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Distribution of Mycobacterium avium subspecies paratuberculosis in the Lower Florida Keys

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ABSTRACT

Johne’s disease, a fatal and contagious gastrointestinal infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), was first diagnosed in an endangered Florida Key deer (*Odocoileus virginianus clavium*) in 1996 and later in six additional Key deer deaths from 1998 to 2004. We investigated the geographic distribution of *Map* in the Lower Florida Keys from February 2005 through May 2006 via collection of blood and fecal pellets from 51 live-captured deer, collection of 550 fecal samples from the ground, and by necropsies of 90 carcasses. Tissue and fecal samples also were submitted from 30 raccoons (*Procyon lotor*), 3 feral cats (*Felis catus*), an opossum (*Didelphis virginiana*), and a Lower Keys marsh rabbit (*Sylvilagus palustris hefneri*). *Mycobacterium avium* subsp. *paratuberculosis* was identified in 23 Key deer fecal samples collected from the ground, tissue samples from two clinically ill Key deer, and from the mesenteric lymph node of a raccoon. The results of this study indicate that Johne’s disease persists in the Key deer population and environment at a low prevalence but its distribution currently is limited to a relatively small geographic area within the range of Key deer.

*Key words*: Florida Keys, Johne’s disease, Key deer, *Mycobacterium avium* subsp. *paratuberculosis*, *Odocoileus virginianus clavium*, paratuberculosis, raccoon

INTRODUCTION

*Mycobacterium avium* subsp. *paratuberculosis* (*Map*) is a hardy and slow-growing microorganism that causes Johne’s disease, a significant economic and health problem for domestic ruminants. Signs of this eventually fatal disease appear many months after infection and include emaciation and diarrhea in some species. Infection
also has been documented in a wide range of wildlife species including white-tailed deer (*Odocoileus virginianus clavium*) (Chiodini and Van Kruiningen, 1983), tule elk (*Cervus elaphus nannodes*) (Jessup et al., 1981), big horn sheep (*Ovis canadensis*) (Williams et al., 1983), wild rabbits (*Oryctolagus cuniculus*) (Greig et al., 1999), fox (*Vulpes vulpes*), stoat (*Mustela erminea*) (Beard et al., 2001), Key deer (*Odocoileus virginianus clavium*) (Quist et al. 2002), raccoons (*Procyon lotor*), opossum (*Didelphis virginiana*), armadillos (*Dasypus novemcinctus*) (Corn et al., 2005), and feral cats (*Felis catus*) (Palmer et al., 2005).

Johne’s disease was first identified in an endangered Florida Key deer (*Odocoileus virginianus clavium*) in 1996 at a private residence on Big Pine Key; a second case was confirmed two years later at the same location (Quist et al., 2002). Based on a subsequent survey of repository serum and fecal samples and live capture, infection prevalence was believed to be low in the Key deer population. However, from 2003 to 2004 five additional deer were diagnosed with Johne’s disease at the same residence and neighboring islands. These reports plus new findings in Johne’s disease research indicating that non-ruminant wildlife are also susceptible to infection on heavily contaminated premises (Beard et al., 2001; Corn et al., 2005) raised the possibility that the infection prevalence had increased or was more extensive than previously thought. Additional concerns included illegal feeding of Key deer and the National Key Deer Refuge policy for translocation of deer to keys previously within the historical range of this species. The purpose of this survey was to determine the geographic distribution of *Map* in the Key deer population.
MATERIALS AND METHODS

Study area

The Florida Keys are a series of islands that extend from the southern tip of the Florida peninsula. Key deer occupy several islands along this chain known as the Lower Keys from Little Pine Key to Sugarloaf Key (Hardin et al. 1984). However, approximately 75% of the population is limited to Big Pine and No Name Keys where fresh water is available (Lopez, 2001).

Capture methods

Key deer were live-trapped at various locations on Big Pine Key. A drop net was used to capture the deer according to methods described by Lopez et al. (1998). Once caught in the net, deer were physically restrained while blood and fecal samples were collected. Sex, age, location, and GPS coordinates were recorded and each animal was either marked with tattoo ink or tattooed prior to release to prevent repeat sampling.

Collection of samples

Samples were collected intermittently from February 2005 through May 2006. Key deer killed by vehicles or other causes were stored in a freezer by National Key Deer Refuge personnel prior to each survey period and were then necropsied as time permitted during the sampling period. Deer killed during the sampling period were necropsied within a few hours of discovery. Fecal pellets and tissue samples including liver, ileum, and mesenteric lymph node were collected from each deer depending on the condition of the animal when found. Blood was collected from freshly killed deer by cardiac puncture; serum was obtained within a few hours of collection and stored in a freezer at 20°F until shipped. Tissue and fecal samples were placed in individual whirl-paks.
Additional sections of each of the tissues were fixed in 10% buffered formalin and stored for histopathological evaluation of tissues testing positive for *Map*. Fecal pellets were collected from the ground at various locations on Big Pine, No Name, Howe, Water, Little Pine, Cudjoe, and Big Torch Keys, and on Munson Island, Little Palm Island and an unnamed offshore island. Samples were refrigerated for no more than 72 hours and shipped on ice packs to the Johne’s Information Center at the University of Wisconsin (Madison, Wisconsin, USA).

During June and July, raccoons and feral cats were captured on Munson Island and the southern end of Big Pine Key using Tomahawk live-traps. Animals were sedated with Telazol® and then euthanized by intracardiac injection of sodium pentobarbital. Necropsies were conducted immediately and fecal and tissue samples including liver, ileum, and mesenteric lymph node were collected from each animal.

**Laboratory methods**

Culture, isolate identification, and serology were conducted by the Johne’s Information Center. Isolation of mycobacteria was performed using the radiometric method of detection (Collins et al. 1990). Acid-fast organisms isolated from the samples were identified as *Map* by an IS900 DNA probe and mycobactin-dependent growth patterns. Sera were tested for antibody to *Map* by a version of an enzyme-linked immunosorbent assay using a protein G antibody conjugate (ELISA, IDEXX, Portland, Maine, USA) (Tryland et al., 2004). Histopathology was conducted at the Southeastern Cooperative Wildlife Disease Study (Athens, Georgia, USA). Tissues from culture-positive animals were embedded in paraffin and sectioned at 3 to 4 µm. Individual
sections were stained with hematoxylin and eosin for routine examination and with Ziehl-Neelsen acid-fast stain to search for acid-fast bacteria.

RESULTS

*Mycobacterium avium* subsp. *paratuberculosis* was isolated from 24 Key deer fecal samples submitted for culture. Twenty-three of the culture-positive fecal samples were collected from the ground: 7 on Munson Island, 11 on Little Palm Island, two on an offshore island, two on the south side of US 1 on Big Pine Key and one from a private residence on Long Beach Road (Figure 1). The other fecal isolate was obtained from a fecal sample collected directly from a clinically ill deer. The 24 culture-positive fecal samples represent (3.6%) of the 669 collected: 536 of which were taken from the ground, 84 from carcasses and 49 from live-captured deer.

Isolates were obtained from 11 of 90 (12%) fecal samples collected from the ground on Little Palm and 7 of 73 (10%) from Munson Island. The next most contaminated site was an offshore island where 2 of 33 (6%) fecal samples were culture positive. On Big Pine Key 3 of 304 (1%) fecal samples were culture-positive; the microorganism was not detected on any of the other nine keys where samples were collected.

A total of 262 Key deer tissue samples collected from 90 deer were submitted for culture and histopathology. The microorganism was isolated from the feces, ileum, mesenteric lymph node, and liver of an adult female Key deer found on Little Palm Island; this animal was also antibody positive and clinically ill. Lesions including severe granulomatous inflammation with intracellular acid-fast organisms were consistent with Johne’s disease. In addition, an emaciated and weak adult female Key deer was found on
Little Palm Island in May 2006. Tissue culture and histopathology of the ileocecal and mesenteric lymph nodes plus the ileum confirmed infection with *Map.

Two of 97 serum samples tested positive for antibodies to *Map*. One sample was collected from a deer found dead on Little Palm Island; corresponding fecal and tissue samples tested positive. The other was from an adult buck found dead on Big Pine Key but corresponding fecal and tissue samples all tested negative.

Tissue and fecal samples were submitted from 30 raccoons, 3 feral cats, an opossum and a Lower Keys marsh rabbit that had been killed by a vehicle (Table 1). The mesenteric lymph node of one raccoon captured on Munson Island was culture positive, but no other tissues were positive and histopathology revealed mild inflammation but no acid-fast bacteria typically observed with *Map* infection. All other non-Key deer tissue samples were culture negative. Fecal pellets from 3 rabbits, 2 silver rice rats, and 9 raccoons were collected from the ground but also tested negative (Table 1).

**DISCUSSION**

Recovery of *Map* from multiple samples confirms the presence and persistence of the microorganism in the Lower Florida Keys. Based on the location of the culture-positive samples, the geographic distribution of *Map* in the Keys currently appears to be limited to Big Pine Key, Munson and Little Palm Islands. All previous cases of Johne’s disease reported since 1996 also occurred in this area (Quist et al. 2002). We did not discover evidence of Johne’s disease in deer sampled north of US 1, but two fecal samples collected from the ground along US 1 were culture positive. Since the actual number of deer represented by the fecal samples collected in this survey is unknown, no infection prevalence can be calculated based on these data.
Key deer comprise most if not all of the ruminants present in the Lower Keys. Although we do not know how or when *Map* was introduced into the Key deer population, it appears that *Map* is being maintained in this population. Furthermore, during collection of fecal pellets on Little Palm Island, a Key deer was observed swimming from Little Palm to another island, suggesting that the potential for spread of the infection on a larger scale exists and that it may not remain limited to the area south of US 1. Supplemental feeding, as occurs on Big Pine Key, Munson, and Little Palm Islands, encourages congregation of Key deer which increases animal density, environmental contamination and the likelihood of transmission of various infectious diseases (Williams, 2001; Nettles et al., 2002). High population density and poor habitat quality as exist in the southern part of Big Pine Key (Harveson et al., 2004) increase the probability of exposure and subsequent infection with *Map*. The urbanization of the Key deer as described by Folk and Klimstra (1991) may perpetuate the microorganism in the environment.

Based on the limited number of samples tested, evidence of infection in non-ruminant species was scant; a single isolate from the mesenteric lymph node of a raccoon captured on Munson Island was culture positive. It is likely that the raccoon became infected through exposure from the contaminated environment since seven of 75 (9%) Key deer fecal samples collected on Munson Island (41 ha) were contaminated with the organism. Previous studies have reported infection in raccoons (Corn et al., 2005) as well as other non-ruminant species that inhabit dairy farms with infected livestock present including feral cats (Palmer et al., 2005), rabbits (Raizman et al., 2005), and birds (Corn et al., 2005).
Key deer mortality caused by Map infection is relatively low in comparison to vehicle related mortality (2002-2004, 72%). However, due to the endangered status of the Key deer and the unknown factors affecting the perpetuation and dispersal of the microorganism in the Lower Florida Keys, it is imperative to minimize the risk of infection and large-scale mortality. Actions that may reduce risk include (1) increased education of tourists and residents about the consequences of supplemental feeding (Lopez et al., 2003) (2) increased enforcement of laws prohibiting illegal feeding of deer (Miller et al., 2003) and (3) continued monitoring of the Key deer population to determine if the disease continues to be maintained and if dissemination to areas north of US 1 occurs. Further studies on the role of environmental contamination in the maintenance and transmission of Map, and studies on the effects of cessation of supplemental feeding of Key deer on dispersal are recommended.

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**LITERATURE CITED**


FIGURE 1. Sites where *Map* positive samples have been collected since 1996.
Table 1. Samples submitted for Johne’s disease testing by location and species

<table>
<thead>
<tr>
<th>Location and species</th>
<th># of <em>Map</em> positive isolates by sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feces</td>
</tr>
<tr>
<td>Big Pine Key – Key deer</td>
<td>3/424</td>
</tr>
<tr>
<td>Big Pine Key – Marsh rabbit</td>
<td>0/1</td>
</tr>
<tr>
<td>Big Pine Key – Raccoon</td>
<td>0/27</td>
</tr>
<tr>
<td>Big Pine Key – Feral cat</td>
<td>0/3</td>
</tr>
<tr>
<td>Big Pine Key – Opossum</td>
<td>0/1</td>
</tr>
<tr>
<td>No Name Key – Key deer</td>
<td>0/15</td>
</tr>
<tr>
<td>Munson Island – Key deer</td>
<td>7/75</td>
</tr>
<tr>
<td>Munson Island – Raccoon</td>
<td>0/5</td>
</tr>
<tr>
<td>Little Palm Island – Key deer</td>
<td>12/91</td>
</tr>
<tr>
<td>Little Palm Island – Raccoon</td>
<td>0/1</td>
</tr>
<tr>
<td>Howe Key – Key deer</td>
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<tr>
<td>Cudjoe Key – Key deer</td>
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<tr>
<td>Ramrod Key- Key deer</td>
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</tr>
<tr>
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<tr>
<td>Water Key – Marsh rabbit</td>
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<td>Big Torch Key – Silver rice rat</td>
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