Tuberculosis is an infectious, granulomatous disease caused by bacilli of the genus *Mycobacterium* that has affected humans since prehistoric times. Tuberculosis caused by *Mycobacterium bovis* affects a number of mammalian species and was a major disease in humans.

Surveillance for *Mycobacterium bovis* transmission from domestic cattle to wild ruminants in a Mexican wildlife-livestock interface area

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**Objective**—To assess the prevalence of *Mycobacterium bovis* infection in cattle and wild ruminants (WRs) in a wildlife-livestock interface area (WLIA) of the Mexican highland plateau.

**Animals**—24,400 cattle from 793 herds (including 17,351 commercially slaughtered cattle) and 142 WRs (110 white-tailed deer [*Odocoileus virginianus*], 20 red deer [*Cervus elaphus*], and 12 North American elk [*Cervus canadensis*]) harvested via controlled hunting.

**Procedures**—Cattle were serially tested for *M. bovis* infection via caudal fold tuberculin and comparative cervical tuberculin tests during field surveillance. Carcasses of cattle and WRs were inspected for gross lesions; samples suggestive of tuberculosis were analyzed via histologic evaluation and mycobacterial culture (HMC). A PCR assay to detect *Mycobacterium tuberculosis* complex organisms was performed to confirm positive results of HMC.

**Results**—WRs had inflammatory lesions in lungs and lymph nodes, although HMC results did not indicate *M. bovis* infection. Eight cattle had positive results for both tuberculin tests, and 31 had positive results for HMC of grossly detected lesions; all were from 7 herds, and ≥1 cow in each herd had positive PCR assay results. These 7 herds were depopulated; adjacent herds and herds related via commerce were quarantined. Calculated true prevalence of *M. bovis* infection was 0.86% (95% confidence interval, 0.24% to 1.49%) in cattle; *M. bovis* was not detected in any WRs.

**Conclusions and Clinical Relevance**—*M. bovis* infection was present in cattle. Although transmission to WRs in this WLIA was not detected, diagnosis and prevention activities should be implemented and consolidated to prevent potential *M. bovis* transmission between cattle and WRs. (Am J Vet Res 2012;73:1617–1625)

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**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCT</td>
<td>Comparative cervical tuberculin</td>
</tr>
<tr>
<td>CFT</td>
<td>Caudal fold tuberculin</td>
</tr>
<tr>
<td>HMC</td>
<td>Histologic evaluation and mycobacterial culture</td>
</tr>
<tr>
<td>PPD</td>
<td>Purified protein derivative</td>
</tr>
<tr>
<td>WCMSU</td>
<td>Wildlife Conservation, Management, and Sustainable Utilization</td>
</tr>
<tr>
<td>WLIA</td>
<td>Wildlife-livestock interface area</td>
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<tr>
<td>WR</td>
<td>Wild ruminant</td>
</tr>
</tbody>
</table>

Tuberculosis is an infectious, granulomatous disease caused by bacilli of the genus *Mycobacterium* that has affected humans since prehistoric times. Tuberculosis caused by *M. bovis* affects a number of mammalian species and was a major disease in humans and domestic animals before control measures were adopted. Tuberculosis caused by *M. bovis* remains an important zoonotic disease in many parts of the world and can affect animal trade and commerce in infected areas.

A WLIA is a region where domestic cattle and WRs coexist and share access to natural resources. One problem in WLIA is the lack of basic field data on the interactions among domestic animals, wild animals, and pathogens. In North America, *M. bovis* infection has
become enzootic in some free-ranging cervid populations, particularly white-tailed deer (Odocoileus virginianus), which have been implicated as a source of infection for domestic cattle herds. Mycobacterium bovis can be transmitted among animals through aerosol, saliva, and nasal secretions; the use of common feeding sites may increase M. bovis transmission by concentrating deer and promoting direct interaction among them as well as with contaminated feed. Tuberculous lesions in infected cattle and deer are sometimes not grossly detectable; therefore, infection must be confirmed by bacteriologic culture of tissue, PCR assay, or both.

In Mexico, M. bovis infection is widely prevalent in cattle. On the basis of herd prevalence of M. bovis infection, the National Tuberculosis Eradication Campaign considers the national territory to include 2 zones: zone A has a < 0.5% herd prevalence of M. bovis infection and is considered to be in an eradication phase, whereas zone B has a 0.5% to 2.5% herd prevalence and is considered to be in a control phase. However, the prevalence of M. bovis infection among WRs in WLIA is unknown. The Sierra Fria is a WLIA, legally recognized as a protected natural area since 1994 by the Government of the State of Aguascalientes; it is located within zone A and contains an important population of WRs, captive ruminants, and other fauna. Inside of the WLIA, WCMSU units are used for hunting. In recent overall estimation, the entire WLIA deer population comprised 2,690 white-tailed deer (Odocoileus virginianus couesi); the deer population density was 4 deer/km², which is considered a low density. Projected production of fawns in the WLIA was 350 fawns/y, with a mean of 45% expected to survive to adult age.

The Aguascalientes Committee for Promoting and Protecting Livestock and the Aguascalientes Produce Foundation are 2 local civil societies concerned with decreasing the incidence of M. bovis infection in domestic livestock through diagnostic tests, slaughter, and the implementation of control measures prescribed by the Mexican Ministry of Agriculture, Livestock, Rural Development, Fishing, and Food. Transmission of M. bovis between cattle and WR populations is considered evidence of loss of efficiency in control measures. Therefore, eradication of tuberculosis caused by M. bovis may not be possible in WLAs where domestic cattle and wildlife are handled without diagnosis, surveillance, and control measures. On the basis of this premise, the purpose of the study reported here was to assess the prevalence of M. bovis infection in cattle and WRs in a WLIA in the Mexican highland plateau by serial use of CFT and CCT tests in cattle and postmortem examination of cattle and WRs with HMC and molecular identification through PCR-based analysis as a means of evaluating the role these species may have in propagation of tuberculosis.

Materials and Methods

Survey area—The study was performed in special WCMSU units intended for legal and sustained hunting inside the WLIA located in the northwestern part of Aguascalientes, Mexico (Figure 1). This area is located approximately at 21°52′50″ and 22°19′46″ North and 102°22′50″ and 102°51′26″ West, and extends over 1,120 km² (276,980 acres). The municipalities of San José de Gracia, Calvillo, Jesús María, Rincón de Romos, and Pabellón de Arteaga are located within the area. The WLIA has a rugged topography with an altitude ranging from 1,800 to 3,050 m above sea level. There are many rocky canyons, ravines, hills, and plateaus. The lithology of the region consists mainly of sandstone, igneous rock, acidic extrusions, alluvial deposits, calcareous tuffs basalt, and rhyolite. Temperate oak (Quercus spp) and pine (Pinus spp) cover nearly 72% of the region.

Traditionally, farmers from the WLIA have developed commercial livestock activities in the same land where free-ranging white-tailed deer are used for cynecetic purposes. There is a registered population of 24,400 cattle of European breeds intended for calf production, distributed in 793 herds, within the WLIA. During the past 2 decades, WCMSU units in which captive WRs (mainly red [Cervus elaphus], white-tailed deer, and elk [Cervus canadensis]) are bred and maintained have been established. Management of these captive WRs ranges from simply erecting a high fence around a tract of land to handling these animals as in livestock operations, including feeding, translocation, and selling individual animals for hunting purposes.

Selection of herds and on-farm testing of cattle—During 2006 and 2007, extraordinary M. bovis infection surveillance activities on all cattle in the described WLIA were conducted in coordination with the Aguascalientes Committee for Promoting and Protecting Livestock. The entire cattle population within this area (24,400 cattle from 793 herds) underwent intradermal CFT testing with M. bovis PPD tuberculina (performed only once for all cattle in each herd), and cattle that had a positive reaction to the CFT test underwent CCT testing with M. bovis and Mycobacterium avium PPDb tuberculin. The diagnostic tests were performed by certified veterinarians according to official Mexican regulations (NOM-031-ZOO-1999). The standard CFT test was the primary antemortem screening test used to detect prior exposure to M. bovis in cattle. The CFT test was performed via ID injection of 0.1 mL of M. bovis AN5 strain PPD (1.0 mg of protein/mL) in the caudal tail fold. Approximately 72 hours (range, 66 to 78 hours) after the PPD was administered, the injection site was inspected, by means of visual examination and palpation, for immune response indicators, such as swelling or discoloration, and results were recorded. Within 7 days after interpretation of CFT test results, all cattle that had a positive reaction to the CFT test were retested via the CCT test. The CCT test was performed with both M. bovis AN5 strain and M. avium D4 strain (0.5 mg protein/mL) PPDs, which were administered (0.1 mL each) ID at the same time in 2 sites on the same side of the neck. Results were interpreted approximately 72 hours (range, 66 to 78 hours) later, and on the basis of the CFT and CCT test results, cattle were classified as negative, suspected, or CFT-CCT test reactors. All reactors, including suspected reactors, were slaughtered, and a follow-up procedure was performed via postmortem inspection and HMC of tissues with grossly identified lesions.
Inspection of slaughtered cattle—During the same surveillance period (2006 to 2007), additional monitoring was also conducted through examination of slaughtered cattle from herds in the WLIA. Including culled, feeder, CFT-CCT test reactor cattle, and cattle from depopulated herds, 17,351 cattle were inspected following procedures indicated by applicable regulations. Inspection was performed by officially accredited veterinarians in 4 slaughterhouses in the Aguascalientes B zone and in 3 other slaughterhouses located in the United States to which cattle were exported from the study area.

Postmortem inspection of cattle was performed as described elsewhere. Each of the inspected organs was subjected to a macroscopic examination to detect possible inflammatory lesions or granulomas. The lymph nodes of head, neck, thorax, and abdomen were incised several times and examined, and the lungs, liver, and spleen were only incised if evident lesions were detected. The lymph nodes or other tissues containing focal or multifocal abscesses, possible granulomas, or other suspected lesions were collected and divided into 3 samples. Two samples were fixed in 10% formalin in PBS solution and embedded in paraffin for histologic evaluation and PCR analysis. The third sample was collected via a sterile technique, placed in a 16.6% sodium borate solution, and used for mycobacterial culture and identification. If several lesions were present in individual animals, all collected samples were divided and processed for HMC. An animal was recorded as infected with M bovis if ≥ 1 specimen tested positive.

Paraffin-embedded tissues for histologic evaluation were sliced, mounted onto glass slides, and stained with HE & E and Ziehl-Neelsen stains; acid-fast bacilli in lesions considered suggestive of tuberculosis were identified according to described methods. Samples were considered to have histologic results compatible with a diagnosis of tuberculosis only if they had granulomatous inflammation associated with central necrosis and no evidence of nonmycobacterial etiologies.

For mycobacterial culture, additional samples from the same tissue were independently macerated via mortar and pestle in 5.0 mL of PBS solution containing phenol red as a pH indicator. Afterward, samples were digested, decontaminated with 0.5N sodium hydroxide, neutralized with 6N hydrochloric acid, and centrifuged at 3,500 g for 20 minutes. The sediment from each sample was cultured at 37°C in Stonebrink medium supplemented with pyruvate, Middlebrook 7H11 medium, Herrold egg yolk medium, or Lowenstein-Jensen medium. After incubation, colonies suspected to be M bovis were cultured again in Proskauer-Beck medium with 5% equine serum for biochemical testing and identification of the genus and species. Cultures that had bacilli growth within a 3-month period were considered to have yielded positive results for M bovis; if growth was not evident, results of the culture were classified as negative.

To confirm infection as well as to identify the species of Mycobacterium isolated from gross lesions, PCR assays were performed serially only on samples from tissues that had positive results for HMC. The following primers were used for mycobacterial identification as described elsewhere: IS6110 for Mycobacterium tuberculosis complex (including M tuberculosis, M bovis, Mycobacterium microti, and Mycobacterium africanum), 16S rRNA for M avium and IS900 for Mycobacterium avium subsp paratuberculosis. All samples that produced a band of the expected size for M tuberculosis complex spp, compared with a DNA control, were considered to have a positive result.

The HMC and PCR tests were used serially for diagnosis. Cattle were classified as infected with M bovis if they tested positive via HMC, PCR assay, or both; a positive test result via ≥ 1 of these methods was considered the gold standard for comparison with other test methods.

Personnel performed the tuberculin tests, postmortem inspections, and laboratory tests without previous knowledge of the exposure status of evaluated cattle. Tissue samples considered suspicious for M bovis infection were analyzed in Mexican or US veterinary laboratories that were specifically approved to conduct official tuberculosis program diagnostic testing (National Veterinary Services Laboratories, Ames, Iowa, and Center of Integrated Diagnostics and Research in Animal Health of the Chihuahua State, Chihuahua, Mexico). Reports of all tuberculosis tests were submitted in accordance with the requirements of Mexican and US animal health officials. Reports included official identification, age, sex, and breed of each animal as well as a test result and interpretation record.

Testing of WRs—Local wildlife authorities, in accordance with applicable laws, issued 160 hunting permits to licensed hunters as part of the WCMSU operation process in the 2006–2007 period. To maintain confidentiality and objectivity in the use of obtained information, diagnostic procedures were implemented only when the hunters and land owners expressed their consent and willingness to provide access to WR carcasses as well as reliable information about the date and location of animal harvesting. Inspections of 142 WRs (75 in 2006 and 67 in 2007) were performed; these included 110 white-tailed deer, 20 red deer, and 12 elk. Ninety of the white-tailed deer and all evaluated red deer and elk were captive animals hunted in the WCMSU units; the remaining white-tailed deer (n = 20) were considered free-ranging animals.

All WRs were > 10 months of age. Age in these animals was determined via assessment of tooth eruption and wear. The WRs were classified into young, middle, and mature age groups (< 3.5, 3.5 to 5, and > 5 years of age, respectively). By use of the same procedures and classifying criteria as in cattle, WRs were individually inspected after death, and samples of tissues considered suspicious for M bovis infection were processed for HMC. If several lesions were present in single WR, all collected samples were divided into pieces and were processed for HMC.

Data analysis—Test sensitivity was defined as the conditional probability of a positive test result, given that the animal was actually infected with M bovis. Specificity was defined as the conditional probability of a negative test result, given that the animal was not actually infected with M bovis. Apparent prevalence...
was calculated as the proportion of tested animals that had positive test results. True prevalence was estimated with apparent prevalence, adjusted for sensitivity and specificity, via the formula \( \frac{AP - Sp - 1}{Se + Sp - 1} \), where AP is apparent prevalence and Se and Sp are the sensitivity and specificity of the CFT-CCT test results. The sensitivity and specificity of the serial CFT-CCT tests were determined by comparison with the gold standard (HMC, PCR assay, or both), which was accepted as determining the true \( M \) \( bovis \) infection status of the cattle. \(^{21}\) Apparent prevalence, sensitivity, and specificity are reported as point estimates with 95\% confidence intervals for binomial proportions. Sex and age group differences in proportions were determined via the uncorrected Pearson \( \chi^2 \) test, with \( P \leq 0.05 \) as the value needed to reject the null hypothesis of no significant differences among evaluated groups.\(^{22}\) Commercially available software was used for all statistical analyses.

The statistical power and level of detection of this study to detect \( M \) \( bovis \) infection in WRs was estimated a posteriori as described in open-source software.\(^{23}\)

**Results**

On-farm testing of cattle—Of 24,400 cattle tested in the WLIA, 191 (132 during 2006 and 59 during 2007) had positive results for the CFT test. All cattle with positive CFT test results were reevaluated with the CCT test; most (181/191 [94.8\%]) had negative CCT test results, 8 were classified as CFT-CCT test reactors (5 during 2006 and 3 during 2007), and 2 were classified as suspected reactors (1 each year). The 8 reactors were from 2 of the 793 herds tested. The CFT-CCT test reactor cattle were immediately sent to slaughterhouses under official inspection. All cattle from herds that had CFT-CCT test reactors underwent special surveillance by means of a precautionary quarantine; when the presence of \( M \) \( bovis \) infection was confirmed, these herds were depopulated and premises were disinfected according to applicable regulations.\(^{13}\)

Slaughtered cattle—Commonly observed histopathologic features in \( M \) \( bovis \)–infected cattle of the present study were similar to those reported,\(^{23}\) with lesions consisting of a caseonecrotic granuloma with peripheral fibrosis and a central mineralized focus surrounded by mixed mononuclear leukocytes and multinucleate giant cells. In addition, acid-fast bacilli were detected in low numbers during histologic examination.

Gross and microscopic lesions compatible with tuberculosis were detected in 7 of the 8 carcasses of CFT-CCT test reactors, and \( M \) \( bovis \) growth was detected in lymph node samples in 5 of these. No lesions were found in the carcasses of 2 cattle classified as suspected CFT-CCT test reactors. Overall, gross lesions consid-

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**Table 1**—Species, sex, and age of cattle (n = 113) and WRs (92) in a WLIA of the highland plateau in central Mexico that had pathological findings detected via histologic analysis of lymph nodes and lung tissues (No. with clinically relevant findings/total).

<table>
<thead>
<tr>
<th>Species</th>
<th>Male</th>
<th>Female</th>
<th>Value*</th>
<th>Young (&lt; 3.5)</th>
<th>Middle (3.5–5.0)</th>
<th>Mature (&gt; 5.0)</th>
<th>Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle WRs</td>
<td>11/17</td>
<td>59/96</td>
<td>0.799</td>
<td>10/13</td>
<td>19/51</td>
<td>41/49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>White-tailed deer (Odocoileus virginianus)</td>
<td>38/38</td>
<td>5/22</td>
<td>&lt; 0.001</td>
<td>5/5</td>
<td>15/20</td>
<td>23/35</td>
<td>0.260</td>
</tr>
<tr>
<td>Red deer (Cervus elaphus)</td>
<td>12/12</td>
<td>1/8</td>
<td>&lt; 0.001</td>
<td>1/2</td>
<td>5/8</td>
<td>7/10</td>
<td>0.846</td>
</tr>
<tr>
<td>North American elk (Cervus canadensis)</td>
<td>9/9</td>
<td>1/3</td>
<td>0.007</td>
<td>0/9</td>
<td>4/4</td>
<td>8/9</td>
<td>0.273</td>
</tr>
<tr>
<td>All WR species</td>
<td>69/59</td>
<td>7/33</td>
<td>&lt; 0.001</td>
<td>6/7</td>
<td>24/32</td>
<td>36/53</td>
<td>0.543</td>
</tr>
</tbody>
</table>

Total for each group represent the number of animals that had gross lesions considered suspicious for tuberculosis involvement (eg, abscesses or possible granulomas). Clinically relevant histologic findings included bronchopneumonia, chronic pneumonia, abscessed lymph node, lymphadenitis, and fibrotic lymph nodes.

*Uncorrected Pearson \( \chi^2 \) test value. ¹One degree of freedom.
ered suspicious for tuberculosis were detected in 113 (0.65%) of the 17,351 carcasses evaluated (8,060 in 2006 and 9,291 in 2007), but of these, only 33 (29.2%) had granulomatous lesions compatible with a diagnosis of tuberculosis detected histologically. Thirty-one of the 33 (93.9%) cattle were identified as infected with M. bovis via isolation of typical acid-fast bacilli; samples from 2 cattle had tuberculosis-compatible lesions with no M. bovis growth.

The M. bovis–infected cattle came from 7 herds in the WLIA (Figure 1). Samples from these 31 cattle were analyzed via PCR assay as a confirmatory test; 24 had positive results, with 1 positive result in each of the 7 herds. Cattle with positive results for the PCR assay were recorded as testing positive for M. tuberculosis complex, presumably because of infection with M. bovis, and testing negative to M. avium and M. avium subsp. paratuberculosis.

The M. bovis–infected cattle were initially detected by means of serial CFT and CCT testing in 2 herds during field surveillance (5/280 tested cattle; apparent prevalence, 1.8%) and in 5 herds during examination of slaughtered culled and feeder cattle (26/392 carcasses examined; apparent prevalence, 6.6%). The 7 herds (672 cattle) in which M. bovis–infected cattle were identified were entirely depopulated. The cattle from these herds were slaughtered in the B zone under official veterinary inspection; in addition, adjacent and related herds (ie, those that had commercial interchange of feed, supplies, or animals with affected herds) were quarantined as a precaution. The quarantine was cancelled when M. bovis infection was not detected via CCT-CFT and HMC tests.

The proportions of cattle with gross lesions considered suspicious for tuberculosis that had clinically relevant histologic findings were not significantly different between sexes (Table 1). Detection of these lesions was significantly (P < 0.001) more frequent in middle-aged and mature cattle (60/100), compared with young cattle (10/13). Clinically relevant histopathologic findings in these cattle included bronchopneumonia, chronic pneumonia, abscessed lymph node, lymphadenitis, or lymphoid depletion in lymph nodes; lesions were primarily in the lungs and mediastinal or tracheobronchial lymph nodes (59/66 [89.4%]), whereas only 7 (10.6%) lesions were found in other organs and lymph nodes. Fourteen of 15 and 45 of 51 (88.2%) lesions evaluated via PCR assay as a confirmatory test; 24 had positive results, with 1 positive result in each of the 7 herds. Cattle with positive results for the PCR assay were recorded as testing positive for M. tuberculosis complex, presumably because of infection with M. bovis, and testing negative to M. avium and M. avium subsp. paratuberculosis.

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Sensitivity and specificity of (combined) serial CFT-CCT tests were calculated as 16.1% and 99.9%, respectively (5 CFT-CCT test reactors/31 cattle with HMC-confirmed M. bovis infection and 24,366 CFT-CCT test–negative cattle/24,369 cattle classified as negative for M. bovis infection). Two cattle initially classified as suspected CFT-CCT test reactors were finally considered noninfected because no lesions were found in their carcasses. The true prevalence of M. bovis in domestic cattle was calculated at 0.86% (95% confidence interval, 0.24% to 1.49%). There was no significant (P = 0.265) difference in the frequency of detection of M. bovis infection in cattle among the municipalities (San José de Gracia, Calvillo, Jesús María, Rincón de Rosom, and Pabellón de Arteaga) of the WLIA. The positive predictive value of CFT-CCT serial testing was calculated at 62.5%, whereas the negative predictive value was 99.9%.

WRs—Ninety-two of 142 (64.8%) WR carcasses had gross lesions considered suspicious for tuberculosis (Table 1). Clinically relevant histologic findings (bronchopneumonia, chronic pneumonia, abscessed lymph node, lymphadenitis, or lymphoid depletion in a lymph node) were detected in all 59 male WRs evaluated but only in 7 of 33 (21.2%) female WRs. Gross lesions appeared to be more frequent in mature and middle-aged WRs, compared with young WRs; however, differences were not significant among age groups.

All WR samples were identified as negative for M. bovis infection on the basis of testing via HMC. Forty-three of the 92 (46.7%) WRs had histologic findings related to inflammation (bronchopneumonia, chronic pneumonia, abscessed lymph node, lymphadenitis, or fibrotic lymph node). Twenty-three (25.0%) animals had evidence of lymphoid depletion in lymph nodes (Table 2). The lesions appeared to be associated with specific anatomic locations because these were detected mainly in the lungs and mediastinal or tracheobronchial lymph nodes (59/66 [89.4%]), whereas only 7 (10.6%) lesions were found in other organs and lymph nodes. Fourteen of 13 and 45 of 51 (88.2%) lesions evaluated in free-range and captive WRs, respectively, were found in the thoracic region.

No significant differences were observed in the frequency of macroscopic lesion identification in free-range WRs (15/20 [75.0%]), compared with captive WRs (77/122 [63.1%]; OR, 1.19; P = 0.642). A large number of captive WRs had clinically relevant lesions detected histologically (51/122), as did free-range WRs (15/20; P = 0.310).

Table 2—Histopathologic findings in the same 113 cattle and 92 WRs in Table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. evaluated</th>
<th>Tuberculosis compatible</th>
<th>Chronic pneumonia</th>
<th>Abscessed lymph node</th>
<th>Lymphadenitis</th>
<th>Fibrotic lymph node</th>
<th>Lymphoid depletion in lymph node</th>
<th>NCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>113</td>
<td>33</td>
<td>9</td>
<td>1</td>
<td>11</td>
<td>0</td>
<td>7</td>
<td>43</td>
</tr>
<tr>
<td>WRs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White-tailed deer</td>
<td>60</td>
<td>0</td>
<td>7</td>
<td>14</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Red deer</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>North American elk</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>33</td>
<td>19</td>
<td>16</td>
<td>21</td>
<td>3</td>
<td>30</td>
<td>69</td>
</tr>
</tbody>
</table>

Histologic findings considered compatible with a diagnosis of tuberculosis included identification of acid-fast bacilli in granulomatous inflammation associated with central necrosis and no evidence of nonmycobacterial etiologies.

NCR = No clinically relevant findings.
Because none of the WRs were identified as infected with \textit{M bovis} via HMC, the PCR assay was not performed in these animals. The apparent prevalence of \textit{M bovis} in WRs from the WLIA was calculated as 0.00%; a posteriori assessment indicated that the statistical power value was inadequate (11.4%) to detect \textit{M bovis}-infected WR for this population. Therefore, the confidence interval for \textit{M bovis} infection prevalence in WRs was estimated in our study as 0.00% to 2.3% with a 95% confidence level for binomial proportions.

\textbf{Discussion}

Even though many epidemiological aspects of \textit{M bovis} infection in Mexican cattle have been described in recent years,\textsuperscript{10} uncertainties about the true prevalence of \textit{M bovis} infection in the WR population have still remained. In our study, none of the 142 inspected WRs were identified as \textit{M bovis} positive; however, \textit{M bovis} infection was confirmed in 31 of 24,400 cattle.

Under natural conditions, WR species have few infectious diseases;\textsuperscript{22} however, through contact with infected domestic species, \textit{M bovis} might eventually become enzootic among these animals in Mexico. This problem has been reported in several countries,\textsuperscript{19} such as New Zealand,\textsuperscript{25} France,\textsuperscript{26} England,\textsuperscript{27} and the United States.\textsuperscript{28} Although tuberculosis caused by \textit{M bovis} has been reported in a variety of animal species, to our knowledge, this report provides the first documented research of this disease in free-ranging and captive WR in any WLIA in Mexico. It is highly relevant for the wildlife and livestock industry to decrease the large and negative impact that this disease has on animal quality and productivity. In addition, these results show that it is possible to perform wildlife epidemiological surveillance in developing countries such as Mexico.

In the United States, the campaign to eradicate tuberculosis caused by \textit{M bovis} infection in cattle has been focused on the identification of infected herds. In 1991, 41 states had achieved tuberculosis-free status by performing actions such as systematic skin testing, detection of suspicious lesions in slaughtered animals, HMC of potentially infected tissues, epidemiological investigations to locate potential sources of infection, and \textit{M tuberculosis} complex bacteria identification via PCR assay in formalin-fixed, paraffin-embedded tissues.\textsuperscript{19} The Mexican national campaign to control \textit{M bovis} infection in cattle is focused on involving farmers by certifying herds as tuberculosis-free herd after 3 consecutive negative intradermal tuberculin test results.\textsuperscript{11} To achieve tuberculosis-free herd status, a series of procedures must be implemented, such as appropriate slaughter of animals with confirmed positive results, movement control, and depopulation of infected herds. However, these measures have been insufficient to eradicate the disease; prevalence of tuberculosis in cattle in Mexico is 2.05% and considerably higher in dairy cattle (16.5%).\textsuperscript{12} In 2008, prevalence of tuberculosis in cattle from zone A, where the WLIA of the present study is located, was estimated at 1.43%; in the adjacent zone B, which has important dairy activity, prevalence of tuberculosis in cattle was calculated at 2.65%. By 2010, prevalence in zone A had decreased to < 0.5%.\textsuperscript{12} The main epidemiological causes of disease propagation are presumed to include incomplete depopulation of infected herds, movement of tuberculosis-exposed animals between herds, and transmission from unidentified wildlife reservoirs.\textsuperscript{23,8,10}

In the study reported here, 24,400 cattle from 793 herds in the WLIA were tested; 191 (0.78%) had positive CFT test results, and 8 (0.03%) of these had positive results for serial CFT and CCT tests (considered CFT-CCT test reactors). Five of 7 herds in which \textit{M bovis}-infected cattle were confirmed by results of HMC had no CFT-CCT reactors among all tested cattle; this can be interpreted as an insufficient sensitivity of serial skin testing to detect infected cattle. The use of intradermal tuberculin tests has been a fundamental tool for detecting \textit{M bovis} infection in cattle; nevertheless, when the prevalence is low, its predictive values are insufficient.\textsuperscript{29} Another problem with CFT and CCT tests is that they cannot detect anergic animals that lack delayed-type hypersensitivity responses to \textit{M bovis} because of depressed cellular immunity, such as may occur with corticosteroid administration, infection with bovine viral diarrhea virus, advanced gestation, and lactation.\textsuperscript{30} Several other explanations have been proposed for the inability of the described intradermal tests to detect \textit{M bovis}-infected animals, such as PPD potency variations with time, erroneous animal identification, and operator errors in the PPD injection.\textsuperscript{39}

Because sanitary status verification could not be performed directly in the whole WLIA cattle population, results from the present study can be considered as an estimation of true \textit{M bovis} infection prevalence in the population (0.86%); however, the results of serial CFT-CCT tests, which were comparatively evaluated against the results of HMC and PCR confirmatory tests, suggest such sanitary status before the herds of origin of the CFT-CCT reactors were depopulated. Confirmatory tests were performed in tissues considered suspicious for tuberculosis during the sanitary inspection of WLIA cattle that were slaughtered for commercial reasons, for culling, or for being from the depopulated herds of origin of CFT-CCT test reactors.

During the 2006–2007 period, 31 of 17,351 (0.18%) slaughtered cattle from WLIA were identified as infected with \textit{M bovis} on the basis of positive results for serial CFT-CCT tests with HMC or PCR assay confirmation for \textit{M tuberculosis} complex organisms, presumably \textit{M bovis}. In our study, the sanitary inspection was performed following applicable official regulations, which indicate that slaughter line inspection is appropriate. However, 1 inspector usually inspects several dozen cattle per shift, and this working pace may cause some lesions to be unnoticed, creating a bias. This bias was not measured in the present study. However, previous studies\textsuperscript{41} have estimated the effectiveness of post-mortem inspection and HMC procedures in Mexican slaughterhouses, with \textit{M bovis} infection confirmed via histologic examination in 87% of cattle with grossly detectable tuberculosis lesions and in 59% via mycobacteriologic culture in tests performed by an approved Mexican veterinary laboratory.

Herd’s of origin for CFT-CCT test reactors, adjacent herds, and herds related via commercial activity...
were depopulated or quarantined during the surveillance period in the present study. As a result of these actions, the USDA and the Bi-National Tuberculosis and Brucellosis Eradication Committee resolved in 2010 to improve the ranking status of zone A, where the WLIA is located, to Accreditation Preparatory; this resolution was reported by the Mexican National Service of Food and Agriculture, Health, Safety and Quality.12

During the 2006 and 2007 regular hunting seasons, free-ranging and captive WR carcasses were examined grossly for lesions considered suspicious for tuberculosis. In 92 of 142 (64.8%) WR carcasses, tissue samples with some type of gross lesion were collected, but all tested negative for M. bovis via HMC. The statistical power of the study to detect at least 1 M. bovis–infected WR according to the calculated true prevalence of the disease in cattle was estimated a posteriori; the statistical power value was inadequate (11.4%) because of the low prevalence (0.86%), the small available sample size (n = 142), and the estimated population of WRs (2,690). For that reason, the confidence interval for M. bovis detection in our study was estimated as 0.00% to 2.3% of M. bovis infection prevalence in WRs with a 95% confidence level. The sample size recommended (230 animals) by Official Mexican Regulations for higher prevalence (20%)11 is larger than the number of WRs that were available for this study; in addition, the difficulty to confirm M. bovis infection eradication in situations where prevalence is very low has been recognized, and it has been proposed that sentinel species should be used as indirect indicators.29 Therefore, we cannot be confident that M. bovis infection does not exist to some degree in the WR population. Considering the maintenance of the wild species as well as their long-term productive potential, local authorities determine the number of hunting permits; therefore, the sample size was smaller than was sufficient for analysis in the present study.

The lack of detection of M. bovis infection in WRs is not rare; in the same manner, in Nebraska, no evidence of tuberculosis was detected in microscopically examined cranial lymph nodes (n = 271) or via PCR assay with M. bovis–specific probes in deer.30 In our study area, the WR population density was low (4 deer/km²)31 and calculated real prevalence of M. bovis infection within the cattle herds was also low (0.86%), which could hinder propagation of the disease to and among the WR population. Tuberculosis transmission among WRs has been associated with a high population density (23 deer/km²) caused by winter feeding motivated by hunting purposes.31

Supplemental feeding is also an important risk factor for the dissemination and maintenance of tuberculosis because it induces an excess population density and increases the number of cattle-deer or deer-deer interactions.32 However, the WCMSU units in our WLIA have participated in commercial controlled hunting, and local residents feed WRs; this may eventually lead to an artificially high WR population density inside these units. This, combined with interactions among cattle herds, may constitute a potential risk for the increase of disease transmission in the WLIA.

In the present study, whole WR carcasses were inspected by professional staff with wide expertise in slaughter activities using a field routine consistent in bilateral examination of the cranial, thoracic, and abdominal organs and lymph nodes, given that partial examinations might have missed some gross lesions.35 Other authors20,36 have proposed that necropsy seems to be a satisfactory tool for routine monitoring of M. bovis in wild deer populations where it may become enzootic. Histologic (typically inflammatory) lesions in lymph nodes and lung tissues were as frequent (P = 0.543) in young (6/77) and middle-aged WRs (24/32 [75.0%]), as in mature WRs (36/53 [67.9%]); however, these were significantly (P < 0.001) more common in males (59/59 [100%]) than in females (7/33 [21.2%]). This suggests that reproductive behavior is a risk factor for the presence of such lesions because the male WR groups are strictly hierarchic and very competitive, whereas the female groups are cooperative in feeding and movement.36,37

In cattle, microscopic examination of H&E-stained sections has been reported as an efficient procedure to identify the characteristic cellular changes of M. bovis infections.30 On the other hand, when M. bovis infects other species, the lesions can be less typical and hence harder to discriminate from other etiologies;32 therefore, bacterial isolation is required to confirm the infection. In our study, microscopic analysis of tissues considered suspicious for tuberculosis in WRs revealed remarkable cellular differences, compared with tuberculosis lesions in cattle; instead of the characteristic granuloma with a caseous necrotic core reported in deer infected with M. bovis,38 abscessed lymph nodes had liquefactive necrosis. In postmortem examination, abscesses are grossly similar to tuberculosis lesions commonly found in deer and other wild animals.39 The origin of these inflammatory lesions remains unknown, but we believe that they may result from stress and injuries acquired in fights during the reproductive periods close to the hunting season. In our study, an association between lesion type and its anatomic location in WRs was detected; our necropsy protocol included not only lymph nodes of the head but all lymph nodes (submaxillary, retropharyngeal, tracheobronchial, mediastinal, mesenteric, hepatic, prescapular, prelumbar, popliteal, and supramammary) as well as lungs, liver, and spleen. Tuberculosis examination for white-tailed deer harvested via hunting commonly includes only lymph nodes of the head; results of such examinations may underestimate disease prevalence by as much as 57%.40 It has been indicated that there is a relationship between lesion location and management circumstances, with thoracic and abdominal lesions being the most common in captive cervids and cranial lesions being the most common in free-ranging cervids.39 In WR, examination of retropharyngeal lymph nodes was shown to be more sensitive in detecting tuberculosis lesions than examination of extracranial lymph nodes or lungs.41,42 Consequently, during tuberculosis surveillance in WR, it seems appropriate to perform a necropsy with a comprehensive review of whole carcasses. In the present study, gross lesions were identified histologically as inflammation and lymphoid depletion in lymph nodes; these lesions were typically detected in the thoracic region, both in free-range (14/15) and captive (43/51
negative to survive in these materials. Current efforts
M. bovis from infected deer could act as a
ated with M. bovis that do not have grossly detectable lesions. The true prevalence of M. bovis in domestic cattle was calculated at 0.86%; there was no difference in the frequency of M. bovis detection in cattle from the 5 municipalities of the WLIA, indicating that the prevalence of M. bovis infection in cattle is low and is evenly distributed. The lack of detection of M. bovis infection in WRs is not conclusive; although M. bovis infection prevalence may have a value very close to zero, this could be increased in the future, suggesting the need to consolidate and expand strategies for diagnosis and prevention of tuberculosis in wildlife.

It has been reported recently that feeds contaminated with M. bovis from infected deer could act as a source of indirect transmission between wild and domestic animals, due to the capability of this bacterium to survive in these materials. Current efforts to ban or control supplemental deer feeding should have a positive effect on decreasing transmission of M. bovis among deer. In the study area, the use of WRs in controlled commercial hunting has only been active for a decade; it is important to consider supplemental feeding practices and to be careful in controlling the access of various species to common feeding sites. Although M. bovis infection prevalence in domestic cattle in the study area was determined to be low, there is a risk that local carnivores such as coyotes might access the remains of dead animals; therefore, to prevent contagion, it is important to ensure the proper disposal of carcasses or discarded internal organs. Carnivores sometimes scavenge on WR and cattle carcasses, providing a plausible mechanism for either cattle- or deer-to-carnivore transmission, but the frequency of such transmissions is unknown. The tradition in Mexico is to harvest internal organs typically used for cooking purposes, which may prevent propagation to other species that might otherwise consume these tissues if they are not destroyed.

The general response of landowners to the proposed field surveillance was positive; most who were contacted for permission to sample within their hunting areas agreed to the request. During the 2-year period, of the total number of authorized WR hunters within the WLIA, only 18 of 160 (11.3%) did not participate in the survey, either because they were not located or did not have any successful hunting activity. The study reported here demonstrated that it is entirely possible to perform wildlife epidemiological surveillance in developing countries such as Mexico, while performing passive surveillance activities in domestic livestock.

Our results suggest that WRs play a limited role in the propagation of tuberculosis in the WLIA evaluated; however, because M. bovis infection is widespread among cattle in Mexico and there are many areas where cattle and WRs coexist and share access to feed and

natural resources, the transmission of M. bovis between cattle and WRs is possible. Therefore, it is desirable to implement and to consolidate activities for diagnosis, prevention, and surveillance of M. bovis infection in WCMSU units.

References


