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Source: Journal of Wildlife Diseases, 33(3) : 635-637

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-33.3.635

Journal of Wildlife Diseases, 33(3), 1997, pp. 635–637

Radiometric culture of *Mycobacterium avium paratuberculosis* from the feces of tule elk

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ABSTRACT: To determine if *Mycobacterium avium paratuberculosis* has persisted in tule elk (*Cervus elaphus nannodes*) at Point Reyes National Seashore (California, USA), 100 fresh fecal samples were collected. Feces were cultured on a modified BACTEC 12B radiometric medium for detection of *M. avium paratuberculosis*. Four samples, coming from two separate groups of elk tested positive for *M. avium paratuberculosis*. Thus, a noninvasive technique was used to document the continued presence of *M. avium paratuberculosis* in elk at Point Reyes National Seashore. These findings document persistence of this organism for a period of at least 13 yr in a free ranging herd of elk, with a 6 yr absence of observed clinical signs.

Key words: *Cervus elaphus nannodes*, feces, Johne's disease, *Mycobacterium avium paratuberculosis*, radiometric culture, tule elk.

Point Reyes National Seashore (PRNS) is a 40,485 ha national park, located approximately 65 km northwest of San Francisco, along the coast of California (USA). The northern tip of this peninsula, known as Tomales Point, is a tule elk (*Cervus elaphus nannodes*) reserve and a popular tourist attraction. The tule elk reserve is about 3 km at its widest point and less than 8 km long. At the southern border of this reserve, a fence prevents the elk from ranging south of Tomales Point. Pursuant to agreements made with the U.S. National Park Service when the park was first established, several dairy farms continue to operate south of this fence.

Tule elk, once plentiful throughout much of California, were decimated almost completely after the Gold Rush of 1849, due to market hunting and habitat loss (McCullough, 1969). The current population of elk on Tomales Point at PRNS derives from animals relocated in 1978 from San Luis National Wildlife Refuge, Merced County, California (Gogan and Barrett, 1987).

Although the tule elk population at Point Reyes began as a small herd of 17 animals, it has grown considerably in the last 15 yr. Two hundred twenty-one elk populated the reserve in 1993 (Bartolome, 1993); the estimate in 1994 was 270. This population growth has caused concern that the elk may soon exceed the maximum sustainable equilibrium density of Tomales Point. Recent estimates suggest that Tomales Point can sustain a maximum of 330 elk (Bartolome, 1993).

Paratuberculosis (Johne's disease) is caused by infection with *M. avium paratuberculosis*, a bacterium that can infect many wild and domestic ruminants (Riemann et al, 1979; Jessup, 1981; Williams et al, 1985). Recently, the taxonomy of this species was amended as a subspecies of *M. avium* (Thorel et al., 1990), but there is much debate on this topic. The clinical signs of Johne's disease in cattle are weight loss and ill thrift in spite of a good appetite, intermittent to chronic diarrhea, and eventually death. Many animals become carriers of the disease and may intermittently shed the organism without showing clinical signs of disease. Clinical symptoms may recur with stress or parturition (Stehman, 1990).

In 1980 and 1981, three elk from PRNS were diagnosed with paratuberculosis. These elk were emaciated, had poor hair coats, and suffered from diarrhea (Jessup et al., 1981). In 1982, five more animals were diagnosed with paratuberculosis (Gogan and Barrett, 1987). In 1984, *M. avium*

paratuberculosis was isolated from tissues of a dead elk (D. Jessup, pers. comm.). Since that time, paratuberculosis has not been diagnosed at PRNS. Because of this, it has been suggested that the elk are no longer infected with *M. avium paratuberculosis* and that it would be safe to relocate excess elk. In order to determine if the elk of Point Reyes are still infected with *M. avium paratuberculosis,* we surveyed the elk population by noninvasive fecal collection and culture.

At dawn on 7 July 1993, about 20 volunteers assembled to collect fresh elk feces. Divided into four groups of five or six individuals, each team covered a specific area of the Tomales Point elk reserve. Each team walked the area on foot until elk were located. The team classified the elk by age and sex, and very slowly herded them away from the area. After the elk had left the area, the teams collected fresh feces in prelabeled plastic bags. We determined fecal freshness by looking for moist feces with a greenish tinge. Fecal samples often were warm when collected.

We collected samples from six different groups of elk found within four separate parts of the park. All of the fresh fecal samples were of normal consistency. We found several older fecal samples which were soft to watery, but they were not selected for culture because they did not appear fresh.

After all the samples were collected, we chose the 25 freshest appearing samples collected by each team (n = 100 samples). We mixed 3 g feces in 30 ml of 1.0% hexadecylcetylpyridinium chloride, then shipped the samples by overnight express to the University of Wisconsin (Madison, Wisconsin, USA), where *M. avium paratuberculosis* would be isolated using a radiometric culture method (Collins et al., 1990). Briefly, the procedure is outlined as follows. The fecal suspensions are filtered through a two-ply thickness of sterile gauze and the filtrate allowed to settle for 24 hr at approximately 22 C. The next day, 10 ml of the suspension is drawn into a syringe and forced through a Swinex filter holder (Millipore Corp., Bedford, Massachusetts, USA) containing a 13 mm diameter 3-μm-pore-size polycarbonate filter membrane (Nuclepore Corp., Pleasanton, California, USA). The filtrate is discarded and filters rinsed with 2 ml of sterile distilled water followed by air to expel excess fluid. Using sterile forceps, the filters are placed into the bottles of radiometric culture medium (BACTEC 12B supplemented with mycobactin, egg yolk suspension, and the antibiotics vancomycin, amphotericin B and naladixic acid). Thereafter, the bottles are incubated at 35 C without agitation and read on a BACTEC 460 (Becton Dickinson, Sparks, Maryland, USA) without CO_2. The BACTEC 460 measures release of radiolabeled CO_2 by microorganisms growing in the culture medium. Bottles are read twice weekly for the first 4 wk and then once weekly through week 10. Isolates are confirmed to be *M. avium paratuberculosis* by use of a PCR-amplified DNA probe for the insertion element IS900 which is specific for the species (IDEXX Laboratories, Inc., Westbrook, Maine, USA) (Vary et al., 1990; Sockett et al., 1992).

Of the 100 samples tested, five fecal cultures could not be evaluated because they were overgrown with normal flora. From the remaining 95 samples, *M. avium paratuberculosis* was isolated from four fecal samples (4%). All four positive samples came from two separate locations in the northern part of the park. Thus, we conclude that the tule elk of PRNS continue to be infected with *M. avium paratuberculosis.* Due to intermittent bacterial shedding patterns characteristic of *M. avium paratuberculosis* and the remote possibility that some animals were sampled twice, we are unable to estimate the prevalence of infection within this elk population.

As long as *M. avium paratuberculosis* exists in the PRNS tule elk population, management options for these animals are limited. None of the animals should be relocated to areas outside the park, because

they might spread the bacteria to other wild or domestic animals. Furthermore, the potential for infection of other native wildlife species and dairy cattle exists if the elk are allowed to roam the park at large. The current management plan for the tule elk of Point Reyes is to allow the elk to self-regulate, and to monitor the herd closely. Culling may be necessary if excess damage to the habitat occurs; however, this is not considered to be a long-term solution. Additionally, baseline data on the feasibility of reproductive control is currently being gathered.

This study was funded by the Committee for the Preservation of Tule Elk. Christina Sigurdson, Cheryl Scott, Melissa Chechowitz, Jennifer Pironis, Jacueline Dieter, Alice Herwell, Denise Driscoll, Bruce Keegan, Barbara Pederson, and Fred Botti assisted in the sample collection. Edith Cook and Elizabeth Williams reviewed this manuscript and gave helpful comments.

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Received for publication 23 September 1996.