et al., 2006). Bucks are much more likely to be infected with *M. bovis* than comparably aged does, and the fact that their risk increases with age suggests they play an important role in disseminating TB (O'Brien et al., 2002). Unfortunately, baiting continues to be widespread, even in banned areas.

In 2006, bovine TB was discovered in white-tailed deer in Minnesota in conjunction with an outbreak of bovine TB in beef cattle (USDA–APHIS–VS–CEAH, 2008). One of 474 hunter-harvested deer tested in the vicinity of affected cattle herds was positive for *M. bovis*, and targeted culling and surveillance identified another positive deer. Epidemiologic linkages between *M. bovis*-infected deer and cattle were supported by the proximity of deer and cattle cases and *M. bovis* strain identity between cattle and deer. The State responded by initiating more rigorous sampling protocols to establish prevalence of *M. bovis* in the deer population. Currently, Minnesota conducts hunter-harvested deer surveillance during the fall hunting season. If *M. bovis*-infected deer are identified, targeted culling is conducted in the spring around areas where infected deer have been found. As of

2009, Minnesota tested more than 4,000 deer in the surveillance zone and identified 18 TB-positive deer and an additional 8 suspects. Based on fall hunter-harvested sampling, the average estimated apparent prevalence of bovine TB in white-tailed deer in the affected area was less than 1 percent, similar to the overall apparent prevalence of *M. bovis* reported for white-tailed deer in Michigan (O'Brien et al., 2002).

6.4.2 Bovidae

Hadwen (1942) documented a severe and prolonged outbreak of TB among bison in Alberta, Canada's Wainwright Buffalo Park during the early to mid-1900s. The bison herd was maintained in a semi free-ranging condition, within a fenced natural area co-inhabited by elk, deer, and moose. More than one-half of approximately 12,000 bison culled between 1923 and 1939 had TB lesions at meat inspection, as did approximately 5 to 6 percent of elk and moose and less than 1 percent of mule deer culled in 1939 and 1940. Epidemiologic investigations suggested that the source of infection could have been bison introduced to the park from another infected herd where a buffalo calf was nursed by a domestic cow. Owners of buffalo herds commonly exchanged calves, many of which were fed milk in transit or fed on nurse cows. The bison herd was eventually destroyed and the park disbanded in 1940 (O'Reilly and Daborn, 1995).

Canada's largest remaining reservoir of bovine TB is the free-ranging bison population in and around Wood Buffalo National Park, bordering northern Alberta and southern Northwest Territories. *M. bovis* infection was introduced to the Park between 1925 and 1928 when more than 6,600 plains bison (*B. b. bison*) were imported from the infected herd at Wainwright (O'Reilly and Daborn, 1995). An unfortunate side effect of this action, in addition to the introduction of TB and brucellosis, was the mixing of plains bison genes into the indigenous wood bison (*B. b. athabascae*) population (Nishi et al., 2006). The bison population in Wood Buffalo Park grew to an estimated 12,000 to 15,000 animals in the late 1940s and then declined to approximately 5,000 by 1968 (Tessaro, 1986). Prevalence of TB lesions was 39 percent among 3,400 bison necropsied in the Park between 1950 and 1967 (Broughton, 1987). Additionally, 22 percent of 192 bison slaughtered in the adjacent Slave River Lowlands during 1964 and 1965 were *M. bovis*-positive (Peterson, 2003). Tessaro et al. (1990) isolated *M. bovis* from 21 percent of 72 bison found in and around Wood Buffalo National Park during 1983 and1985. They concluded that bovine TB was endemic in the bison population and was a growing threat to uninfected bison and cattle.

6.4.3 Other species

Bruning-Fann et al. (2001) conducted necropsies of 294 carnivores from the *M. bovis*-endemic area of Michigan. Seven animals had microscopic lesions suggestive of TB and nine had lymph node cultures positive for *M. bovis*; six coyotes (*Canis latrans*), two raccoons (*Procyon lotor*), one red fox (*Vulpes vulpes*), and one black bear (*Ursus americanus*). RFLP patterns of *M. bovis* isolates were identical in carnivores and deer, indicating that both groups were infected with the same strain. Moreover, most of the lesions in affected carnivores were in mesenteric lymph nodes, suggesting exposure through ingestion of scavenged material. The location of lesions, variety of species involved, and geographic spacing of cases are indicative of disease spillover rather than endemic bovine TB in these carnivores (Bruning-Fann et al., 2001).

A broader survey of 175 coyotes in Michigan found that 58 (33 percent) were either culture-positive for *M. bovis* or had granulomatous lesions. Prevalence of *M. bovis* in coyotes varied regionally from 19 to 52 percent. Lesions occurred most commonly in the gastrointestinal tract; however, one coyote had advanced disease with lesions occurring in the lung and liver (VerCauteren, 2008). A study by Johnson et al. (2009) found that captive coyotes orally inoculated with 1×10^5 CFU of *M. bovis* did not become infected or shed *M. bovis* in feces or oronasally. Similarly, researchers at the USDA APHIS National Wildlife Research Center (NWRC) found no evidence of shedding of *M. bovis* from naturally infected coyotes from Michigan (M. Dunbar, personal communication).

Birds may also be involved in the transmission cycle of *M. bovis,* but their relative importance is not well understood (Butler et al. 2001, Clark et al., 2003, Clarke et al., 2006). In the El Paso, Texas,

milkshed, pigeons, blackbirds, and other species were thought to be responsible for introducing bovine TB into U.S. dairies from dairies in Cuidad Juarez, Mexico, through mechanical transport of contaminated material or infected birds shedding *M. bovis* into cattle feed (Figure 6.2) (Pillai et al., 2000). However, follow-up surveys of tissues from 252 pigeons, 9 European starlings, and 1 common grackle from the 14 affected dairies in El Paso failed to identify *M. bovis* (Pillai et al., 2000). Blackbirds were not sampled in the survey. Currently, this is the only reported survey of wild avian species for bovine TB. The study did not address the potential of birds to mechanically transport *M. bovis*-contaminated material.

Figure 6.2 Blackbirds feeding with dairy cattle in the southwestern United States

Photos: USDA/APHIS/Wildlife Services

Limited research concerning the ability of avian species to become infected and subsequently shed bacterium has been conducted. Experimental infections of pigeons resulted in 2 of 12 developing microscopic lesions after a high dose inoculation (Fitzgerald et al, 2003). Pigeons were also shown to shed *M. bovis* in feces. Intraperitoneal inoculations of European starlings and American crows resulted in three of four starlings and two of four crows developing histologic lesions suggestive of mycobacteriosis, without the presence of acid-fast bacilli (Butler et al., 2001). In separate studies, wild turkeys and mallard ducks were shown to be resistant to *M. bovis* (Fitzgerald et al., 2005a; Clarke et al., 2006).

6.5 Bovine TB in Wildlife of Europe, Africa, and Oceania

6.5.1 Cervidae

Contact with domestic animals has resulted in bovine TB becoming endemic in a few wild populations of deer outside North America, including New Zealand, Ireland, the UK, Hungary, Switzerland, and Spain (O'Reilly and Daborn, 1995; Hermoso et al., 2006; Parra et al., 2006). Bovine TB has been recognized as an important animal and public health problem in New Zealand since the early 1900s and probably was introduced through the importation of infected cattle (Palmer, 2007). *M. bovis* was first isolated from feral red deer (*C. elaphus*) in the 1970s (O'Reilly and Daborn, 1995) and spread through the movement of farmed and captured wild deer (de Lisle et al. 2001). Red deer in New Zealand appear to be a spillover host infected by contact with brushtail possums, which are the primary wildlife reservoirs for this disease (O'Brien et al., 2002).

O'Reilly and Daborn (1995) highlighted several reports of *M. bovis* isolation among free-ranging deer in Europe, including red deer in Hungary, roe deer (*Capreolus capreolus*) in Switzerland, fallow deer (*Dama dama*) in Ireland, and both Sika (*C nippon*) and roe deer in England. Despite the fact that wild deer in the UK were found in proximity to infected cattle, crossover infection from wild deer to cattle

was rare (O'Reilly and Daborn, 1995). Parra et al., (2006) examined more than 50,000 carcasses of red deer in western Spain over a 5-year period and noted a doubling of apparent bovine TB prevalence from 0.8 to 1.7 percent. They attributed this increase to management practices that led to artificially high numbers of wild game. Sawa et al. (1974) reported *M. bovis* infection from wild axis deer (*Axis axis*) and feral cattle on Hawaii's Molokai Island. They concluded that the deer contracted *M. bovis* from infected cattle, as deer were not allowed to be transported onto or off of the island.

6.5.2 Bovidae

The African (Cape) buffalo is the most studied maintenance host of *M. bovis* on the African continent (de Lisle et al., 2001). Guilbride et al. (1963) published the first report of bovine TB in this species after finding eight cases among buffalo shot in Uganda's Ruwenzori-Queen Elizabeth National Park. At the time of the study, the authors estimated that TB caused an annual mortality of 1 percent of the Park's buffalo. Three decades later, Bengis et al. (1996) reported a case of bovine TB in a free-ranging Cape buffalo in Kruger National Park, South Africa. A young bull, found sick and emaciated in 1990, was euthanized for necropsy. After researchers confirmed *M. bovis* in the animal, they conducted a survey by culling 57 buffalo from 2 herds. Nine of the animals (16 percent) had lesions compatible with bovine TB, suggesting that the disease had been in the herd for several years or perhaps decades. The authors considered TB-infected cattle along the southern boundary of the Park the most likely source of infection.

De Vos et al. (2001) stated that while *M. bovis* probably was imported to South Africa with European cattle breeds sometime during the late 1700s, it likely entered the Kruger National Park ecosystem after 1960. Once there, it became established in an immunologically naïve and susceptible population of African buffalo. The authors believed the infection originated along the Park's southern boundary where bovine TB-infected cattle were found beginning in the early 1960s. After a slow start, the disease gained a foothold and spread northward in the buffalo population at a rate of approximately 6 km/yr. Although bovine TB has occasionally infected other species including chacma baboons (*Papio ursinus*), lions (*Panthera leo*), cheetahs (*Acinonyx jubatus*), leopards (*Panthera pardus*), and kudu, none is considered a maintenance host. Surprisingly, bovine TB has not been found in the Park's population of warthogs (*Phacochoerus africanus*), which were highly susceptible to bovine TB in Queen Elizabeth National Park (de Vos et al., 2001).

Mean herd prevalence of bovine TB among buffalo during a survey in Kruger National Park was approximately 24 percent, with a range of 2 to 76 percent (de Vos et al. 2001). Herds in southern regions of the Park, where *M. bovis* presumably gained entrance, had higher prevalence than northern herds farther away from the origin of the outbreak. Coughing was a prominent clinical feature of highly infected buffalo herds, although clinical signs in early stages of the disease were often subtle (as in cattle). Older animals were more likely to be infected, similar to bison in Wood Buffalo National Park, Canada, and African buffalo in Queen Elizabeth National Park, Uganda (de Vos et al., 2001). The majority of *M. bovis* isolates were the same genotype found within cattle near the Park's southern boundary, supporting the theory that cattle were the main source of the outbreak in buffalo. Guilbride et al. (1963) likewise concluded that cattle were the source of the bovine TB outbreak among buffalo in Queen Elizabeth National Park. Given the wide distribution and status of the buffalo as a maintenance host, bovine TB should be considered endemic in wildlife within Kruger National Park (de Vos et al., 2001).

Other potential maintenance hosts of *M. bovis* in Africa include the lechwe antelope (*Kobus leche*) and greater kudu. High levels of *M. bovis* infection have been observed among lechwe in Lochinvar National Park, Zambia since 1956 (Gallagher et al., 1974; Clancey, 1997). Before the park was created, thousands of cattle (some infected with bovine TB) grazed the park and surrounding rangelands. Lechwe most likely acquired *M. bovis* while sharing these rangelands and now appear to be maintenance hosts of bovine TB (Gallagher et al., 1974; Stafford, 1991). A recent study (Munyeme et al. 2009) found that cattle commingling with lechwe in the Kafue Basin region of Zambia were nearly seven times more likely to be infected with bovine TB than cattle grazed in a region where there was little contact with infected wildlife.

The greater kudu was among the first African wildlife species known to be infected with bovine TB (de Lisle et al., 2001). Cases were recognized in the 1920s in the Eastern Cape Province of South Africa in an area shared with infected cattle (Paine and Martinaglia, 1928). Kudu are unique in that TB-infected animals can be visually identified by mumps-like swellings of their parotid lymph nodes (Keet et al., 2001). While kudu are more solitary than typical maintenance hosts, they may nevertheless sustain *M. bovis* at low levels (de Lisle et al., 2001). Kudu feed principally by browsing on shrubs, and infectious purulent material draining from facial abscesses may contaminate thorns and leaves during feeding activities where it can be passed on to other browsers (de Lisle et al., 2001).

Australia's Northern Territory is inhabited by a large population of feral Asian water buffalo (*Bubalus bubalus*) in which bovine TB is endemic. Numbering as many as 250,000, these animals descended from domestic stock imported in the early 1800s. In the 1970s, TB was endemic over much of the buffalo range and prevalence exceeded 25 percent in some areas. While cattle and buffalo grazing ranges overlapped extensively during the dry season, the two species did not interact and there was little opportunity for transmission (Corner, 2006). Australia received its bovine TB-free status in 1997 after eradicating the disease in domestic cattle (de Lisle et al., 2001). Test-and-slaughter was the predominant control method, although some chronically infected herds were depopulated. Inspection of feral buffalo meat between 1959 and 1979 revealed a prevalence of TB between 1.7 and 16.4 percent. Thoracic lesions and generalized cases of TB indicated that the buffalo were a likely maintenance host of *M. bovis*. Although TB infection in buffalo apparently cycled independently from that in cattle, the buffalo were extensively culled as part of Australia's bovine TB eradication program (de Lisle et al., 2001). Depopulation was easily justified, as the animals were an introduced species with little economic value and caused significant damage to ecologically sensitive wetlands (Corner, 2006).

6.5.3 Suidae

M. bovis has been isolated from feral swine (*Sus scrofa*) in many regions including Australia, New Zealand, Hawaii, and several European countries (Aranaz et al., 1996; Corner et al., 1981; Essey et al., 1981; Letts, 1964; O'Reilly and Daborn, 1995; Palmer, 2007; Serraino et al., 1999). In northern Australia, a relatively high prevalence of bovine TB infection in feral swine was attributed to close association with infected feral water buffalo, which are *M. bovis* maintenance hosts (Letts, 1964). Hundreds of buffalo died at the end of each dry season, providing food and a source of *M. bovis* infection for scavenging swine. Researchers concluded that these pigs were spillover hosts, rarely transmitting the infection to other species (Corner et al., 1981). This conclusion was evidenced by a major decline in prevalence of *M. bovis* infection among feral swine (from 19 percent in 1972 to less than 1 percent in 1992). The decline coincided with the culling of water buffalo and a marked reduction of bovine TB among cattle (de Lisle et al., 2001). In New Zealand, *M. bovis*-infected feral swine were also considered spillover hosts, contracting the disease by scavenging carcasses of infected brushtail possums (Wakelin et al., 1991).

On Molokai, Hawaii, the epidemiologic role of feral pigs in a multispecies outbreak of bovine TB was investigated but never clearly defined (de Lisle et al., 2001). Although *M. bovis* was efficiently transmitted among pigs (estimated prevalence 20 percent), the disease was controlled in cattle by depopulating infected cattle and culling infected pigs and axis deer (Essey et al., 1981; 1983). Post-culling bovine TB prevalence in feral swine was 3.2 percent (Essey et al., 1983) and although TB continues to be identified in swine, spillover into cattle on the island has been mitigated by maintaining spatial separation of swine from cattle.

Recently, *M. bovis* was isolated from 15 of 63 wild boars in northern Italy, an area where the disease existed in cattle (Serraino et al., 1999). A common *M. bovis* isolate was found in both cattle and wild boar, leading the authors to conclude that feral pigs were probably infected by commingling with pastured cattle. In Spain, Aranaz et al. (1996) used DNA fingerprinting of *M. bovis* isolates to demonstrate transmission of bovine TB among cattle, deer, and wild boar. Parra et al. (2006) noted an increase in the apparent prevalence of bovine TB (from 1.4 to 2.3 percent) among feral swine in western Spain, and Hermoso et al. (2006) reported that *M. bovis* persisted in free-ranging Iberian wild boar where cattle and deer were absent. Naranjo et al. (2008) presented several lines of evidence

indicating that free-ranging Mediterranean wild boar are true maintenance hosts of bovine TB, able to maintain and transmit infection to other species.

6.5.4 Other species

The European badger has been implicated as a source of bovine TB infection among British cattle, as incidence of the disease has increased over the past decade (Cheeseman et al., 1989; Delahay et al., 2000; Palmer, 2007). *M. bovis* was first isolated from badgers in Switzerland in 1957, where it is believed they were infected by contact with TB-infected roe deer (Bouvier, 1963). The first TB-infected badger in Britain was identified in 1971 (Muirhead et al., 1974) on a farm that had reactor cattle with TB lesions (Muirhead et al., 1974). Three years later, *M. bovis*-infected badgers were confirmed in Ireland (Noonan et al., 1975). Woodroffe et al. (2005) reported overall prevalence of 12 percent *M. bovis* infection (n=2,692) among adult badgers in southwestern England. Adult males were at greater risk of infection (prevalence 14 percent) than adult females (prevalence 10 percent). In another study, Delahay et al. (2000) calculated an annual bovine TB prevalence of 10 to 18 percent in badgers; between 1 and 8 percent of animals were culture-positive and excreting bacilli. It is thought that badgers in Britain became infected with *M. bovis* during the late 1800s and early 1900s when the disease spilled over from British cattle, many of which were infected with bovine TB (Palmer et al., 2007).

Woodroffe et al. (2005) provided the first clear evidence of an association between *M. bovis* infection in cattle and badgers. Not only were patterns of infection in the two species spatially correlated, but there were also close linkages in the distribution of *M. bovis* strain types in the two species. The data did not allow an assessment of the relative importance of badger-to-cattle vs. cattle-to-badger *M. bovis* transmission.

Culling badgers has been a central component in attempts to control bovine TB in British cattle for many years (Vicente et al., 2007). A large-scale field experiment (the Randomized Badger Culling Trial) was initiated in 1998 to compare the effects of different methods of bovine TB control. Three different treatments for badgers were tested; no culling, targeted culling in areas that TB had been identified in cattle, and proactive culling designed to reduce badger densities to low levels across entire trial areas. After 5 years, it was determined that reactive culling was causing increased levels of TB in cattle, and this portion of the trial was terminated (Donnelly et al., 2003). The failure of reactive culling to reduce TB incidence may have been caused by disruption of badger social organization, resulting in increased movement and greater contact between groups (Tuyttens et al., 2000). Social restructuring of *M. bovis*-infected badger populations has been linked to increased disease transmission among badgers (Rogers et al., 1998) and could have similar effects on disease transmission between badgers and cattle (Palmer, 2007; Woodroffe et al., 2005).

New Zealand has a comparable TB situation with the brushtail possum, an exotic species imported from Australia to establish a fur industry. Between 1837 and 1922, at least 30 groups of possums were brought to New Zealand, bred in captivity, and released in 160 sites around the country. Possums thrived because of abundant food and lack of predators and competition; thereby quickly expanding their range. There are an estimated 60 to 70 million possums nationwide and the species occupies more than 90 percent of New Zealand's land area. Possum density estimates in some areas are 20 times that of their native habitats in Australia (O'Neill and Pharo, 1995, cited by Palmer, 2007).

Bovine TB was probably introduced to New Zealand through the importation of cattle in the 1800s. By the early 1900s, bovine TB was seen as a serious animal and public health problem (Palmer, 2007). The first reported case of TB in a wild possum in New Zealand was in 1967 (Ekdahl et al., 1970). However, the susceptibility of brushtail possums to infection with *M. bovis* had been established much earlier (Bolliger et al., 1948). Apparent prevalence estimates of TB in possums, based solely on lesions, are usually quite low (less than 5 percent). However, true prevalence may be much higher because possums usually die within months of showing clinical signs (de Lisle et al., 2001). Possums in New Zealand probably acquired *M. bovis* from cattle or other wildlife, as *M. bovis* infection has never been found among possums in Australia (Palmer, 2007).

TB in possums and cattle has been epidemiologically linked (Collins et al., 1988) and there is ample evidence supporting the role of possums as maintenance hosts of bovine TB in New Zealand (Morris and Pfeiffer, 1995). This evidence includes: clear spatial and temporal association between TB infection in possums and incidence of infection in domestic stock; persistent TB infection in possums without any evidence of spillback infection from domestic animals or wildlife; prolonged reduction in bovine TB incidence in cattle following control programs to reduce densities of tuberculous possums; and an epidemiologic pattern, verified through *M. bovis* DNA analysis, consistent with long-term maintenance of bovine TB infection among groups of possums with repeated infection of other species, including cattle.

Bovine TB occurs in wild ferrets (*Mustela putorius*) in New Zealand, and tuberculous ferrets occupy many areas inhabited by other infected wildlife. Caley and Hone (2005) concluded that ferrets in some areas were at high enough densities to be maintenance hosts of bovine TB. Since rabbit hemorrhagic disease was introduced in 1997, rabbit densities have declined by an average of 50 percent on New Zealand's South Island. It is likely that this decline in rabbits has also led to a reduction in ferret densities, which lessens their chances of acting as maintenance hosts for TB. The role of ferrets as a source of bovine TB infection for domestic livestock remains undetermined (O'Reilly and Daborn, 1995).

In Africa, one of the more serious effects of bovine TB may be on spillover hosts, particularly top predators and scavengers (de Lisle et al., 2001). Predators are apt to target TB-debilitated prey species like buffalo or kudu, or may scavenge infected carcasses, thus receiving frequent high exposures to *M. bovis*. Keet (1998) found that lions in Kruger National Park became infected with TB as a result of spillover from infected buffalo. In some parts of the Park, more than three-fourths of randomly-tested lions had positive tuberculin skin tests. TB has also been diagnosed in cheetah, leopard, and chacma baboon in the Park. Baboons most likely became infected by scavenging the remains of infected buffalo. Similarly, TB was observed in olive baboons (*P. anubis*) in the Masai Mara Game Reserve, where the likely source of infection was *M. bovis*-infected waste material from a cattle abattoir (Keet et al., 2000). The greater the prevalence of TB in maintenance hosts, the greater the exposure and infection rate among predators and scavengers. Bovine TB may affect these low-density populations by reducing longevity and altering social structures (de Lisle et al., 2001).

6.6 Mitigating bovine TB in wildlife populations

6.6.1 Vaccination

Although disease eradication may be the ultimate goal of wildlife immunization, lowering the rate of pathogen transmission is a more practical and immediate aim (Palmer et al. 2007). This objective can be achieved with a vaccine that reduces infection and bacterial shedding, rather than preventing primary infection (Buddle et al., 2006).

BCG has been used as an anti-TB (*M. tuberculosis*) vaccine in humans since the early 1920s. Despite its variable effectiveness, it is still one of the most widely used vaccines throughout the world. BCG has been tried as a means of controlling bovine TB in cattle in many countries but has been abandoned because it is poorly efficacious and induces sensitivity in vaccinated animals that interferes with tuberculin testing. Nevertheless, the search for a more effective TB vaccine continues, and BCG remains the gold standard against which all experimental TB vaccines are compared. Currently, there are no accepted TB vaccination programs for livestock (Nol et al., 2008).

Several countries are conducting research on BCG vaccine efficacy in wildlife, including New Zealand, the UK, the Republic of Ireland, the United States, and South Africa. Griffin et al. (2001, 2006) demonstrated that BCG, administered subcutaneously to red deer, provided significant protection against intratonsillar *M. bovis*. Likewise, Palmer et al. (2007) found that subcutaneous BCG yielded moderate protection to white-tailed deer against bovine TB. Especially promising are recent studies showing the efficacy of orally-delivered BCG in wildlife. Nol et al. (2008) found that a lipid-formulated oral bait preparation containing BCG offered significant protection to white-tailed deer against *M. bovis*. Similar bait preparations have induced protection against bovine TB in brushtail

possums and European badgers (Buddle et al., 2006; Corner, 2006). These studies bring closer the possibility of an oral vaccine that is both effective and practical for controlling bovine TB in free-ranging animal populations.

Oral immunization of wildlife presents many challenges but has several advantages over traditional, hands-on vaccination. Benefits include the ease of vaccine distribution at a relatively low cost, the capacity for multiple dosing, a lower rate of disturbance and trauma to animals, and the potential stimulation of mucosal immunity. Improved levels of delivery and efficacy can be achieved by developing selective baiting techniques, optimizing the frequency of vaccine and bait distribution, and improving vaccine efficacy through genetic engineering. The disadvantages of oral vaccination are the difficulties in controlling vaccine exposure of target and non-target animals (including humans) and preserving vaccine efficacy and stability in the environment. Oral immunization using recombinant Vaccinia-rabies glycoprotein has achieved widespread success in controlling rabies epizootics in wildlife, including canids and raccoons (Slate et al., 2005). This success could provide a model for managing TB and other wildlife diseases.

6.6.2 Nonlethal deterrents

Historically, lethal control (i.e., culling or hunting) has been the primary method for controlling wildlife-disease conflicts with humans and livestock. However, in most situations, the public supports nonlethal methods to control wildlife conflicts (Reiter et al., 1999; Loker et al., 1999). Extensive research has been devoted to developing methods to mitigate contact between livestock and free-ranging cervids. Mitigations to reduce or prevent livestock interactions are generally grouped into four categories: 1) fencing, 2) frightening devices, 3) biological methods, and 4) livestock management.

Fencing: To prevent cervids from accessing cattle feed, physical barriers are generally employed as fencing around stored cattle feed or cattle dry lots (winter yards) (Figure 6.3). Fencing that is effective at preventing deer from accessing stored cattle feed has been developed (Beringer et al., 2003; VerCauteren and Lavelle 2003; VerCauteren et al., 2006). However, for these methods to be successful, producers must maintain fencing and ensure that gates to stored feed are kept closed. The data suggest that up to 50 percent of landowners actively using fences to protect stored cattle feed do not close the gate to feed-storage areas, resulting in potential sharing of feed sources between deer and cattle (Berentsen, personal communication).

The NWRC is developing new methods to prevent deer from accessing stored cattle feed. These include replacing conventional gates with bump gates that will allow farmers to drive machinery into and out of feed-storage areas without manually closing the gates. However, recent testing of bump gates combined with deer guards (e.g., cattle guards) found that over time the gates failed and deer learned to walk or jump across deer guards (VerCauteren,et al., 2009). Additional research is being conducted to test the use of polyvinyl chloride (PVC) curtains to prevent deer from accessing stored feed areas. PVC curtains are used in place of gates and are designed to allow producers access to stored feed without relying on gates (Berentsen, personal communication).

Figure 6.3 Examples of fencing to prevent deer from accessing cattle feed

Good design: No gaps in gate door and material without rungs prevent entry to stored hay.

Photo by USDA-APHIS-WS-NWRC

Poor design: Large gap in gate and between rungs of gate allow deer entry to stored hay.

Photo by USDA-APHIS-VS-CEAH

Frightening devices: Frightening devices are used to create psychological stimuli that train wildlife to avoid areas where the deterrents are used. Available deterrents include propane exploders, lasers, motion-activated yard guards, and bio-acoustic devices. Many studies have evaluated the effectiveness of these devices (Belant et al., 1996; VerCauteren et al., 2003; VerCauteren,et al,, 2009; Beringer, 2003; Gilsdorf et al., 2002). However, developing a system that is consistently effective has proven difficult. Most devices are effective only for a short duration (a few days to weeks) until deer become habituated to the device. Currently, the most promising stimuli for frightening deer are animal-activated devices that stimulate vision and hearing (Gilsdorf et al., 2002).

Biological management**:** Guard dogs have been used for centuries to protect domestic sheep from predators (Green and Woodruff, 1990; Andelt, 1992). More recently, dogs have successfully prevented deer from damaging tree plantations (Beringer, 1994). This concept is currently being evaluated by NWRC as a tool to prevent deer from visiting areas of stored cattle feed and farm yards, specifically to mitigate transmission of *M. bovis*. Preliminary results from studies in Michigan indicate that dogs may prevent deer from utilizing stored cattle feed (VerCauteren et al., 2008).

6.6.3 Lethal control of Cervidae

It is uncertain what effect intensive culling efforts has on the control or reduction of *M. bovis* in free-ranging cervid populations. Nonspecific culling and increased hunter-harvest has often been used in an attempt to control or eliminate disease from free-ranging populations, based on the assumption that lower density means less transmission of disease (Barlow, 1996; Essey et al.; 1983; Wobeser, 2002). However, this assumption is rarely tested, and it is unclear if *M. bovis* transmission in free-ranging deer populations is primarily density dependent, frequency dependent, or some combination of the two. Partial nonspecific culling of host populations can cause eradication of a pathogen with primarily density-dependent, but not frequency-dependent transmission, unless selective culling of known infected individuals in a population is conducted (Schauber and Woolf, 2003; Gross and Miller, 2001). Additionally, public support for lethal control methods can be controversial and often difficult to implement long term from a social perspective (Reiter et al. 1999; Loker et al., 1999).

Nonselective partial culling of a free-ranging population assumed to have density-dependent disease transmission is optimally employed when the basic reproductive rate (R_0) of the pathogen can be estimated. Knowledge of R_0 for a population is not required for culling to be successful, but it does provide valuable information related to the number of individuals that must be removed to maximize

the probability that a pathogen is forced to extinction. R_0 has not been estimated for *M. bovis* infection in free-ranging cervid populations in North America. Estimates of R_0 not only are important for efforts to control infection by culling but are also necessary for other control measures (e.g., vaccination) that attempt to reduce or eliminate a pathogen from a host population.

The efficacy of culling strategies for controlling *M. bovis* infection in North American cervid species has not been reported in the research literature. Efforts to eradicate *M. bovis* from white-tailed deer populations by culling and hunter-harvest in Minnesota and Michigan have been unsuccessful (USDA, 2008, Payeur et al., 2002). An effort to eradicate *M. bovis* from feral swine on Hawaii's Molokai Island reduced apparent prevalence of *M. bovis* in feral swine populations from 20 to 3.2 percent, but failed to completely eradicate the pathogen from the population (Essey et al., 1983). Nonspecific culling of badgers in the UK has often resulted in increased spread of *M. bovis* in both badger and cattle populations and further complicates control efforts (Tuyttens et al., 2000; Donnelly et al., 2006, 2007). Increased hunting of elk in Riding Mountain National Park, Canada, along with test-and-cull strategies intended to control *M. bovis* infection also failed to eradicate this pathogen (Thoen, 2006).

Research is sparse on the effectiveness that culling and associated strategies (nonspecific or targeted) have in eradicating pathogens from free-ranging cervid populations in North America. Currently, the only published field studies on culling strategies in North American cervid populations focus on CWD in Colorado mule deer (Wolfe, 2004; Conner, et al., 2007), brucellosis in Rocky Mountain elk in the Wyoming portion of the Greater Yellowstone ecosystem (Scurlock, 2006, 2007, 2008), bovine TB in elk in Riding Mountain National Park (Thoen, 2006), and bovine TB in white-tailed deer in Michigan (Schmitt, 2004). Except for Conner et al. (2007), all of these studies have addressed only test-and-cull strategies for control of pathogens in wild populations and have not tested more targeted culling strategies (e.g. removing high-risk individuals, removing family groups, and focal culling).

In cases in which testing and culling have been studied and reported in North American Cervidae, this strategy has not been shown to be very effective in eradicating a pathogen from a population (Wolfe, 2004; Scurlock, 2006, 2007, 2008; Schmitt, 2004). Trapping, marking (e.g. radio collaring or GPS collaring) and testing wildlife populations is exceedingly expensive and difficult to implement at the landscape or population scale. Moreover, bovine TB tests with adequate sensitivity and specificity for use in live cervids are currently unavailable; thereby complicating identification of TB-positive individuals. Recapturing and removing test-positive animals is also expensive, labor intensive, and often fails (Schmitt, 2004). An accurate trapside test for TB in cervids would be helpful to avoid the need to recapture and handle infected animals more than once.

Models of culling strategies for CWD in mule deer have indicated that nonselective culling may not eliminate CWD from free-ranging populations (Gross and Miller, 2001). These models suggest that early and aggressive targeted culling shows the best promise of preventing the establishment of new endemic foci of infection. However, the models have been widely debated, largely because of assumptions regarding transmission (density vs. frequency dependent) (Schauber and Woolf, 2003, Wasserberg, 2009). While CWD differs biologically from bovine TB, it possesses similar epidemiologic characteristics, including an extended latency period, environmental persistence, and tolerance to environmental exposure. Several culling strategies designed to control CWD in mule deer are being tested (M.W. Miller, personal communication). Research on culling strategies related to control of CWD may provide some insight for management of *M. bovis* infection in free-ranging deer populations.

The effects of intensive culling and hunter-harvest on cervid movements and dispersal are unknown. Numerous studies have shown that recreational hunting has an effect on animal behavior and may increase or alter animal movements at both the population and individual animal level (Roland et al., 1988; Root et al., 1988; VerCauteren et al., 1998; Kilpatrick et al., 1999; Conner et al., 2001). Changes in animal behavior resulting in increased movement or dispersal have been reported for a wide range of disturbances for many wild ungulate populations in North America. Culling or increased

hunting of cervids in response to disease problems is generally more intensive than recreational hunting and may result in greater or more pronounced animal movements. It is currently unclear if intensive culling and increased hunting of infected populations may serve primarily as a population sink (i.e., removing infected animals from a population) or if these practices serve to disperse potentially infected animals outside of the area of infection, further exacerbating the problem.

6.6.4 Risk Identification

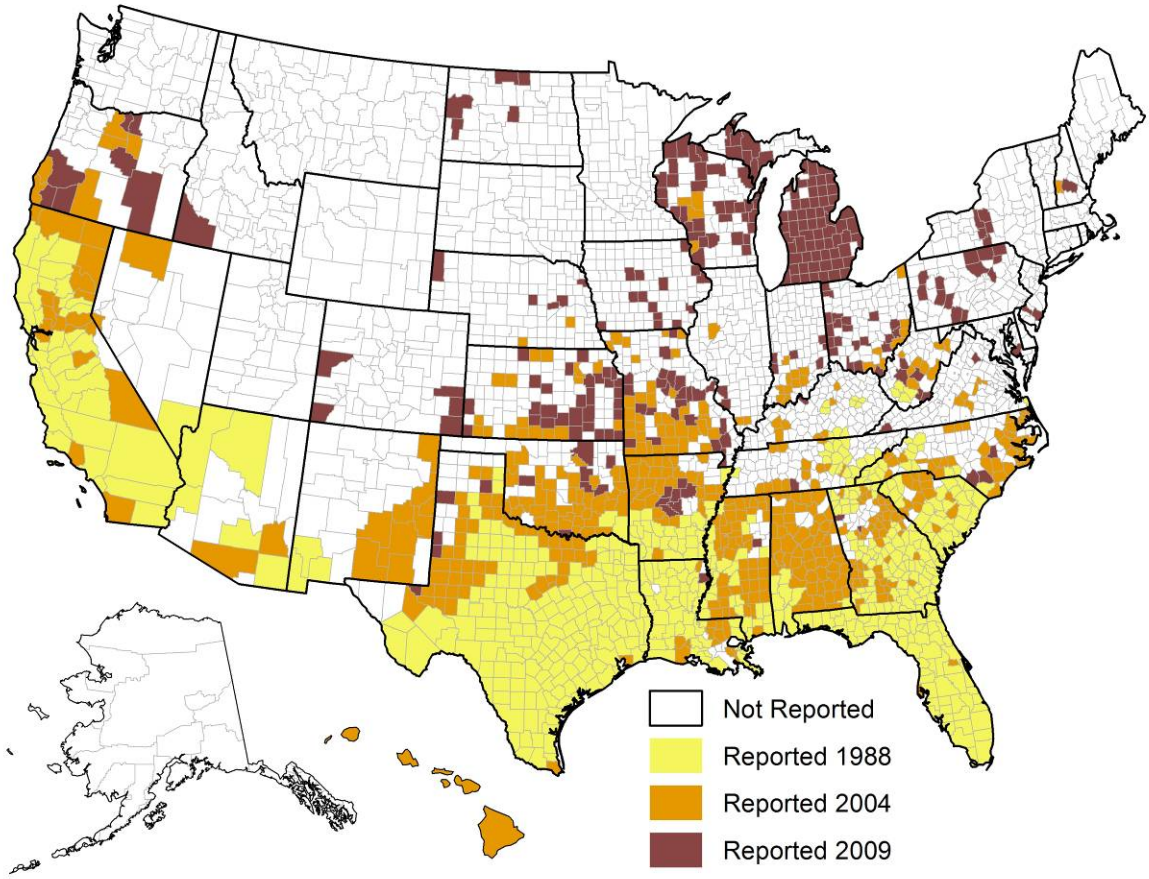
Risks from wildlife trade: Illegal wildlife trade presents a serious risk of introducing foreign animal diseases into the United States. Entry and feral establishment of a new host species, particularly the brushtail possum, would have disastrous ecologic and economic consequences, all but nullifying efforts to control *M. bovis* in North America. After it was introduced to New Zealand, the brushtail possum rapidly expanded its range and now occupies nearly the entire country. Due to its abundance, susceptibility to TB, and shared habitat with farm animals, the brushtail possum is the most important wildlife disease reservoir in New Zealand (de Lisle et al., 2001). The U.S. Fish and Wildlife Service and VS have prohibited the import of the brushtail possum since 2002.

Risks from feral swine: There is conflicting scientific evidence regarding the role of feral swine in the epidemiology of bovine TB. In Australia and New Zealand, wild pigs are spillover hosts, contracting TB through consumption of infected buffalo and possums (Corner, 2006). While *M. bovis* was highly prevalent (>40 percent) among feral pigs in some parts of northern Australia, the predominant lesions were confined to mandibular lymph nodes, indicating oral infection through scavenging (Corner et al., 1981). Therefore, even though bovine TB infection could be transmitted between pigs by cannibalism, there was no significant shedding of the bacteria and the animals did not play a significant role in the epidemiology of the disease. This was confirmed when bovine TB disappeared from swine after it was controlled in cattle and water buffalo (McInerney et al., 1995; Corner, 2006).

In contrast, Naranjo et al. (2008) reported that wild boar in Mediterranean ecosystems sustained TB infection and appeared capable of transmitting the disease to other species. Supporting evidence included high TB prevalence among wild boar fenced from contact with other species, lesions in thoracic lymph nodes and lungs suggesting respiratory infection and transmission, and extensive lesions in juvenile pigs that likely represented the main shedders of *M. bovis*. Likewise, Aranaz et al. (2004) and Hermoso et al. (2006) concluded that feral swine in Spain were maintenance hosts of *M. bovis* based on ecological factors and lesion characteristics. Circumstances favoring *M. bovis* transmission between wildlife and livestock included artificial increases in wild game populations stimulated by a robust hunting industry, lack of natural predators, and intensive cattle grazing in game preserves with susceptible wildlife hosts. These findings contrasted with other studies of wild boar in Spain (Gortazar et al., 2003) and in Italy (Serraino et al., 1999) that found TB lesions were more localized to the alimentary system. The predominance of well-encapsulated and calcified lesions in wild boar indicated a prolonged and chronic infection in this species (Parra et al., 2006).

Given the range expansion of feral swine in the United States (Figure 6.4), there is a great deal of concern about the animals' almost limitless capacity for carrying diseases that impact agriculture and human health. Feral swine may carry more than 30 bacterial and viral diseases in addition to numerous parasites that affect people, pets, other farm animals, and wildlife. Feral pigs have been documented in at least 44 States, nearly doubling the number occupied since 1988 (Hutton et al., 2006). Particularly worrisome is the recent appearance of feral swine in Michigan, where the potential exists for interaction with TB-infected white-tailed deer and cattle. Once a population of wild pigs becomes established, it is next to impossible to eradicate. Prevention of escapes or releases and timely elimination of new populations are the best management practices known at this time. In view of the risks presented by these animals, Hutton et al. (2006) argued for a federally- coordinated, comprehensive feral swine control program. To succeed, such a program would require legislation and regulatory changes, a sustained multidimensional public education effort, overt and covert law enforcement, and an aggressive, adequately funded control effort.

Figure 6.4 Current range of feral swine in the United States



Source: Data compiled and provided by APHIS Wildlife Service's National Wildlife Disease and Emergency Response Program, Southeastern Cooperative Wildlife Disease Study and State reports.

Risks from birds: Although the potential role of birds in the transmission of *M. bovis* has not been extensively studied, published and unpublished surveillance and clinical data suggest that birds are less susceptible than mammals to *M. bovis* infection and do not play a large role in the transmission of bovine TB. This assessment is supported by the observation that despite the abundance and mobility of wild birds and their extensive contact with other animals and humans, the distribution of bovine TB is relatively circumscribed and can be explained on the basis of terrestrial animal movements, both natural and anthropogenic. There may be special situations in which birds do play a role in the epidemiology of bovine TB. There has been considerable interest, for example, in wild birds commonly associated with cattle production (i.e., farm visitation), and research has demonstrated a wide range of susceptibility to *M. bovis* infection among such peridomestic species (Butler et al., 2001; Fitzgerald et al., 2005b; Clarke et al., 2006). Data on *M. bovis* susceptibility are still lacking for some species, such as European starlings which are believed to transmit *E. coli O157* between dairies (LeJeune et al., 2008) and frequently move, sometimes long distances, between feedlots (Gaukler et al., 2008). Similarly, blackbirds often congregate in very large numbers on feedlots and dairies but have not been investigated for their possible role in TB transmission (Clark et al., 2003). Research is particularly needed on the role of such birds in mechanical transport of *M. bovis*-contaminated feces, feed, or other material between farms, feedlots, and dairies in regions with TB-infected cattle populations.

Areas at risk due to historic M. bovis infection in wildlife: Bovine TB infections in free-ranging wildlife may be silent, existing for years or even decades before being detected in hunter-killed animals or emerging or re-emerging in local cattle populations. Examples include Michigan, where TB is thought

to have been spilled over from cattle to white-tailed deer 40 to 50 years before it was recognized as a serious threat, and on Hawaii's Molokai Island, where TB apparently smoldered for years in feral swine before spilling back into cattle. In Riding Mountain National Park, Manitoba, bovine TB was identified in 1937 in plains bison and again in the 1970s in wolf pups (Tabulenas, 1983; Carbyn, 1982). During the 1950s and 1960s, *M. bovis* was endemic in Canadian cattle herds and several outbreaks occurred in cattle surrounding the Park (Thoen, 2006). Manitoba was declared TB-free in 1986, but tuberculosis resurfaced 5 years later in cattle and elk, more than 50 years after TB was first discovered in wildlife. Lack of surveillance, poor diagnostic tests, and the long latency period for bovine TB may pose risks for re-emergence of this pathogen in areas where it has historically existed.

Bovine TB was reported in free-ranging white-tailed deer in New York in 1933, 1937, and again in 1961 (Levine, 1934; LeDune, 1937; Friend, 1963; Wagner, 1993). These reports occurred from hunters reporting unusual findings in deer after harvest. Moreover New York has been plagued by periodic outbreaks of bovine TB in cattle and in confined deer herds—the most recent in 2008. Extensive surveys of New York's wild deer populations have not been conducted to determine if a wildlife reservoir is present. The periodic identification of bovine TB in wild deer and the continued occurrence of bovine TB in confined populations raise the likelihood that bovine TB is present in New York's deer population.

In 1994, bovine TB was identified in free-ranging mule deer and coyotes surrounding an infected, confined elk farm in southern Montana (Thoen, 1992; Rhyan, 1995). The mule deer and coyote populations surrounding the farm were culled and surveyed for the presence of bovine TB, resulting in the identification of bovine TB in both populations. Elk populations near the farm were also surveyed with no findings of bovine TB (Rhyan, 1997). At the time, it was assumed that the infection would not persist in wild populations without a domestic livestock source for bovine TB. However, recent evidence from Michigan and other areas with endemic bovine TB in wildlife populations indicates that it may be possible for deer populations to maintain the disease.

Risks from unregulated game ranching and transport: Exotic game ranching is a rapidly growing segment of the U.S. animal agriculture industry. This expanding industry is largely unregulated in the United States and poses a risk for introducing and establishing additional areas of endemic wildlife diseases, including bovine TB. In some locations, exotic hoofstock or native cervids are released or baited into a semi-free-ranging environment (maintained by three-sided fencing) for recreational hunting. The animals are fed corn or other protein and often commingle with cattle and feral swine (Figure 6.5). This is an ideal environment for pathogen exchange. In south Texas, exotic and native hoofstock game ranching is often combined with cattle production, and in some areas these nontraditional ranching operations have replaced cattle production as a form of animal agriculture. The exotic game ranching industry is also expanding to other southern and Midwestern States (Teer, 1991, Mungall, 2000; 2007).

Figure 6.5 Example of livestock and wildlife use of a protein feeder in South Texas.

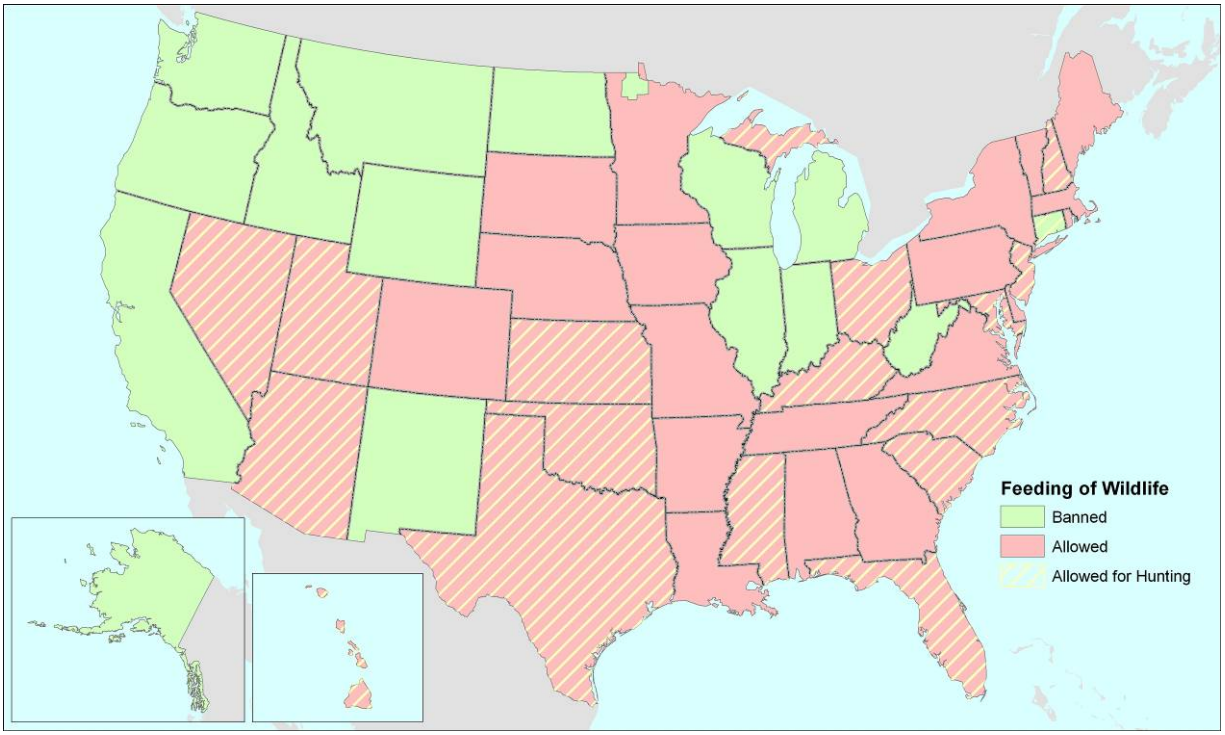
Source: Anonymous private ranch in South Texas.

Because game ranching has limited regulation, little is known about species locations, numbers, how animals are managed and moved, or what diseases or parasites they may carry. Few animals on game ranches are routinely tested for TB or other endemic or foreign animal diseases, and unregulated transport of animals between farms or movement of potentially infected carcasses by hunters present a risk for spread and introduction of pathogens (Jennelle, 2009; VerCauteren, 2008). It has been estimated that Texas has more than 70 species of exotic ungulates, numbering close to 200,000 animals (Traweek, 1995). Incomplete fencing and poor maintenance of high fences has allowed many animals to escape. At least five species of exotic ungulates exist as free-ranging populations in Texas and other regions of the United States where they are commonly farmed or have been intentionally released (Traweek, 1995; Gray, 1980; Mungall, 2000; 2007; Teer, 1991; Witmer and Lewis, 2001; Chapman, 1995; Feldhamer, 1988; Leslie, 2008).

The extensive unregulated interaction of domestic livestock with native and exotic game animals (e.g., along fence-lines, at sales, and at feeding stations), raises the risk of bovine TB introduction and may result in serious economic and ecological consequences. Additionally, Texas and other southern and Midwestern States frequently receive cattle from Mexico that are often infected with bovine TB. Because of the unregulated and unsurveyed nature of exotic ungulates and the potential introduction of bovine TB from Mexico, States with exotic livestock may be at risk for the emergence of disease in free-ranging wildlife populations. To address this situation, a national survey of exotic ruminants, both ranched and free-ranging populations, should be considered as well as a compilation of State and Federal regulatory requirements for keeping and moving exotic game. Estimating contact rates between domestic ruminants, exotic animals, and native wildlife would require a more extensive research effort, but may also be useful.

Risks from recreational and institutional feeding: Recreational and institutional feeding of deer and elk may also contribute to the establishment of bovine TB in wildlife. Feeding and baiting of white-tailed deer in Michigan helped to establish bovine TB in that State and other States that use this practice would be at risk if bovine TB were introduced (Miller, 2003; Schmitt, 1997; Hickling, 2002; Palmer, 2004). Recreational feeding of wildlife for wildlife viewing or hunting is common throughout North America and has only been banned or restricted in 16 States. The remaining 34 States have limited or lax regulations for wildlife feeding (Figure 6.6).

Figure 6.6 State regulations allowing recreational feeding of wildlife



The Federal and State feeding grounds in Wyoming (where brucellosis is an ongoing problem in elk) are of particular concern regarding feeding of wildlife. Winter feeding of elk in Wyoming has a long history. In 1912, the National Elk Refuge was the first feedground created to mitigate damage to hay stored for wintering cattle. The number of feedgrounds has continued to increase, comprising 1 federally- and 22 State-operated feeding grounds in 3 counties. On average, these feeding grounds support 20,500 elk annually, and an estimated 80 percent of the total elk population winters on or near these grounds (Dean, 2004; Smith, 2001; Boyce, 1989). Social and political constraints have allowed the continued operation of the Wyoming feeding grounds, which have become a source of brucellosis infection (Smith, 2001). If bovine TB were introduced to these areas by infected livestock or other sources, it could quickly become established and spread to other populations, where it would be very difficult to eradicate (Peterson, 2003). During severe winters when animal mortality is expected to be high, many other western States also periodically feed populations of elk, deer, and sheep (Smith 2001). Although less of a risk because of the infrequent nature of feeding programs, these practices pose a risk of spreading of bovine TB if introduced.

6.7 Discussion

6.7.1 Disease surveillance

Bovine TB has been identified in nine geographically distinct wildlife populations in North America and is endemic in at least five of these populations. *M. bovis* has been detected in free-ranging wildlife often decades before it has re-emerged in local cattle populations. An example is Riding Mountain National Park in Manitoba, Canada, where bovine TB was identified in plains bison in 1937 and again in the 1970s in wolf (*Canis lupus*) pups (Tabulenas, 1983; Carbyn, 1982). During the 1950s and 1960s, bovine TB was endemic in Canadian cattle herds and several outbreaks occurred in cattle surrounding the Park (Thoen, 2006). Manitoba was declared TB-free in 1986, but 5 years later TB resurfaced in cattle and elk, more than 50 years after TB was first discovered in wildlife. Recent evidence suggests that the same strain of *M. bovis* has been circulating in Cervidae, cattle, and wolves for at least three decades (Lutze-Wallace, 2005).

A similar situation exists in the United States. In 1975, a white-tailed deer with TB was killed by a hunter in Michigan. No systematic surveys were conducted after this event and it was nearly two decades before an endemic focus of bovine TB became evident in free-ranging deer and had spread to the State's cattle herds (Thoen, 2008). New York has been plagued by periodic outbreaks of bovine TB in cattle and in confined deer herds, the most recent in 2008. Bovine TB was reported in free-ranging white-tailed deer in the State in 1933, 1937, and again in 1961 (Levine, 1934; LeDune, 1937; Friend, 1963; Wagner, 1993). These events highlight the risk of TB re-emergence in areas where it has historically existed in wildlife and cattle. This risk is exacerbated by poor diagnostic tools and a lack of sufficient funds for robust wildlife disease surveys.

6.7.2 Population and behavior management

The development of effective mitigation measures to prevent or reduce contact between livestock and free-ranging cervid populations has been challenging. In addition to technical difficulties, public participation and compliance have impeded progress. More research is needed to develop effective, practical, and economic mitigations. Some of the best solutions may prove to be the simplest, e.g., the use of livestock protection dogs.

Although culling is sometimes controversial, it can be an important management tool to reduce disease spread. Research has not addressed issues related to optimal culling strategies for disease control in North American free-ranging cervid populations. Studies on the behavioral effects of intensive culling or hunting, specifically for reducing disease transmission, are needed to better determine the effects on deer populations and the dynamics of *M. bovis* infection in these populations. Selective culling strategies that target high-risk animals or groups should be investigated to design practical and effective culling programs, such as deer that frequent farms where they might contact cattle, or targeting cohorts (e.g., males or family groups) that are more likely to be infected and spread disease to herd-mates.

6.7.3 Vaccination

Progress has been made in the search for new TB vaccines, including vaccines designed for humans and others intended for use in cattle and wildlife (Buddle et al., 2006). Some vaccine strategies involve supplementing the human TB vaccine (BCG) with DNA or protein subunits. The complete genomes of *M. tuberculosis* and *M. bovis* have been sequenced, and since these pathogens are so closely related a vaccine effective against one organism may also be effective against the other. Significant challenges remain, including a better understanding of wildlife immune responses to different vaccines and vaccination methods, as well as more effective delivery methods and adjuvants that stimulate immunity (Buddle et al., 2006). Results of recent oral vaccination studies in deer and other wildlife are promising. In the United States, further development and licensing of an oral BCG vaccine for white-tailed deer may be a valuable tool for addressing bovine TB issues in Michigan and Minnesota.

6.8 Summary

Bovine TB is a readily controlled disease when there is no reservoir of *M. bovis* infection in wildlife (O'Reilly and Daborn, 1995). Conversely, once bovine TB has been introduced into an ecosystem with free-ranging maintenance hosts, the infection is almost impossible to eradicate without extreme measures. The most economical and effective control measure for bovine TB in wildlife may be mass oral vaccination with progressive reduction in susceptibility of target species. A robust program of feral swine control and better regulation and surveillance of the game ranching industry is also needed. The 2008 outbreak of wildebeest-associated malignant catarrhal fever in Texas cattle is a clear indication that changes are necessary to protect animal agriculture from further spread of bovine TB and other diseases in the United States.

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7. Zoo, Exotic, and Pet Animals

7.1 Introduction

The last assessment of risk factors for *M. bovis* in the United States was performed in 1992 (USDA, 1992). At that time, it was noted that failure to eradicate bovine TB from exotic hoofstock was a concern, and the presence of *M. bovis* in exotic species represented a reservoir of infection for cattle, other domestic animals, and was a public health hazard. *M. bovis* has still not been eradicated from exotic and wild animals. The status and risk factors of *M. bovis* in captive exotic animals, wild animals, and pets are discussed in this chapter.

7.2 Current tuberculosis situation in captive exotic, wild, and pet animals

Many captive exotic animals are susceptible to *M. bovis,* such as members of the Bovidae, Giraffidae, Cervidae, and Camelidae families, and other animals including primates, rhinoceros, hippopotamus, elephants, tapirs, and swine. The transmission of TB is facilitated by conditions of captivity including crowding, common feed and water sources, and facilities that are difficult and expensive to sanitize. The first case of TB in captive exotic, wild, and pet animals was recorded in a chimpanzee (Thoen et al., 1976) only 10 years after the first modern zoo was established in London in 1826. .

7.2.1 Captive exotic animals in zoos, exhibits, and private collections

In the United States, NVSL records show that *M. bovis* was diagnosed in zoo and captive animals between 1993 and 2008 for antelope, goat, gerenuk, sitatunga, llama, snow leopard, elephant, and rhinoceros.[11] Recently, outbreaks of *M.* bovis have occurred in zoos or wild animal parks worldwide. An epizootic of *M. bovis* in a zoological park in Louisiana affected four white rhinoceroses and two Colobus monkeys. Personnel at the park who were exposed to the affected animals were found TB-positive by intradermal skin test results (Stetter et al., 1995). A report from France showed *M. bovis* was diagnosed in baboons, leopards, and a sea lion (Thorel et al., 1998). In Australia, *M. bovis* was found in three seals with documented transmission to one of the trainers (Thompson et al., 1993). A German wild animal park detected *M. bovis* in three pot-bellied pigs, a red deer, a buffalo, and two European lynxes, but sentinel animals (fox, badger, ferret, and rodents) were negative (Schmidbauer et al., 2007). Other mycobacterial species within the *M. tuberculosis* complex also cause outbreaks in zoological parks. For example, six animals (two Asian elephants, three Rocky Mountain goats, and one black rhinoceros) were infected with *M. tuberculosis* at the Los Angeles Zoo*;* (Oh et al., 2002).

TB in zoo and circus elephants is a concern for public health and animal welfare. Most cases are caused by *M. tuberculosis* (Shimshony, 2008; Gavier-widen et al., 2006)[12] and only rarely by *M. bovis* (Lyashchenko et al., 2006). There is one report of *Mycobacterium szulgai* as a cause of atypical TB in African elephants at Lincoln Park Zoo in Chicago, Illinois (Lacasse et al., 2007). One strain of *M. tuberculosis* was thought to spread between humans and elephants (Michalak et al., 1998.)

Camelids, including llamas, alpacas, guanacos, vicunas, dromedary, and Bactrian camels are present in relatively small numbers in the United States. Reports of *M. bovis* in camelids are rare; however, an outbreak of *M. bovis* in a dromedary racing herd was reported in 2005 in Dubai (Wernery et al., 2007). Llamas and alpacas are “common exotic” animals and are usually kept as hobby or working animals. Increased numbers and decreased value for llamas and alpacas has slowed their once explosive growth as breeding animals, and exports from South America are no longer allowed. Since 1992, there were only three cases of *M. bovis* in llamas reported by NVSL and a case of generalized tuberculosis in a llama in Switzerland caused by *Mycobacterium microti* (Oevermann et al., 2004). Few cases of TB are diagnosed in camelids, and testing procedures for these species are not validated and rarely utilized.

[11] National Veterinary Services Laboratories, unpublished official microbiology records, 2009.
[12] http://www.elephantcare.org/TBrefs.htm

7.2.2 Pet animals

Bovine TB is occasionally reported in pet animals. *M. bovis* was isolated by NVSL from a cat that resided on a feed yard in Texas and experimental infection is known to occur in guinea pigs. Disseminated *M. bovis* was also detected in a cat in Michigan, likely a result of exposure to infected wildlife (Kaneene, 2002). Two unusual cases of *M. bovis* in dogs exist; in New Zealand (Gay et al., 2000) *M. bovis* was isolated from a farm dog, and in the UK, a dog apparently became infected with *M. bovis* from its owner, who may have contracted the disease from handling a badger (Shrikrishna et al., 2009). These findings are likely exceptional, however. In a 2002 study from Michigan, 18 cats and 5 dogs residing on 9 bovine TB-affected farms were tested for *M. bovis* and all were negative (Wilkins et al., 2008).

7.3 Applicable regulations and testing

The Federal government and all State governments regulate interstate animal movement, usually by permit and testing. Domestic Bovidae and Cervidae are regulated under the APHIS regulations for required TB testing; however TB tests for other species are not required.

Approved antibody tests are available only for elephants and nonhuman primates. For all other animals, most diagnoses originate from culture or necropsy. Although not approved in many exotic species, skin tests may still be useful indicators, albeit with greatly reduced sensitivity and specificity. Private sales and public auctions are not obligated to require TB test results for transfer of ownership or for presence on sale grounds. Routine tests of exotic animals on private reserves, game parks, or wild animal exhibits are also not required. As a part of APHIS-Animal Care (AC) requirements, elephants held by licensees or registrants are required to be TB tested annually. For elephants, a triple sample trunk wash and microbiological testing along with the antibody test are the recommended diagnostic tests.

Members of the American Association of Zoos and Aquariums (AZA) often follow additional testing and movement guidelines, such as the “Tuberculosis Surveillance Plan for Non-Domestic Hoofstock” (National Tuberculosis Working Group, 2001). This document was updated and approved by the United States Animal Health Association (USAHA) in 2008 (National Tuberculosis Working Group 2008). It establishes a surveillance plan, which in turn, sets a standard for intradermal tuberculin testing of exotic hoofstock, collecting data, estimating the prevalence and incidence of TB in zoological collections, guiding personnel exposed to TB-infected animals, and preventing transmission of TB between zoological collections. The National Association of State Public Health Veterinarians also publishes a compendium outlining measures to reduce disease and injury associated with animals in public settings (NASPHV, 2005).

7.4 Summary

M. bovis is important in animal and public health because it is the second most pathogenic mycobacterium, has a wide host range, and many of the animals it affects can become sources of infection and live in the human environment (Isaza, 2003; Corner, 2006). Risk of *M. bovis* transmission from captive exotic animals to domestic cattle is difficult to estimate because of a lack of uniform surveillance. However, this risk is perceived to be low because captive exotic animals are not usually located near domestic cattle.

In addition to elephant testing requirements, non-AZA-member exotic animal exhibitors should be regulated, either by separate legislation or by requiring membership in AZA to obtain an exhibitor’s license for other species. Points of public sale and aggregation are difficult to regulate without approved tests; therefore, methods of quarantine, movement restriction, or limits on the number of animals in contact with each other may be appropriate. An effective vaccine may assist in reducing the prevalence of *M. bovis*.

The risk from pet animals is extremely low to negligible. Very few case reports exist of any strain of mycobacteria in dogs and cats, even when they reside on affected premises. It appears that the primary risk of TB in dogs and cats may be by infected owners (a reverse zoonosis).

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8. Conclusions

This assessment evaluated five pathways for *M. bovis* spread into and within the United States. Of the pathways explored, additional understanding of each is needed to truly recognize the extent of risk. Additional pathways, such as the U.S. bison industry, human interaction, and fomites should also be considered as potential pathways; however, data limitations have prevented further exploration of these pathways.

Legal and Illegal Imports: Additional investigation is needed to determine where Mexican-origin cattle commingle with U.S. cattle. Genotyping analyses continue to suggest a relationship between infection in cattle from Mexico and U.S. cattle, particularly in the Southwest United States. Limitations of the current antemortem tests indicate the need for mitigations on both sides of the U.S.-Mexico border.

U.S. Cattle Industry: The outbreaks in California and New Mexico highlight the risk of bovine TB spread through current industry practices. The risk factors identified were commingling with Mexican-origin steers, offsight heifer-raising, and the large number of purchased additions introduced to herds. Many producers are not familiar with bovine TB or do not perceive a risk of bovine TB transmission. Therefore, current management practices are not geared toward minimizing the risk of TB transmission.

U.S. Captive Cervid Industry: In the past decade, APHIS has implemented actions to reduce the risk of bovine TB spread through the captive cervid industry, such as movement restrictions associated with the CWD eradication program and development of the 1999 UM&R for bovine TB. However, surveillance of captive cervids is minimal, and little is known about the true prevalence of bovine TB in the captive cervid industry. Captive cervids may pose a risk of bovine TB transmission to local wildlife and potentially cattle.

Wildlife: It is likely that wildlife in the United States will continue to serve as a risk factor for bovine TB transmission. The disease has been identified in white-tailed deer in Michigan and Minnesota and has been found intermittently throughout other areas of the United States. The lack of surveillance in wildlife makes it difficult to understand the extent of this pathway for bovine TB spread throughout the country (e.g., surveillance of wildlife around affected herds remains inconsistent). Eradication of bovine TB in other countries that have wildlife reservoirs has proven to be almost impossible.

Zoo, exotic, and pet animals: Tuberculosis has been a concern in elephants throughout the zoological community. Testing has been implemented in elephants, but several other zoo specimens are also susceptible to mycobacterial organisms. While these species may have little exposure to domestic cattle, they do pose a public health risk. Game ranches are also a sector in which little to no bovine TB surveillance has been done. If infected, this sector poses a risk to wildlife, cattle, and captive cervids. Examples of *M. bovis* in cats and dogs have been reported; however, these animals are usually infected as a result of exposure to infected cattle or humans. It is possible that cats and dogs pose a public health risk, but they are inefficient hosts and likely demonstrate little risk to domestic cattle.

Of the pathways evaluated in this assessment, all call for additional research and mitigation measures. Without better surveillance throughout these pathways, the true risk to U.S. cattle cannot be estimated. It is likely that some level of bovine TB will continue to circulate through the U.S. cattle industry based on current industry practices, the large amount of mixing that occurs, and exposure from wildlife and Mexican-origin animals. Current efforts are underway to implement changes to the bovine TB program and address the risks associated with the pathways outlined in this assessment.

Appendix I: State regulations for captive and free-ranging hoofstock

Figure A3. States that allow exotic hoofstock

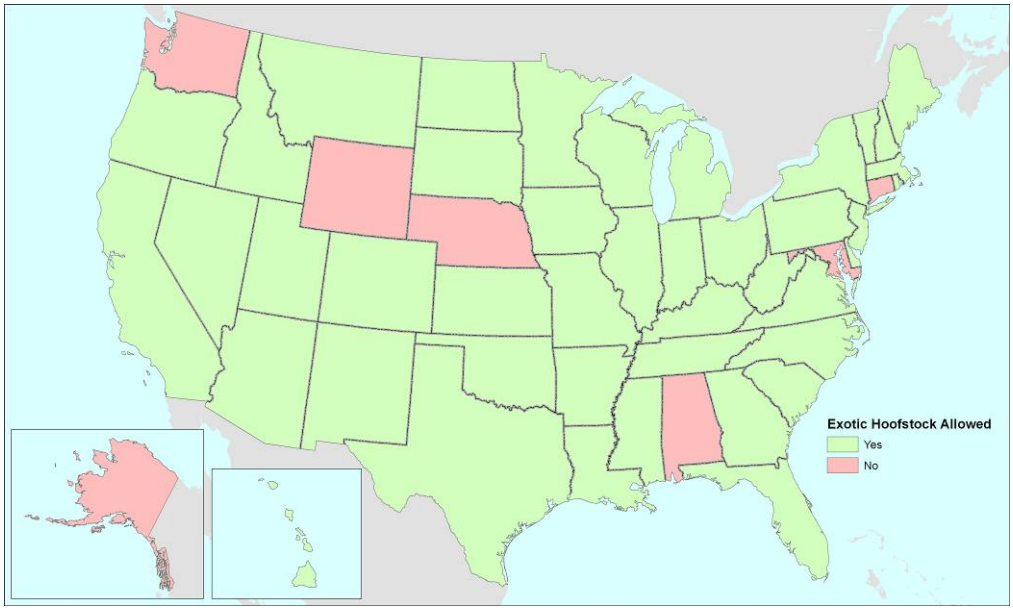

Figure A4. States that require bovine TB testing for movement of wildlife or exotic hoofstock

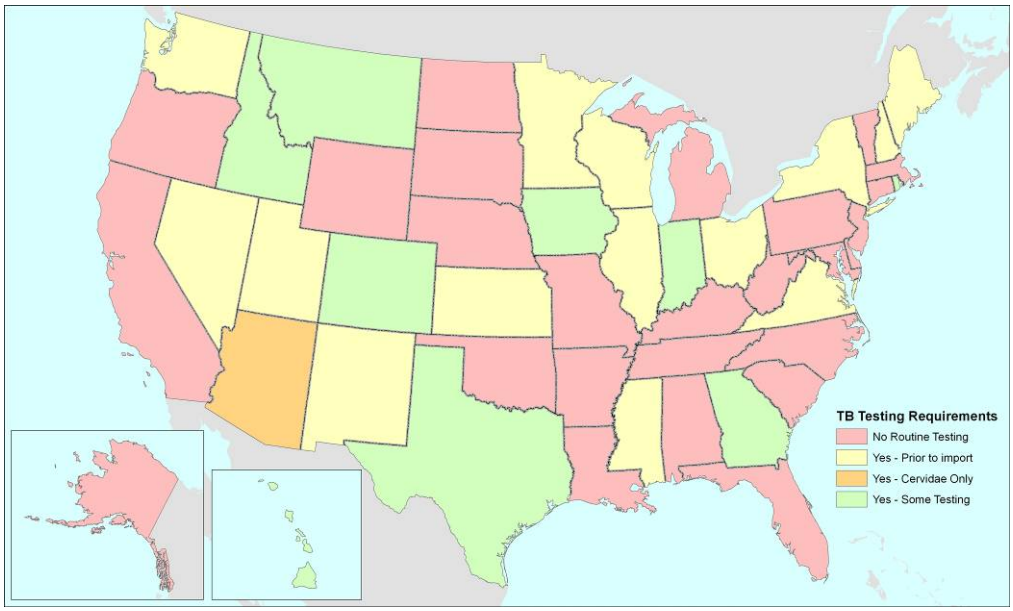

Figure A5. States that allow recreational feeding of wildlife

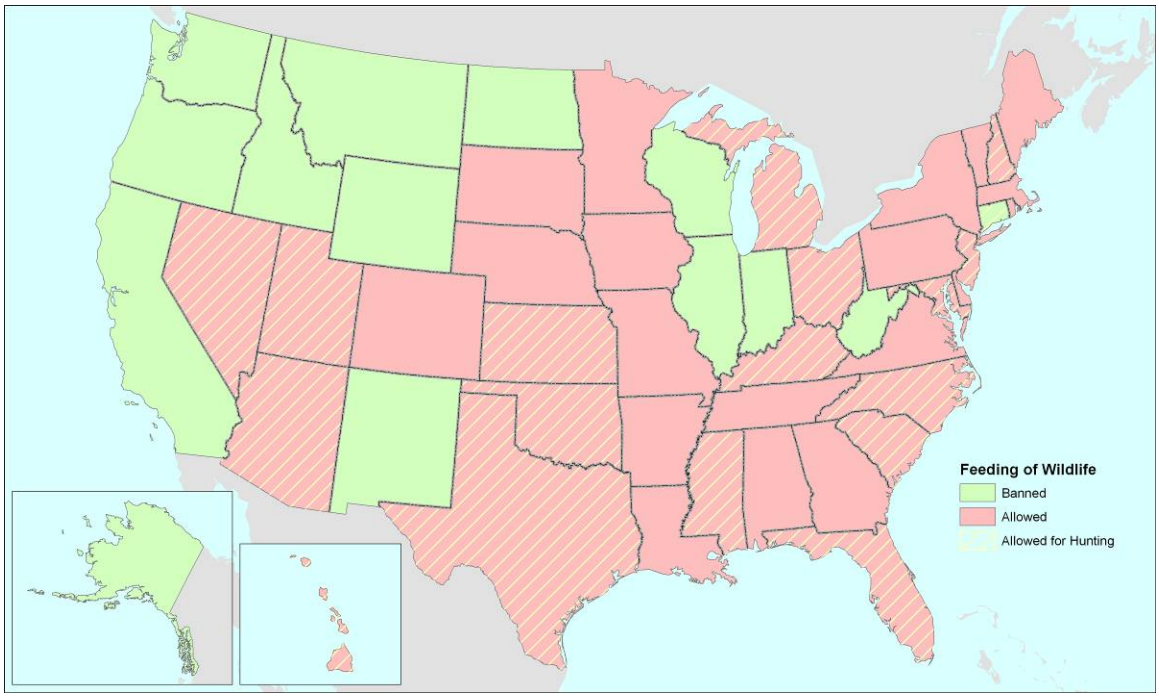

Figure A6. States with high fence requirements for exotic hoofstock or cervids

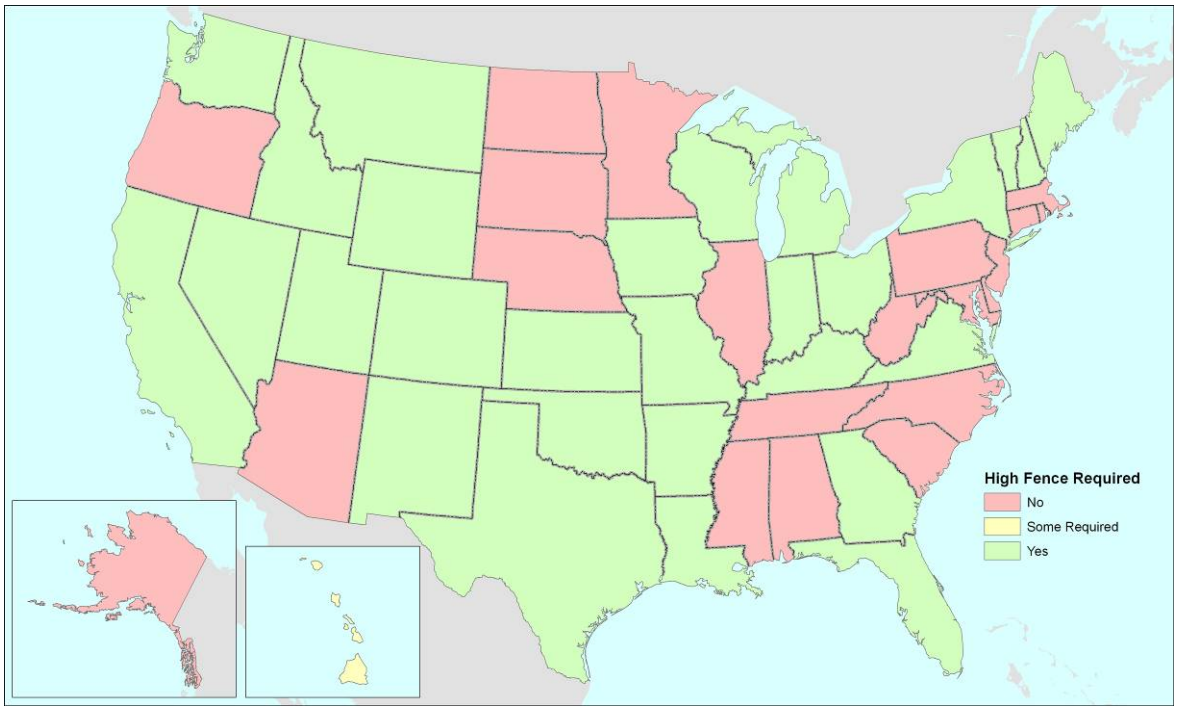

Figure A7. States with confined hunting regulations