Bovine tuberculosis is caused by *Mycobacterium bovis*, which is a zoonotic pathogen. In 1917, the USDA initiated a state-federal-industry eradication program to address bovine tuberculosis in cattle. Consequently, bovine tuberculosis has been nearly eliminated from US cattle herds, but intermittent *M. bovis* infections continue to occur. From 2002 to 2011, national surveillance detected *M. bovis*-infected cattle in 80 herds, including 25 (31%) dairy, 53 (66%) beef, and 2 (3%) mixed purpose herds. During this same period, *M. bovis*-infected animals were detected in 9 captive cervid herds, including 1 each in 2003 and 2008, and 5 in 2009. National surveillance for bovine tuberculosis consists of antemortem surveillance, which includes CFT tests performed by USDA-accredited veterinarians, and slaughter inspection performed at federally inspected slaughter establishments.

The purpose of an epidemiological investigation is to determine the origin of an infected individual and the potential spread of infection. In regard to livestock production, these investigations focus primarily on the origin and disposition of all animals that have resided at an affected premises for the 5 years prior to the discovery of the infected animal or as far back as producer records allow. During such an investigation, the tracing of sexually intact animals is emphasized because these animals typically live longer and thereby pose a greater risk for disease transmission than do neutered animals.

Traditionally, it has been assumed that neonatal dairy bull calves, which are typically sold at 1 to 3 days old, pose a low risk for transmission of bovine tuberculosis because of the limited time they spend in the herd of origin and the fact that they are generally housed with similar calves that are destined for slaughter with minimal exposure to sexually intact cattle. However, this assumption is potentially flawed because neonatal calves are often exposed, albeit for a brief time, to colostrum

**Objective**—To describe an epidemiological investigation of a bovine tuberculosis outbreak on a Colorado dairy operation.

**Procedures**—A culled dairy cow infected with *Mycobacterium bovis* (index cow) was detected at a Texas abattoir during routine slaughter surveillance and subsequent diagnostic testing. This initiated an epidemiological investigation that was performed in accordance with USDA regulations.

**Results**—The index cow was traced back to a Colorado dairy (index herd). Of the 908 cattle in the index herd, 101 (11.1%; 86 adult cattle > 2 years old and 15 immature cattle ≤ 2 years old) were infected with *M. bovis*. Fourteen *M. bovis*-infected cattle ≤ 2 years old were identified on 5 additional premises that had purchased cattle from the index herd directly or indirectly. All 115 affected cattle were infected with the same genetic type (spoligotype) of *M. bovis*. A substantial proportion of cattle that left the index herd during the 5 years previous to the identification of the index cow were untraceable because of a lack of unique animal identification and inadequate records.

**Conclusions and Clinical Relevance**—Results indicated that neonatal calves can have an important role in the transmission of *M. bovis*. Also, this report highlights the exigent need for unique individual identification of livestock, including neonatal animals, so that thorough epidemiological investigations of reportable (zoonotic or foreign animal) diseases can be conducted when necessary. (J Am Vet Med Assoc 2014;244:805–812)
and milk from adult cows, which could be contaminated with *M. bovis*. Thus, these calves could become infected with *M. bovis* prior to their sale at 1 to 3 days of age. Unfortunately, neonatal bull calves are frequently moved with little to no official or permanent identification from their birthplace to calf-raising operations or other non-commercial facilities. Lack of identification complicates tracking animal movement and limits the ability to determine the extent of animal exposure to the pathogen in question. The purpose of this report was to describe an epidemiological investigation of a bovine tuberculosis outbreak that began in a Colorado dairy herd in 2010 and resulted in *M. bovis*-infected cattle being identified on 5 additional premises, presumably because of disease transmission by young calves.

**Index Cow**

In March 2010, a 4-year-old Holstein cow was culled because of low production and subsequently slaughtered at a Texas abattoir. During postmortem examination, USDA Food Safety and Inspection Service inspectors observed bronchial and mediastinal lesions suggestive of *M. bovis* infection and submitted specimens of those lesions to the NVSL in Ames, Iowa. The carcass was retained at the abattoir until laboratory examination of the submitted specimens could be completed. Histologic examination revealed that the lesions were compatible with bovine tuberculosis, and PCR assay of the tissue specimens yielded positive results for *Mycobacterium tuberculosis* complex IS6110, resulting in a presumptive diagnosis of bovine tuberculosis. Culture of the specimens resulted in growth of *M. bovis*, which was then subjected to spoligotyping, a type of genotype testing, so that the isolate could be compared genetically with other *M. bovis* isolates in the NVSL database. All diagnostic test results from the NVSL and identification information collected at the Texas abattoir were forwarded to the USDA APHIS Veterinary Services area office in Colorado for initiation of an epidemiological investigation.

**Testing of Cattle Suspected of Being Exposed to *M. bovis***

The epidemiological investigation was conducted in accordance with all applicable USDA APHIS Veterinary Services regulations. Briefly, a CFT test was administered to all cattle ≥2 months old that were suspected of being infected with *M. bovis* or potentially exposed directly or indirectly to the index cow. Cattle that developed a visible or palpable response to the CFT test (ie, suspects or responders) were then administered a secondary test such as the CCT test or IFN-γ assay. Alternatively, when necessary because of logistic or time constraints, initial screening of cattle for *M. bovis* in herds with an unknown *M. bovis* status can be performed by administration of the CFT test in parallel with the IFN-γ assay. Initial screening of cattle in *M. bovis*-affected herds can be performed by administration of the CFT test in parallel with the IFN-γ assay. The CCT test can only be performed by state or federal regulatory veterinarians. For the CCT test, *M. bovis* PPD and *Mycobacterium avium* PPD were injected intradermally at separate sites in the midcervical region and 72 ± 6 hours later, the thickness of the skin at the respective injection sites was measured, and these measurements were compared. For the IFN-γ assay, blood was collected from cattle classified as suspect by the CFT test between 3 and 30 days after the *M. bovis* PPD was injected for the CFT test or from cattle at the time the *M. bovis* PPD was injected for the CFT test. The sensitivity of the CFT and CCT tests is estimated to range between 74.0% and 93.6%, and the specificity is estimated to range between 96.0% and 99.9%. Use of the IFN-γ assay in parallel with the CCT test in cattle experimentally infected with *M. bovis* resulted in a sensitivity and specificity of 81.8% and 99.1%, respectively. For the purpose of the epidemiological investigation reported here, results of the IFN-γ assay were classified as positive or negative in accordance with serum concentration cutoffs recommended by the manufacturer of the assay.

Cattle from an *M. bovis*-affected herd and classified as a suspect or reactor on the basis of CFT test results or with a positive IFN-γ assay result were euthanized and necropsed with a regulatory veterinarian in attendance. Alternatively, all cattle from a confirmed *M. bovis*-affected herd could be euthanized and necropsed without any ante-mortem testing performed. During necropsy of these cattle, specimens (fresh and those fixed in neutral-buffered 10% formalin) of any tissue with granulomatous-appearing lesions as well as representative lymph nodes from the head and thorax were submitted for laboratory examination, which consisted of histologic examination and culture. For specimens with histologic lesions consistent with bovine tuberculosis and in which acid-fast bacilli were detected, PCR assay was performed. Fresh specimens were cultured for *M. bovis* as described with at least 1 liquid media system and 2 tubes of a modified Middlebrook 7H11 agar supplemented with calf serum, hemolyzed blood, pyruvate, and malachite green. For the epidemiological investigation reported here, *M. bovis* infection was confirmed on the basis of the presence of histologic lesions in any examined tissue compatible with bovine tuberculosis, growth of *M. bovis* from culture of a submitted specimen, or positive results for DNA sequences consistent with *M. bovis* in a submitted specimen as determined by PCR assay.

**Identification of the Herd of Origin for the Index Cow and Screening of That Herd for Other *M. bovis*-Infected Cattle**

The first phase of the epidemiological investigation involved the identification of the herd of origin for the index cow and screening of that herd for other *M. bovis*-infected cattle. Animal identification was critically important for tracing the index cow back to the herd of origin. Back tags placed on cattle at livestock markets provide a temporary method of animal identification and are collected at slaughter. The back tag number for the index cow was matched with sale records from the livestock market that had consigned the cow to the abattoir, and it was determined that the cow had originated from a Colorado dairy. Review of tissue specimens submitted to the NVSL from other cattle that were slaughtered on the same day as the index cow at the Texas abattoir revealed that none of those specimens had lesions consistent with *M. bovis* infection.
The herd of origin for the index cow (ie, index herd) was a fourth-generation, family-owned operation that was geographically isolated from other dairies. At the time the investigation was initiated in 2010, there were 908 cattle on the premises, including 185 neonatal calves (0 to 3 days old), 225 replacement heifers (> 3 days and ≤ 2 years of age), and 498 adult cows (> 2 years of age). The standard practice for this herd was to allow calves to suckle milk from their dams for the first 3 days after birth and then both heifer and bull calves were offered for sale. In 2009, all adult cows (> 2 years old) were screened for M. bovis infection by means of the CFT test to satisfy the M. bovis testing requirements for Colorado dairy herds in accordance with the Pasteurized Milk Ordinance.

For the epidemiological investigation, all cattle ≥ 2 months old in the index herd were tested for M. bovis infection in accordance with the USDA’s uniform methods and rules for eradication of bovine tuberculosis. Because of the index herd owner’s concerns about the time required for testing and the potential for inaccurate test results, it was decided to test all adult cows (> 2 years old) that responded to the CFT test with the CCT test and IFN-γ assay in parallel to increase the overall sensitivity of the testing protocol, compared with the sensitivity of the CFT test when used alone. Of the 498 adult cows tested, 160 (32%) responded (ie, were classified as suspects) to the CFT test; 90 (56.3%) of those cows subsequently responded to the CCT test, and 105 (65.6%) had positive results on the IFN-γ assay.

Because of the high proportion of adult cows in the herd that were classified as responders by the CCT test (18%) and that had positive IFN-γ results (21%), an agreement was reached with the herd owner to initially submit the 4 cows with the highest IFN-γ serum concentrations to the Colorado State University Veterinary Diagnostic Laboratory for necropsy. During necropsy examination of those 4 cows, all personnel working on the necropsy floor wore personal protective equipment in accordance with CDC recommendations. All 4 of those cows had extensive granulomatous lesions in the retropharyngeal, mediastinal, or supramammary lymph nodes. Results of diagnostic examination (microbial culture, histologic evaluation, and PCR assay) of tissue specimens obtained from those cows and performed by the NVSL confirmed that all 4 cows were infected with M. bovis. Ultimately, the index herd was depopulated and 101 of the 908 (11.1%) cattle were confirmed to be infected with M. bovis, including 15 of 410 (3.7%) cattle ≤ 2 years old and 86 of 498 (17.3%) cows > 2 years old.

**Tracing of Cattle Movement to and from the Index Herd**

The second phase of the epidemiological investigation involved tracing the movement of all cattle to and from the index herd (Figure 1). According to herd documents, in 2005, adult cows were purchased from dairies or cattle traders in Oregon (n = 88), Oklahoma (100), and Nebraska (24) and 100 pregnant heifers were leased from a calf-raising operation in Colorado. All cattle in those 4 source herds were screened for M. bovis by means of the CFT test, CCT test, or IFN-γ assay, and none were identified as infected.

Calves born to the leased heifers were transferred to another Colorado dairy soon after birth without individual animal identification. As part of the lease agreement, 100 heifers were returned to the heifer raising operation in 2008; these were not the same animals that were originally leased. Thus, all cattle at the dairy that received calves born to the leased heifers and the heifer raising operation to which 100 heifers from the index herd were transferred were screened for M. bovis by the CFT test and IFN-γ assay. On the basis of the test results, none of those cattle were infected with M. bovis.

Documentation for other cattle leaving the index herd was available beginning in 2008. Evaluation of the herd records revealed that from 2008 until initiation of the epidemiological investigation in 2010, 301 adult cows and heifers and 259 neonatal calves had left the herd. The adult cows and heifers that left the herd were traced through 3 Colorado livestock markets and were subsequently slaughtered at various abattoirs with or without an intermediate stay at a feedlot. Review of the Food Safety and Inspection Service reports from the abattoirs to which the cows and heifers from the index herd were traced revealed no evidence that any of those cattle were infected with M. bovis. Unfortunately, the neonatal calves that left the herd were not individually identified. Of those 259 calves, 18 were sold through a southeast Colorado livestock market and the remaining 241 were sold to private buyers directly from the index herd. Those buyers frequently raised the calves for 3 to 12 months and then sold them through various Colorado livestock markets. On the basis of livestock market sales receipts and the recollection of the owner of the index herd, 58 of those 259 (22%) calves were presumptively traced to 34 Colorado livestock operations; the remaining 201 calves could not be traced.

The owners of the 34 livestock operations to which calves from the index herd were traced were contacted. For each of those operations, the USDA paid the owner indemnity for any calf from the index herd that was still present on the operation. Those calves were then euthanized and necropsied by state or federal veterinarians. The remaining animals on each operation were placed under a hold order (ie, quarantined) until necropsy results for the euthanized calves became available. If the calf from the index herd had no lesions compatible with bovine tuberculosis, the remaining animals were screened for M. bovis by means of a CFT test, and if all animals had negative results, the hold order was released. If the calf from the index herd was confirmed as infected with M. bovis, the owner was paid indemnity for the remaining animals on the operation and those animals were euthanized and necropsied as described. That premise was then cleaned and disinfected under the supervision of USDAAPHS Veterinary Services personnel and could not be restocked with any livestock for at least 30 days. Of the 34 livestock premises that had purchased calves from the index herd, M. bovis–infected cattle were identified on 5, and the investigation for each of those operations is described.

**Additional Herds to Which Cattle From the Index Herd Were Traced and in Which M. bovis–Infected Cattle Were Subsequently Identified**

Herd A—The owner of herd A purchased 2 calves directly from the index herd in 2009. In the fall and winter of 2009, all the cattle in herd A were liquidated and the owner moved to another state. State and federal ani-
mal health personnel visited the premises, which consisted only of pasture, and could not locate any animals. Because no livestock were present on the premises, no regulatory action was imposed on the new owner. Records from a southeastern Colorado livestock market revealed that the owner of herd A had consigned 8 calves to the market in late 2009. It could not be determined whether any of those 8 calves were from the index herd. Of those 8 calves, 1 was sold to herd E, 1 was sold to herd F, and the remaining 6 were untraceable.

Herd B—Herd B was a cow-calf operation, the owner of which had purchased a single bull calf from the index herd in March 2010. That calf was purchased as a surrogate to be raised by a beef cow that had a calf that died and was subsequently confirmed as being infected with *M. bovis*. A cohort of 31 adult cows that were exposed to the calf from the index herd were euthanized and necropsied, and none had lesions consistent with bovine tuberculosis. An additional group of 59 cow-calf pairs that were not exposed to the calf from the index herd (ie, were housed at a geographically separate location) were screened for *M. bovis* with the CFT test. Four of those cattle responded to the CFT test; however, all 4 had negative results on the subsequent IFN-γ assay and none of those cattle were euthanized. Regardless, the herd was quarantined and the barn area was cleaned and disinfected in accordance with the USDA protocol. The quarantine was released 30 days after the final cleaning and disinfection of the barn area.

In April 2010, 2 calves from the cohort of cattle that were exposed to the calf from the index herd were...
sold to another operation, which had 19 adult cows; 1 calf died shortly after being purchased, and the other was still present on that premises at the time of the trace back. All the cattle on that premises were screened for *M bovis* with the CFT test. All results were negative; and thus no regulatory action was taken, although the owner was educated about the risk of introducing animals with an unknown disease status into his herd.

**Herd C**—Herd C was a small feedlot operation that had purchased 36 calves directly from the index herd beginning in 2009. At the time of the trace back, 5 of those 36 calves were still in the herd, of which 4 were subsequently confirmed as being infected with *M bovis*. Twenty-three calves were sold to feedlot operations from which cattle are only sold direct to slaughter (ie, terminal feedlots). The owner indicated that he occasionally slaughtered some cattle for personal use; however, it could not be determined how many cattle he had slaughtered since 2009 and whether any of those cattle were from the index herd. Herd C was depopulated and quarantined for 30 days following cleaning and disinfection of all cattle holding areas.

**Herd D**—Herd D was once a dairy operation but was a dairy calf raising operation at the time of the trace back. Eight to 12 adult dairy cows were maintained at the facility to provide milk for the calves. The owner routinely purchased 1-day-old calves from dairies in Colorado and New Mexico, raised them for 5 to 6 months, and then sold them through a northern Colorado livestock market. Records from the index herd indicated that 47 calves from the index herd had been purchased by herd D since 2008; however, the owner of herd D did not have any records to confirm those purchases. The owner was able to identify 1 calf on the premises that had originated from the index herd, but was unable to account for the other 46 calves. The calf identified as originating from the index herd was euthanized and necropsied and had no gross lesions consistent with bovine tuberculosis. The remaining 140 cattle (8 adult cows, 1 adult bull, 12 heifers > 6 months old, and 119 bull and heifer calves ≤ 6 months old) on the premises were screened for *M bovis* with the CFT test, of which 10 calves ≤ 6 months old were classified as suspects. The IFN-γ assay was performed on those 10 calves, and 9 yielded positive results, of which 7 were subsequently confirmed as being infected with *M bovis*. The herd was then depopulated. All cattle were necropsied and determined to not be infected with *M bovis*.

Cattle sold from herd D since the beginning of 2008 were traced through 2 Colorado livestock markets and subsequently to 20 additional Colorado livestock operations as well as livestock operations in Minnesota, Nebraska, Oklahoma, Pennsylvania, Texas, Wisconsin, and Wyoming. Unfortunately, a large proportion of those cattle were untraceable because of the lack of individual animal identification. All cattle that could be traced from herd D were indemnified and necropsied, and none were confirmed as infected with *M bovis*.

**Herd E**—The owner of herd E had purchased 2 calves from a southeastern Colorado livestock market on the day that the 8 calves from herd A were sold through that market. These calves were purchased to be raised for personal consumption and were still present on the premises at the time of the trace back. Unfortunately, because of a lack of individual animal identification, it could not be determined whether either calf had originated from the index herd. Thus, the herd was depopulated, and the purchased calves were subsequently confirmed as being infected with *M bovis*. No cattle had been sold from this herd.

**Herd F**—The owner of herd F had purchased 1 calf from a southeastern Colorado livestock market on the day that the 8 calves from herd A were sold through that market. The calf was purchased for use in roping practice and eventually for personal consumption. Herd F was depopulated, and the purchased calf was confirmed as being infected with *M bovis*. No cattle had been sold from this herd.

**Other Aspects of the Epidemiological Investigation**

Animal health officials were notified in the states to which cattle from the index herd and the other 5 herds with *M bovis*-infected cattle were traced. Producers who had purchased cattle from one of the *M bovis*-affected herds were contacted, and their herds were screened for cattle infected with *M bovis* in accordance with USDA regulations. No *M bovis*-infected cattle linked to the index herd were identified outside of Colorado.

Throughout 2010, the Colorado Department of Natural Resources, Parks, and Wildlife surveyed wildlife in the area surrounding the index herd for *M bovis* infection. Lymph nodes from the carcasses of 16 mule deer (*Odocoileus hemionus*) and 1 elk (*Cervus canadensis*) were submitted to the Colorado State University Veterinary Diagnostic Laboratory for microbial culture and histologic evaluation. Results of all diagnostic testing were negative for *M bovis*. Tissue specimens from wild cervids submitted by hunters were to continue to be monitored for bovine tuberculosis through 2015.

Within Colorado, the state veterinarian notified the Colorado Department of Public Health and Environment of the location of each premises on which *M bovis*-infected cattle were identified. A local public health nurse then visited each premises, and all individuals associated with the livestock operation were offered tuberculosis skin testing. Fifteen individuals underwent tuberculosis skin testing, of which 2 were classified as responders. No further information about these individuals was available because of the confidentiality clause of the Health Insurance Portability and Accountability Act of 1996.

**Epidemiological Investigation Summary**

At the conclusion of the epidemiological investigation, 908 cattle from the index herd and 220 cattle, sheep, goats, and pigs on the 5 other *M bovis*-affected premises in Colorado were euthanized in an effort to eradicate bovine tuberculosis. Of those animals, 115 cattle were confirmed to be infected with *M bovis*. Results of spoligotyping revealed that the *M bovis* isolates from those cattle were indistinguishable, which sug-
gested that all animals were infected with the same strain of the bacterium that originated from a common source.

Interestingly, 29 of the 115 (25%) M. bovis–infected cattle were ≤2 years old, and all identified transmission of bovine tuberculosis outside of the index herd was associated with the movement of neonatal calves that were presumably infected with M. bovis from the index herd. The location and extent of tuberculosis lesions in M. bovis–infected calves varied and included granulomatous lesions in the retropharyngeal lymph nodes (n = 5 calves) and thoracic lymph nodes (7 calves) and abscesses in the lungs (6 calves).

In the index herd, proportionately more cattle > 2 years old (86/498 [17.3%]) were infected with M. bovis, compared with the proportion of cattle ≤2 years old (15/410 [3.7%]) that were infected with M. bovis. However, of the cattle that left the index herd and were located, a greater proportion of cattle ≤2 years old (14/58 [24.1%]) were infected with M. bovis, compared with the proportion of cattle > 2 years old (1/276 [0.4%]; index cow) that were infected with M. bovis.

## Discussion

This epidemiological investigation highlights several important issues that USDA-accredited veterinarians should share with dairy producers. The first is the importance of unique individual animal identification and the maintenance of adequate records so that each animal can be traced throughout its lifetime when necessary. Many US dairy operations send neonatal heifer calves off-site to be raised for a period of time and sell neonatal bull calves, and many of these calves leave the operations where they were born without individual identification. Although it was not documented during this investigation, USDA and state animal health officials involved in previous epidemiological investigations of M. bovis–affected dairies have documented that replacement dairy heifers are occasionally raised in feedlots, where they are exposed to and commingled with replacement dairy heifers or other cattle. This practice might increase the risk of M. bovis transmission to replacement dairy heifers and contribute to the ongoing challenge of eradicating bovine tuberculosis from US dairy operations.

The application of unique identification to each animal would allow animal health officials to track an animal’s movements throughout its lifetime and more efficiently identify the origins of disease outbreaks and animals that have been exposed to diseases of interest. At the time of this investigation, movement of neonatal calves between premises within the state of Colorado required only that the calves be described, not individually identified, on a Colorado No Brand Required permit, which the owner of the index herd had acquired. Consequently, only 58 of 259 (22%) calves that left the index herd were located, despite thousands of hours spent by state and federal personnel trying to track those animals. Of the 58 calves that were located, 14 (24%) were infected with M. bovis. Assuming a similar proportion of the 201 calves that could not be traced were infected with M. bovis, as many as 50 additional M. bovis–infected calves were unidentified. Conventionally, it is assumed that most, if not all, dairy bull calves from commercial dairy operations are sold to terminal feedlot operations; however, during the present investigation, M. bovis–infected bull calves were found in herds with dairy and beef breeding cattle. Had the calves leaving the index herd had unique individual identification and had adequate records been kept to document the movements of those calves, it is likely that more cattle from the index herd would have been located.

During the present epidemiological investigation, various methods were attempted to trace cattle from the index herd, including official USDA metal ear tags when they were used and recorded by a herd or livestock market that was being investigated. Ideally, in a state such as Colorado that uses registered fire brands as a component of their animal identification program, a record of the application of official USDA ear tags would be kept in a brand inspection book or on a certificate of veterinary inspection as well as by the practitioner who dispensed or applied the ear tags. Some of the adult cattle from the index herd that were sold through livestock markets had fire brands that were recorded along with the back tag numbers that were applied by market personnel on the livestock market intake card. This information was valuable for tracing an animal to a feedlot or abattoir; however, brands were not recorded at the abattoirs.

On herd D, animal health officials observed calves with plastic ear tags with writing similar to that observed on ear tags in calves at the index herd, which led them to believe those calves might have originated from the index herd. Seven of the 10 calves that responded to the CFT test had those plastic ear tags, and all 7 were subsequently confirmed to be infected with M. bovis. It was surmised those calves were obtained from the index herd but had not yet spread the infection to other animals on the premises.

Bovine tuberculosis is still present in the United States, and the disease can spread quickly and affect a high proportion of animals within a herd. Many producers are reluctant to acknowledge that cattle within their herds might be infected with M. bovis, especially if their cattle have been previously screened for the infection. In the index herd of the present investigation, the prevalence of M. bovis–infected cattle was 11.1%, which was substantially higher than that typically found in affected US cattle herds during the previous decade. Most US cattle herds affected by bovine tuberculosis had a within-herd prevalence of M. bovis–infected cattle < 2%. In 2007, epidemiological investigations of bovine tuberculosis on 2 New Mexico dairies indicated that the prevalence of M. bovis–infected cattle in those herds was 0.055% and 1.23%. Results of an unpublished epidemiological investigation of bovine tuberculosis in a California dairy indicated that only 1 M. bovis–infected cow was detected during slaughter inspection of several thousand cows when that herd was depopulated.

The within-herd prevalence of M. bovis–infected cows on affected large confinement dairies between 1982 and 1993 was approximately 1%. More recently, the within-herd prevalence of bovine tuberculosis in 3

---


Unauthenticated | Downloaded 12/20/23 02:17 AM UTC
small (< 100 cows) Michigan beef herds ranged from 14% to 56%. 8

In 2009, adult lactating cattle in the index herd were screened for *M. bovis* to meet requirements established by the Pasteurized Milk Ordinance. Review of those test results revealed that only 2 cows were classified as reactors on the CCT test and were euthanized and necropsied, and neither had gross lesions consistent with bovine tuberculosis, nor was *M. bovis* cultured from representative lymph node specimens from those cows that were submitted to the NVSL. Cattle not infected with *M. bovis* can have false-positive CCT test results because of exposure to other mycobacterial or bacterial organisms. This suggested that bovine tuberculosis began to spread rapidly within the herd sometime after the 2009 herd test, although it is important to note that not all cows were tested during the 2009 herd test, including the index cow. It is possible that cows that were not tested during the 2009 test were infected with *M. bovis* or that cows that were tested and were infected were anergic and therefore did not respond to the CFT and CCT tests. Alternatively, it is possible that the index cow was not lactating at the time of the 2009 herd test and began shedding *M. bovis* in colostrum and milk during the subsequent lactation. Calves born in 2009 were confirmed to be infected with *M. bovis*, which suggested that those calves were exposed to the bacterium in utero or soon after birth. *Mycobacterium bovis* can be inactivated by pasteurization, and the index herd did have a pasteurizer for milk fed to calves, but it had not been in service for an indeterminant period of time before the initiation of the epidemiological investigation. Thus, it is likely the calves were exposed to *M. bovis* via aerosol as well as unpasteurized colostrum and milk. This supposition is supported by the finding of extensive *M. bovis* lesions in the supramammary lymph nodes in 1 of the 4 initial cows from the index herd that were necropsied at the Colorado State University Veterinary Diagnostic Laboratory.

During a bovine tuberculosis outbreak investigation, samples of colostrum and milk fed to calves are not routinely screened for *M. bovis*. Cows with bovine tuberculosis can shed *M. bovis* in milk, regardless of the location of lesions. 13,15 Results of another study17 indicate that *M. bovis* DNA can be identified in both colostrum and milk samples from cows with bovine tuberculosis. In a prospective study,18 feeding pooled unpasteurized colostrum and milk to calves was associated with those calves subsequently developing bovine tuberculosis. Thus, screening colostrum and milk samples from primiparous cows for *M. bovis* should be considered for inclusion as a standard operating protocol for bovine tuberculosis epidemiological investigations.

Sporadic outbreaks of bovine tuberculosis in the United States suggest the need for continued vigilance for the disease by regulatory animal health officials and accredited veterinarians. Ongoing national surveillance for bovine tuberculosis includes antemortem screening of individual animals for *M. bovis* by accredited veterinarians and slaughter surveillance at federally inspected abattoirs. Antemortem CFT tests can only be performed by USDA-accredited veterinarians, who meet certain performance standards outlined in *Bovine tuberculosis eradication: uniform methods and rules*. 9 For herds without *M. bovis*-infected cattle, it is assumed that at least 1% of cattle will respond to the CFT test (ie, have false-positive results). Multiple factors are associated with false-positive CFT test results, including improper storage and injection of PPD and incorrect test interpretation. Thus, accredited veterinarians who are properly performing the CFT test will generally have a response, or suspect, rate of 2% to 5% in the NVSL. Slaughter surveillance is also an effective method for detection of cattle with bovine tuberculosis. Inspectors at federally inspected abattoirs are encouraged to submit at least 1 *M. bovis*-suspect lesion/2,000 adult cattle slaughtered. 8,19 According to the USDA, in 2009, 16 *M. bovis*-infected cattle from 3 herds were detected during routine slaughter surveillance at federally inspected abattoirs. From 2001 through 2011, 92 herds with cattle infected with *M. bovis* were identified in the United States, of which 22 (24%) were detected by slaughter surveillance, 34 (37%) were detected by routine screening of herds in areas where *M. bovis* is endemic in wildlife, and 36 were detected by CFT testing of cattle for various reasons other than previous indication of infection (ie, sale or interstate transport requirements). 19,6 The present investigation supports the importance of continued monitoring for bovine tuberculosis by antemortem testing and slaughter surveillance in abattoirs.

Historically, herds in which *M. bovis*-infected cattle are identified are depopulated. This approach to controlling bovine tuberculosis is difficult and costly for the producer. Although producers of affected herds are paid indemnity (fair market value minus slaughter value) for their animals, they incur other substantial costs associated with depopulation such asso the loss of production income and genetically valuable animals and the cost to clean and disinfect the premises. The USDA Centers for Epidemiology and Animal Health has developed a mathematical model to determine whether depopulation or repeated screening of cattle for *M. bovis* with removal of test-positive cattle (ie, test and removal) is the most cost-effective method of controlling bovine tuberculosis for each affected herd. Inputs for the model include the estimated prevalence of *M. bovis*-infected cattle in the herd and current cattle prices. Regardless of the method chosen, eradication of bovine tuberculosis from an affected herd requires a substantial investment from both the producer and government. For the index herd of this investigation, state and federal animal health officials discussed the mathematical model results, availability of federal funds for indemnity, and other logistic and financial factors with the producer to decide which bovine tuberculosis control approach would be used. The mathematical model estimated that eradication of bovine tuberculosis from the index herd with the test and removal approach would require > 7 years, primarily because of the high prevalence of *M. bovis*-infected cattle in the herd. During this period, the producer would be unable to sell milk or cattle and a whole herd test for *M. bovis* would be required approximately every 60 days. The producer did not consider this practi-
cal. Additionally, the state and federal officials did not believe the test and removal approach was the best option for disease containment; therefore, the index herd was depopulated.

The source of *M. bovis* infection for the index herd was not determined in the present epidemiological investigation. The spoligotype did not match that of any other *M. bovis* isolates in the NVSL database. Possible sources of *M. bovis* exposure for the index herd include the purchase or leasing of infected cattle, introduction of infected material such as colostrum (which may not have been reported to the owner), zoonotic transmission from an infected employee, and transmission from infected sympatric livestock or wildlife.21 The Colorado Department of Natural Resources, Parks, and Wildlife will continue surveillance of wildlife for bovine tuberculosis in the area surrounding the index herd for 3 years after the initiation of the epidemiological investigation.

Epidemiological investigations of bovine tuberculosis outbreaks require joint cooperation among state and federal animal health officials and livestock producers, markets, and abattoirs. The present report highlights the important role neonatal calves can have in the transmission of bovine tuberculosis. In this investigation, the transmission of bovine tuberculosis from the index herd to the other 5 affected herds was associated with the movement of neonatal calves that were presumably infected with *M. bovis* from the index herd. Furthermore, this report highlights the need for unique individual identification of all livestock, including neonatal calves. Because of a lack of individual animal identification and corroborating records, only a small percentage of cattle that left the index herd were able to be traced and located. Given the relatively high prevalence of *M. bovis*-infected cattle in the index herd and large proportion of cattle that were untraceable, it is likely that bovine tuberculosis was spread to additional herds that remained unidentified during the investigation. Consequently, ongoing surveillance efforts for bovine tuberculosis are warranted. Continued spoligotyping of *M. bovis* isolates will aid in the tracking and eradication of bovine tuberculosis from US livestock operations. In the meantime, livestock producers should implement biosecurity practices such as not purchasing animals or only purchasing animals with a known health status and being knowledgeable about premises (ie, off-site heifer raising operations and show arenas or fairgrounds) to which their animals will be exposed to reduce the risk of introducing bovine tuberculosis or any other infectious disease into their herds.

References

1. Meyers JA. Man’s greatest victory over tuberculosis. Springfield, Ill: Charles C Thomas Publisher Ltd, 1940.