

# Epizootic of paratuberculosis in farmed elk

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- ▶ Elk infected with *Mycobacterium paratuberculosis* have clinical signs that are similar to those in infected cattle, but elk may die from the disease at a younger age than is commonly reported in cattle. Histologic lesions in elk are similar to classic lesions of paratuberculosis in cattle.
- ▶ Diagnostic techniques such as bacterial culture of feces or tissues and microscopic examination of tissues are useful in confirming a diagnosis made by the agar gel immunodiffusion test.
- ▶ Husbandry methods that can limit transmission of the infection include use of feed and water troughs that can be cleaned with tuberculocidal disinfectant, fencing off or draining areas of standing water, removal of manure from feeding areas, and prompt and continued isolation of elk with clinical signs consistent with paratuberculosis.
- ▶ The elk farming industry, characterized by frequent exchange and sale of elk, may benefit from increased veterinary attention to detection and control of paratuberculosis.

Three yearlings and 1 adult elk (*Cervus elaphus*) with histories of chronic weight loss and diarrhea were brought to the University of Wisconsin School of Veterinary Medicine for euthanasia and necropsy. On arrival, the elk were gaunt and weak but alert and able to stand. Their coats were dull, dry, and rough; the perineum of each elk was stained with liquid green feces. Each elk was euthanized by intravenous administration of sodium pentobarbital.<sup>3</sup> On examination at necropsy, each was severely emaciated with almost complete depletion of adipose stores. Abscesses and areas of congestion and hemorrhage consistent with mild to moderate verminous pneumonia were found in the lungs of all 4 elk. Gross findings in the yearlings were similar: the mucosa of the small intestine (especially jejunum) was irregularly roughened, raised, and thickened. The mucosae of the colon and cecum were also thickened and nodular. Serosal lymphatics of the small intestine were prominent, pale, and somewhat firm. Mesenteric lymph nodes were pale and enlarged, with indistinct corticomedullary borders.

On histologic examination, lamina propria of the jejunum, ileum, cecum, and colon from 2 of 3 yearlings were moderately to massively infiltrated with epithelioid macrophages packed with acid-fast bacilli.

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Scattered foci of lymphocytes and plasma cells were also found. A granulomatous reaction, also characterized by epithelioid macrophages containing many intracytoplasmic acid-fast bacteria, displaced and replaced typical architecture in mesenteric, colic, and hepatic lymph nodes. All 3 yearlings had several bile ducts that were thickened and surrounded by white fibrous tissue and epithelioid macrophages, consistent with mild to severe chronic multifocal granulomatous cholangiohepatitis. An adult fluke, identified as *Fasciola hepatica*<sup>b</sup> (vs the more common *F magna*), was retrieved from the thickened bile duct of 1 yearling. In the adult elk, hepatic trematodiasis with severe peritonitis and verminous pneumonia were found. Severe and diffuse necrosis with vacuolization of neurons, primarily in the gray matter of both sides of the brain, was also noted. Slides of 14 organs from each elk were stained with Ziehl-Neelsen stain and examined for acid-fast bacilli. Acid-fast bacilli were found in 9 of 14 organs examined from each of 2 yearlings and 7 of 14 organs from the third yearling. Gross and microscopic lesions characteristic of infection with *Mycobacterium paratuberculosis* were not detected in the adult elk, and acid-fast organisms were not observed.

Bacterial culture of feces and tissue samples from the same 14 organs was performed using radiometric methods to isolate *M paratuberculosis*.<sup>1,2</sup> Isolates were identified as *M paratuberculosis* by use of a DNA probe.<sup>5</sup> *Mycobacterium paratuberculosis* was isolated from the feces of 2 yearlings and from 11 of 14 and 13 of 14 organs examined from these same 2 yearlings but not from tissues or feces from the third yearling. In contrast, organisms were not isolated from feces of the adult elk and were isolated from only 2 of 14 tissues (mesenteric lymph node and uterus). Microscopic examination and bacterial culture were not equally successful at detecting the organism in each case. For example, *M paratuberculosis* organisms were isolated through bacterial culture from lung, kidney, testes or uterus, and muscle but were not observed microscopically in these tissues. Conversely, microscopic examination revealed acid-fast organisms in the ileum, jejunum, cecum, ileocecal lymph node, and tonsil in 1 elk, whereas radiometric culture techniques did not isolate the organisms from the same tissues. Given that each radiometric bacterial culture isolate was subsequently identified by DNA probe as *M paratuberculosis*, the authors believe that the acid-fast rods observed, using special stains and microscopic examination, in tissue sections were also *M paratuberculosis*.

On the basis of the test's efficacy in sheep, goats, and cattle,<sup>3,4</sup> the agar gel immunodiffusion (AGID) test was used to detect serum antibody produced in response to infection with *M paratuberculosis*. Visible precipitin lines were interpreted as a positive result.

The 2 yearlings with the most disseminated infection (as indicated by the extensive number of organs from which the organism was isolated) had produced antibody to *M paratuberculosis* at the time of necropsy. The adult elk and remaining yearling had not produced serum antibodies detectable by the AGID test.

In the 3 months before these elk were referred for euthanasia and necropsy, 7 other yearlings from the same herd with similar clinical signs died or were euthanized. Similar lesions of the gastrointestinal tract were noticed in these elk; the gross and histologic diagnoses were paratuberculosis. To the best of our knowledge, this is the first report of a fulminant epizootic of paratuberculosis in farmed elk in the United States. The epizootic occurred in a facility built in the early 1990s for elk farming on land that had not housed livestock for more than 20 years. Eighty elk were purchased from various herds in the United States and Canada. Unusual or extensive health problems were not noticed in the herd until 1994. In December 1994, a 3-year-old female elk from a Midwestern herd was introduced into the herd without a quarantine period. During the next 4 months, this elk had signs of lameness, weight loss, and diarrhea. Weight loss and diarrhea were not affected by several treatments for parasitism of the gastrointestinal tract. Results of a CBC and chemistry panel were not diagnostic. Results of a complement-fixation test for paratuberculosis were negative. The elk was transferred several times from a 16-acre group pen to a pen used to isolate sick elk. While in the group pen, the elk was reportedly excluded from mingling with the adult herd by females with calves. During the spring of 1995, the elk acted as a nursemaid to calves born in the group pen. (Cross-fostering is not uncommon in this and other cervid species). The elk died in June 1995, a month after delivering a stillborn calf. Paratuberculosis was diagnosed at necropsy and confirmed by isolation of *M paratuberculosis* by radiometric bacterial culture and identification of *M paratuberculosis* isolates by DNA probe.

We believe that the source of infection in the epizootic was this particular female elk. During the spring of 1995, the elk had advanced clinical paratuberculosis. It is likely that *M paratuberculosis* was being shed in her feces and perhaps in her milk. Because the weather that spring had been wet, a shallow, muddy wallow had formed at one end of the group pen. Calves drank from and lay in this wallow; it was also frequently used as a resting place by this female elk, according to the owner's observations. It is likely that the organism was spread to calves via fecal contamination of the wallow by this female elk and possibly via milk during cross-fostering. Of 31 calves born in the group pen in 1995, 11 (35%) died or were euthanized because of paratuberculosis before reaching 2 years of age. Of the remaining 20 calves, 1 died of unrecorded causes, and 19 were sold from the farm at 2 years of age. Their health status is unknown.

This theory of source of infection is complicated by findings of extensive lesions unrelated to infection with *M paratuberculosis* in the adult 10-year-old female elk of this report. Because there were few lesions characteristic of paratuberculosis and organisms isolated

were few in number (ie,  $10^{0.25}$  organisms/g of uterus,  $10^3$  organisms/g of mesenteric lymph node) and found in an atypical location (ie, uterus), we believe that infection in the adult elk of this report developed shortly before death from other causes. The elk had been in the group pen with infected yearlings for several weeks. Because the elk was debilitated by other diseases, it could have been sufficiently immunosuppressed to become infected with *M paratuberculosis* as an adult. Spongiform encephalopathy was ruled out in this elk by use of immunohistochemical techniques for examination of brain tissue for a modified protease resistant protein. Grey matter lesions were most likely attributable to a hepatoencephalopathy secondary to massive, severe, long-standing hepatic trematodiasis with secondary abscesses and bacterial infections.<sup>5</sup>

After paratuberculosis was diagnosed in the yearlings of this report, the group pen became the quarantine site for any elk with clinical signs of paratuberculosis. Once yearlings had been removed from the premises, the soil in the pen was turned, and the pen remained empty for 1 year. It will be used for adult elk on the presumption that otherwise healthy, mature elk would be less susceptible to infection should *M paratuberculosis* organisms remain, as is the case in cattle. Clinical signs of paratuberculosis in other elk have not been evident at the farm to date. Cows with calves that had been affected during the epizootic and 5 resident bulls were tested 1 year after the epizootic. Results of radiometric bacterial culture of fecal samples were negative. Multiple negative annual tests will be necessary, however, before we can confidently conclude that remaining elk on the premises are not infected with *M paratuberculosis*.

*Mycobacterium paratuberculosis* is an acid-fast gram-positive obligate parasite that causes paratuberculosis (Johne's disease) in cattle, sheep, goats, deer, elk, llamas and other ruminants.<sup>6,7</sup> Rabbits and primates have also been infected.<sup>8,9</sup> Infection causes a slowly developing granulomatous disease of the small intestine and associated lymphoid tissue. The infected animal has chronic weight loss and, in some species, diarrhea. Paratuberculosis is contagious and incurable. *Mycobacterium paratuberculosis* is shed intermittently in feces of infected animals and can also be detected in milk or colostrum. In utero infection has been confirmed in cattle and is suspected in other species.<sup>10</sup> Cattle typically acquire the infection while young and manifest clinical signs of periodic diarrhea and progressive weight loss 1 to 5 years later. Common techniques used to diagnose paratuberculosis in cattle include detection of serum antibodies by AGID and ELISA and isolation of the organism by culture (radiometric or conventional) of feces or tissue. Other methods include microscopic examination of mesenteric lymph nodes or the distal portion of the small intestine for histologic lesions characteristic of the disease and use of Ziehl-Neelsen stain to reveal the short acid-fast bacilli.

Paratuberculosis in the elk of this report was similar in many aspects to the disease in cattle. Both species have an apparently increased susceptibility to infection in young animals and have similar clinical signs (eg,

weight loss and diarrhea) and histologic lesions (ie, granulomatous inflammation primarily in the gastrointestinal tract). Aspects of paratuberculosis that differed between the elk of this report and cattle were more rapid progression of the disease in young elk, as evidenced by severe clinical signs, than is commonly seen in young cattle<sup>11</sup> and a more proximal distribution of granulomatous lesions in the small intestine, with the jejunum being as or more affected than the ileum. Others<sup>12-16</sup> have reported a similarity in lesions (especially caseous necrosis and mineral deposition) in elk infected with *M bovis*, *M avium*, and *M paratuberculosis*. Lesions in the elk of this report were neither caseated nor mineralized; instead, they closely resembled typical granulomatous lesions caused by *M paratuberculosis* in cattle.

*Mycobacterium paratuberculosis* can be detected in elk by bacterial culture (radiometric or conventional) of feces or tissue, but the number of organisms isolated with these techniques is expected to be higher in samples taken from elk that are in later stages of the disease when the organism may be disseminated. The number of organisms isolated per gram of tissue may indicate severity of infection. In these elk, more than  $1 \times 10^6$  organisms/g were isolated from 85% of samples positive by radiometric bacterial culture (as determined by a quantification method).<sup>5</sup> This heavy bacterial burden in a multiplicity of tissues from the young elk of this report are in contrast to what is typically seen in cattle with paratuberculosis. We conclude that elk are a species that is permissive to infection or that elk of this report were exposed to massive numbers of organisms during a period of susceptibility. The method that was most successful in detecting acid-fast organisms in a yearling of this report was microscopic examination of tissue sections, whereas in the adult elk of this report, use of radiometric bacterial culture alone confirmed infection. These findings underscore the importance of using both diagnostic methods to increase the probability of detecting infection.

Two elk developed antibodies to infection with *M paratuberculosis* that were detected by use of the AGID test. It is likely that antibodies are produced late in the course of the disease in elk as in cattle. The AGID test can be used in elk with clinical signs of paratuberculosis as it is in sheep, goats, and cattle. However, the AGID test has yet to be validated in elk; its sensitivity and specificity are unknown.

This report highlights the need for increased veterinary attention to detection and control of an infectious disease in a growing agricultural industry that is characterized by frequent sale and exchange of elk. Paratuberculosis does not appear to be a common problem for the elk farming industry, but it can be economically ruinous to individual herd owners. Husbandry methods that may limit transmission of the infection include use of feed and water troughs that

can be cleaned with tuberculocidal disinfectant, fencing off or draining areas of standing water, removal of manure from feeding areas, and prompt and continued isolation of elk with clinical signs consistent with paratuberculosis. The impact of this disease epizootic emphasizes the importance of limiting the spread of *M paratuberculosis* by knowing the paratuberculosis status of the source herd and testing elk for the organism or antibody to the organism before they are introduced into a new herd.

<sup>a</sup>Beuthanasia-D, Schering-Plough Animal Health, Kenilworth, NJ.

<sup>b</sup>Identification performed by William Foreyt, Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, Wash.

<sup>c</sup>IDEXX probe, IDEXX Laboratories Inc, Portland, Me.

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