POTENTIAL CONTAMINATION OF ENDANGERED FLORIDA KEY DEER HABITAT WITH *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*

by

HEIDI LYNN MURRAY

(Under the Direction of Michael J. Yabsley)

ABSTRACT

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease, a progressive wasting disease of domestic and wild ruminants. Johne's disease was first detected in the endangered Florida Key deer population in 1996 on Big Pine Key, Florida. Since previous studies have shown that MAP is persistent in contaminated areas, I hypothesized that MAP is present in the environment on Big Pine Key and the Newfound Harbor Keys. Between November 2009 and July 2011, a total of 675 environmental samples, 249 fecal samples, and tissue and fecal samples from 43 necropsied Key deer were collected and cultured for MAP. All environmental and fecal samples collected were negative for MAP but MAP was isolated from three of 43 (7%) sampled Key deer. Results of this study indicate that MAP is at a very low prevalence in the environment within the Key deer range.

INDEX WORDS: *Mycobacterium avium* subspecies *paratuberculosis*, Johne's disease, Key deer, *Odocoileus virginianus clavium*, environmental contamination

POTENTIAL CONTAMINATION OF ENDANGERED FLORIDA KEY DEER HABITAT WITH *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*

by

HEIDI LYNN MURRAY

BS, University of Maine, 2006

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2012

POTENTIAL CONTAMINATION OF ENDANGERED FLORIDA KEY DEER HABITAT

WITH *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*

by

HEIDI LYNN MURRAY

Major Professor: Michael J. Yabsley

Committee: Robert J. Warren
M. Kevin Keel
Joseph L. Corn

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2012

ACKNOWLEDGEMENTS

My project was fully funded by the U.S. Fish & Wildlife Service. I want to thank Anne Morkill and the National Key Deer Refuge for providing me with housing, office space, and a vehicle while conducting field work. Specifically, I want to thank Chad Anderson, Kate Perry, Philip Hughes, Dana Cohen, Jim Bell, Tom Wilmers, Steve Nelms, Mike Gallagher, and Steve Berger for helping me collect Key deer for necropsy. Thank you for putting up with the fire ants, the late hours, and the stench. I want to thank Kristie Killum for helping me find my natural freshwater sites and Tom Wilmers for sharing his extensive knowledge of Key deer and their habitat with me. I want to thank Steve Nelms for helping me with boat maintenance and Mike Gallagher for teaching me how to use power tools and heavy machinery. Steve and Mike are great friends and mentors and have taught me more about wildlife management than they can possibly realize.

I thank the local residents and business owners in the Lower Florida Keys for their knowledge and guidance. A special thanks to Deer Run Bed & Breakfast, Barnacle Bed & Breakfast, Boy Scouts of America, Little Palm Island Resort & Spa, and the residents of Long Beach Road on Big Pine Key for allowing me to collect samples from your property. Thank you to Big Pine Shipping for going out of your way to help me with my shipping needs.

I want to thank my committee, Michael Yabsley, Joe Corn, Kevin Keel, and Bob Warren, for all of their guidance and support. Special thanks to Joe Corn for visiting me in the Keys and helping me to design my study and collect samples for my project. Also, special thanks to Kevin

Keel for answering my impromptu calls and giving me guidance for tissue collection during my necropsies.

Thank you to all the staff and students at Southeastern Cooperative Wildlife Disease Study and Warnell School of Forestry and Natural Resources for all your support and guidance. A special thanks to Jess Gonyor and Emily Blizzard for your continued support and friendship even though you are miles away. Thank you to Josh Paris and Joe Slusher for helping me with my field gear and sending me supplies during my field season.

I want to thank the personnel at the Johne's Testing Center, especially Elizabeth Manning and Brenna Kunkel, for processing my samples, sending me relevant articles, and for allowing me to visit the testing center and see first hand how samples are processed.

Finally, I want to thank my family and friends for all of their love and support. You have provided me with the strength and encouragement necessary to succeed.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

The Key deer (*Odocoileus virginianus clavium*) is a subspecies of the white-tailed deer that is endemic to approximately 22 islands in the Lower Florida Keys and is currently listed as endangered (U.S. Fish & Wildlife Service, 2012). Approximately 65-75% of the population resides on Big Pine and No Name Keys (Lopez 2001). While the population size has increased over the past five decades from 25-80 deer to approximately 800, the available area of habitat has not increased (Gallant and Soliz 2009). Ecological consequences associated with high population densities, including disease, have become a concern for Key deer management.

One of the diseases emerging in the Key deer population is Johne's disease, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Johne's disease is a progressive wasting disease found mainly in domestic ruminants (White et al. 2010) but has been reported in numerous wild species including bovids, cervids, camelids, and lagomorphs (Williams 2001). Transmission usually occurs through the fecal-oral route and clinical signs can take years to develop. The disease is associated with intestinal lesions, chronic diarrhea, and weight loss, eventually leading to emaciation and death (Williams 2001).

In North America, Johne's disease is rare among free-ranging ruminants but has been reported from Rocky Mountain bighorn sheep (*Ovis canadensis*) and a Rocky Mountain goat (*Oreamnos americanus*) in Colorado (Williams, et al., 1979, Jessup and Williams, 1999), tule elk (*Cervus elaphus nannodes*) (Manning et al., 2003), Key deer, and exotic fallow deer (*Dama*

dama) (Marco et al., 2002) and axis deer (*Axis axis*) (Riemann et al., 1979). Johne's disease was first reported in Florida Key deer in 1996 at a private residence on Big Pine Key (Quist et al. 2002). From 1998 to 2008, eight additional infected Key deer were detected on Big Pine Key and the nearby Newfound Harbor Keys (Pederson et al. 2008). Since 1996, MAP has been found in tissue and/or fecal samples south of U.S. Highway 1 on Big Pine Key and on the Newfound Harbor Keys (Big Munson Island, Little Palm Island, and Hopkins Island). Importantly, MAP has not been discovered north of U.S. 1 on Big Pine Key where the majority of the remaining Key deer population currently resides (Pederson et al. 2008).

Previous studies have detected MAP in Key deer fecal samples and tissues (Pederson et al. 2008), but no surveys have been conducted to determine if the general environment in Key deer habitat is contaminated with MAP. Other studies conducted at MAP-infected domestic ruminant farms and ranches indicate that soil, water, and artificial food and water containers can become contaminated with MAP (Pickup et al. 2006, Florou et al. 2008). For this reason, it is important to consider the environment as a potential vector for transmission of MAP in the Key deer population. Thus, this study was conducted to determine if natural freshwater sources, artificial water sources, and artificial feeding sites are contaminated with MAP. Some sites were selected based on locations of positive Key deer tissue and fecal samples.

Hypothesis: MAP is present in the environment (water and/or soil) south of U.S. 1 on Big Pine Key and on the Newfound Harbor Keys in Monroe County, Florida. This contamination would serve as a continued source of infection for Key deer.

Specific Objectives:

1. To identify freshwater sources, artificial water sources, and artificial feeding sites used by Key deer within the known distribution of MAP-infected Key deer.
2. To determine if MAP is present in identified freshwater sources, artificial water sources, and/or artificial feeding sites used by Key deer.
3. To determine if MAP has spread from the Newfound Harbor Keys and Long Beach Road on Big Pine Key to the main population of Key deer on Big Pine Key north of U.S. 1.

Key deer (*Odocoileus virginianus clavium*)

The Key deer is the smallest subspecies of the white-tailed deer in North America (Lopez 2001). Barbour and Allen (1922) first described the Key deer as a distinct race based on length of row of molariform teeth. Since then, multiple characteristics have been used to separate the Key deer from other white-tailed deer by more than just race. In general, the Key deer is smaller than other eastern white-tailed deer. They are stockier, have shorter legs, and have an average shoulder height of 61-81 cm and average weights of 29 kg for females and 38 kg for males (Lopez 2001). Fawns weigh about 1.5 kg at birth (Hardin et al. 1984). Females usually reach their maximum size at 4-5 years while males usually reach their maximum size after 8 years (Maffei et al. 1988). Key deer skulls are noticeably shorter and relatively wider than other deer, especially in males (Maffei et al. 1988, Klimstra et al. 1991). Their coats are variable in color ranging from a deep reddish-brown to a grizzled gray; the gray deer often appearing older. A distinct black cross or mask is commonly found between the eyes and across the brow line (Klimstra 1992). Bucks typically develop a spike at 2 years, a fork at 3 years, 6 points at 4 years, and 8 points at 5 years. However, data suggest much variation to this pattern (Klimstra et al.

1978, 1980, 1982). Key deer also differ from other white-tailed deer by having a high tolerance to saltwater (Jacobson 1974), low birth rates, low productivity (Folk and Klimstra 1991), more solitary habits, and weak family bonds (Hardin 1974).

The historical range of the Key deer extended from Vaca Key to Key West (Klimstra et al. 1978) prior to the rise in sea level from the retreat of the Wisconsin glacier (Hoffmeister and Muller 1968). Currently, the Key deer is restricted to approximately 22 islands in the Lower Florida Keys ranging from Big Johnson to Sugarloaf Keys within the National Key Deer Refuge and Great White Heron NWR; however, the majority of the Key deer, approximately 65-75%, reside on Big Pine and No Name Keys (Lopez 2001).

Due to uncontrolled hunting and disturbances to their habitat, Key deer numbers reached a low of 25 to 80 individuals in the 1950's (Folk 1991). In 1957, The National Key Deer Refuge was established to provide protection for the deer and their habitat through regulations on hunting, fire management, and habitat conservation (Lopez 2001). A decade later, the Key deer was listed as endangered by the U.S. Fish and Wildlife Service (Folk 1991). By 1974, the Key deer population had grown to an estimated 300-400 individuals (Klimstra et al. 1974). While there were fluctuations in the population during the last 30 years, currently the population is estimated between 500 and 600 Key deer on Big Pine and No Name Keys and between 100-150 deer on the outer islands (Gallant and Soliz, 2009).

Urban Development in the Lower Florida Keys

There is significant concern regarding urban development in the Lower Florida Keys. Road mortality is the most important threat to the Key deer population and currently accounts for over half of all mortality (Galant and Soliz, 2009). Most of these vehicle collisions occur on U.S. 1, the only road connecting the Keys to the mainland (Lopez 2001). To address this cause of mortality, speed limits were reduced, law enforcement was increased, and Key deer crossing signs, flashing lights, and no-passing zones were added on Big Pine and No Name Keys. These interventions have only had limited success in decreasing mortality (Lopez 2003).

The National Key Deer Refuge property is interspersed between private and public ownership parcels. This integration of property creates serious problems for refuge management especially concerning automobile traffic, accessibility for management activities, and increased human interaction with Key deer (Folk 1991). Developed properties place constraints on deer movement through yard fencing and human interaction which can lead to isolated groups of the population (Folk 1991).

Urban development has specifically posed a threat to the southern population of Big Pine Key. A source-sink system seems to exist between the deer that reside on the northern side of U.S. 1 and those deer on the southern side of the highway. Haverson et al. (2004) reported that the deer south of U.S. 1 are an absolute sink, requiring immigration from deer north of U.S. 1 to prevent extinction. Haverson et al. (2004) also found that despite deer emigrating from the north side of U.S. 1 to the south, the population north of U.S. 1 is still increasing, indicating a source.

Urbanization and Artificial Feeding of Key deer

Feeding of Key deer is prohibited by state [F. A. C. 39-27.002 (5)] and federal (16 U. S. C. 1531) laws. However, Folk and Klimstra (1991) reported that some Key deer have become reliant on food and water sources provided by residents and tourists. While some deer in developed areas still predominantly forage for native flora in vacant lots and abutting refuge property, others concentrate on artificial plantings and garbage (Folk and Klimstra 1991). These behaviors are still present in the Key deer population (personal observation, 2010-2011). During the course of this study, I regularly saw group of Key deer overturning garbage cans and feeding on the contents. I also observed Key deer climbing steps and waiting at front doors in multiple neighborhoods on Big Pine Key. I also saw local residents throwing dinner scraps, such as watermelon rinds and bread, on their lawns in the evenings (personal observation, 2010-2011).

A reduction in Key deer home range size has been observed in developed areas (Folk and Klimstra 1991). In one study area on Big Pine Key, Folk and Klimstra (1991) found deer density to be 20x what was considered typical in the early 1970's (1 deer/12 ha; Silvy 1975). Hardin et al. (1976) found that during the rutting season males were never found together without aggression between them or a female present. Interestingly, Folk and Klimstra (1991) witnessed four bucks during rutting season feeding in the same area with no sign of aggression. Females have also become more tolerant of each other because in the late 1970's does required dominance-submission displays when meeting an unknown deer, whereas females in developed areas in the early 1990's showed little evidence of a matriarchal hierarchy and moved about randomly (Folk and Klimstra 1991). In one extreme instance of tolerance, Key deer were seen foraging on commercial feed provided on a concrete patio where they stood next to each other in a long line, often touching. These deer were described as moving like cattle, walking with heads down without any specific leader (Folk and Klimstra 1991).

Urbanization has apparently also resulted in a loss of flight response by Key deer. Loud noises such as barking dogs, passing vehicles, circular saws, leaf blowers, and wood chippers provoked no more than a passing glance from the deer in Folk and Klimstra's study (1991). Deer were regularly observed in open areas bedded down within 2 meters of the road, undisturbed by motorists, pedestrians, or cyclists. Some deer were even tolerant of being petted (Folk and Klimstra 1991). There is a strong concern that the loss of alarm and flight response will make these deer more susceptible to harassment from neighborhood dogs and the public.

For example, there was an instance in the early 1990's on No Name Key where a Key deer was beaten to death by a person using a baseball bat (Folk and Klimstra 1991).

Water Availability

Freshwater is considered a limiting factor for Key deer (Hardin, 1974). Basins and holes that occur naturally in rocky pinelands provide the most common natural water sources. Active alligator holes are also a significant source for permanent freshwater, especially in times of drought. During the wet season, there are usually ample freshwater sources throughout the Key deer range, which allows them to expand their range to neighboring islands. However, in the dry season, water availability decreases and the salinity levels rise which results in a reduction of their range (Folk, 1991). During the past 30 years, urban development has provided an additional source of freshwater (e.g., bird baths, pet dishes, ornamental ponds, and water provided by residents specifically for the deer) (Peterson et al. 2004).

The salinity level of a water source is an important factor when determining its value for wildlife. Based on previous radio-tracking studies, the distribution of Key deer is directly affected by freshwater availability (Folk, 1991). Key deer depend on the permanent freshwater sources available on Big Pine Key because many of the water sources on surrounding Keys dry up, especially in times of drought (Klimstra, 1973) and in the months following hurricane season (Lopez, 1990). When freshwater is not available or rare, Key deer have been observed drinking water with salinity levels as high as 14 ppt and tracks leading to water with even higher salinity levels have been recorded (Folk, 1991).

Mycobacterium avium* subspecies *paratuberculosis

Mycobacterium avium subspecies *paraturberculosis (*MAP*)* is a small (0.5 x 1.5 micron), Gram-positive, facultative, acid-fast bacillus (Chiodini et al., 1984). MAP is the causative agent for Johne's disease or paratuberculosis, a progressive wasting disease of ruminants. The distribution of MAP is worldwide and it is an economically important pathogen of cattle, sheep, and goats (White et al., 2010). In addition, many other ruminants and non-ruminant species, including zoological specimens and wildlife, are susceptible (Davidson et al., 2004). Infection with detection of shedding has been reported in white-tailed deer (*Odocoileus virginianus*) (Chiodini and Kruiningen, 1983), tule elk (*Cervus elaphus nannodes*) (Jessup et al., 1981), big horn sheep (*Ovis Canadensis*) (Williams, 1983), rabbits (*Oryctolagus cuniculus*) (Greig et al., 1999), red fox (*Vulpes vulpes*), stoat (*Mustela erminea*) (Beard et al., 2001), Key deer (Quist et al., 2002), raccoons (*Procyon lotor*), opossum (*Didelphis virginiana*), armadillo (*Dasypus novemcinctus*) (Corn et al., 2005), and feral cats (*Felis catus*) (Palmer et al., 2005).

MAP infects cells in the lower portion of the small intestine and results in granulomatous inflammation and thickening of the intestinal wall. This thickening interferes with the proper function of the intestines causing diarrhea and poor nutrient absorption (Chiodini et al., 1984). Infected animals can become emaciated even though they are eating normally. Clinical signs, including diarrhea and emaciation, usually take months to develop after initial infection (Williams, 2001). Because of the slow growth of MAP, signs are not often seen until individuals become adults (Chiodini et al., 1984). Since diarrhea and emaciation are common signs for other wildlife diseases, laboratory tests are necessary to confirm MAP as the causative agent of disease.

The main source of transmission is the fecal-oral route. Clinically ill animals can shed up to 5 x 10^{12} bacilli daily (Dukes, 1942). Ingestion of MAP most commonly occurs when young animals nurse from teats that have come into contact with contaminated feces. MAP bacteria have also been detected in the testes (Tunkl and Aleraj, 1965), semen, bulbourethral gland, prostate, seminal vesicles (Larsen and Kopecky, 1970), mammary gland (Doyle, 1954), uterus, and fetuses (Pearson and McCelland, 1955). Since MAP has been recovered from semen and the male reproductive tract, there is concern that venereal transmission can occur. However, numbers of bacilli in semen is very low (Merkal et al., 1982). In addition, during experimental studies, MAP placed in the uterus is eliminated within 3 weeks and when MAP is placed in the uterus after implantation of the zygote, the bacilli remain viable but do not multiply or infect the fetus (Merkal et al., 1982). Studies have also found that as many as 7% of clinically infected cows excrete MAP in milk (Hole, 1958). MAP has also been isolated from as many as 85% of fetuses from terminally infected animals (Doyle, 1954). However, intrauterine transmission does not seem to be a significant cause of natural infection as 75% of infected calves in one study were born to noninfected dams (Merkal et al., 1975). When the bacillus is passed to the fetus, the organism is found in the visceral organs, not in the gastrointestinal tract (Lawrence, 1956). Development of disease from intrauterine transmission has never been documented. Overall, these extraintestinal infections likely do not contribute to the spread of the bacteria (Chiodini et al., 1984).

MAP is an environmentally resistant organism and can remain viable for 163 days in river water (Hole, 1958), 270 days in pond water (Jorgensen, 1977), 11 months in bovine feces and black soil (Larsen et al., 1956), 17-19 months in a saline environment, 24 weeks on grass in complete shade, 9 weeks on grass in 70% shade (Whittington et al., 2004), but only 7 days in urine (Lovell et al., 1944). MAP has also been shown to survive freezing at -14 ºC for at least a year (Richards and Thoen, 1977). However, the bacterium is not able to grow and multiply in the environment due to a lack of sufficient mycobactin, a protein required to transport iron (Chiodini et al., 1984). Any paratuberculosis bacilli found in the environment are considered dormant. Previously, MAP has been detected in puddles, troughs, streams, ponds, water tanks, and hose and pipe biofilms from farms and ranches with infected domestic animals (Pickup et al., 2006).

Johne's disease in the Lower Florida Keys

Johne's disease was first documented in the Florida Keys in 1996, when it was diagnosed in an emaciated Key deer doe at a private residence on Long Beach Road on Big Pine Key. The 2.5-year-old deer was found in a residential area and belonged to a herd that was frequently fed by locals and tourists (Quist et al., 2002). The initial diagnosis was based on an acid-fast staining of the bacteria from a sample of the intestine preserved in formalin. The results were confirmed by PCR (Quist et al., 2002). This result was surprising as MAP infection of free-ranging white-tailed deer is rare; most cases of MAP detected in non-domestic ruminants have occurred in captive herds. Cattle and sheep are not present on Big Pine Key; however, according to National Key Deer Refuge staff, there are domestic goats present between U.S. 1 and Watson Boulevard on Big Pine Key. A second confirmed case was detected two years later at the same residence (Quist et al. 2002). Between 2003 and 2004, five additional MAP-positive Key deer were detected on Big Pine Key and neighboring islands. Between 2005 and 2006, a large ecological study of the distribution of MAP and other possible hosts was conducted by Pederson et al. (2008). Tissue and/or fecal samples were collected from Key deer, 30 raccoons, 3 feral cats, 2 Silver rice rats (*Oryzomys argentatus*), an opossum, and a Lower Keys marsh rabbit

(*Sylvilagus palustris hefneri*) for MAP culture. Positives were only detected in tissue samples from two clinically ill Key deer, 23 Key deer fecal samples collected from the ground, and the mesenteric lymph node of a single raccoon that was captured on Big Munson Island (Pederson et al. 2008). Collectively, these studies indicate that MAP is found south of U.S. 1 on Big Pine Key and on Big Munson and Little Palm Islands.

Thesis Format

My thesis is presented in manuscript format. Chapter 1 is an introduction and a literature review of previous studies addressing similar research topics. Chapter 2 is the manuscript chapter that will be submitted to a peer-reviewed scientific journal for publication. It describes the evaluation of environmental contamination of endangered Florida Key deer habitat in the Lower Florida Keys with *Mycobacterium avium* subspecies *paratuberculosis*. Chapter 3 presents conclusions and the management implications of the findings of my study.

Literature Cited

Barbour, T. and G. M. Allen. 1922. The White-Tailed Deer of Eastern United States. Journal of Mammalogy 3: 65-78.

Beard, P. M., M. J. Daniels, D. Henderson, A. Pirie, K. Rudge, D. Buxton, S. Rhind, A. Greig, M. R. Hutchings, I. McKendrick, K. Stevenson, and J. M. Sharp. 2001. Paratuberculosis infection of nonruminant wildlife in Scotland. Journal of Clinical Microbiology 39: 1517-1521.

Chiodini, R. J., and H. J. Van Kruiningen. 1983. Eastern white-tailed deer as a reservoir of ruminant paratuberculosis. Journal of American Veterinary Medical Association 182: 168-169.

Chiodini, R. J., H. J. Van Kruiningen, and R. Merkal. 1984. Ruminant paratuberculosis (Johne's disease): The current status and future prospects. The Cornell Veterinarian 74: 218-262.

Corn, J. L., E. J. B. Manning, S. Sreevatsan, and J. R. Fischer. 2005. Isolation of *Mycobacterium avium* subspecies *paratuberculosis* from free-ranging birds and mammals on livestock premises. Applied and Environmental Microbiology 71: 6963-6967.

Davidson, W. R., E. J. B. Manning, and V. F. Nettles. 2004. Culture and serologic survey for *Mycobacterium avium* subsp. *paratuberculosis* infection among southeastern white-tailed deer (*Odocoileus virginianus*). Journal of Wildlife Diseases 40: 301-306.

Doyle, T. M. 1954. Isolation of Johne's bacilli from the udders of clinically infected cows. British Veterinary Journal 110: 215-218.

Dukes, H. H. 1942. *The Physiology of Domestic Animals*. Comstock Publishing Co., Ithaca, NY

Folk, M. L. 1991. Habitat of the Key deer. Dissertation, Southern Illinois University, Carbondale, Illinois, USA.

Folk, M. J. and W. D. Klimstra. 1991. Urbanization and domestication of the Key deer (*Odocoileus virginianus clavium*). Florida Field Naturalist 19:1-32

Galant, D.L. and L. Soliz. 2009. Population ecology of Florida Key deer on outer keys. Final Report to U.S. Fish and Wildlife Service, Big Pine Key, Florida, USA.

Greig, A., K. Stevenson, D. Henderson, V. Perez, V. Hughes, I. Pavlik, E. Hines 2nd, I. McKendrick, and J. M. Sharp. 1999. Epidemiological study of paratuberculosis in wild rabbits in Scotland. Journal of Clinical Microbiology 37: 1746-1751.

Hardin, J. W. 1974. Behavior, socio-biology, and reproductive life history of the Florida Key deer, *Odocoileus virginianus clavium.* Dissertation, Southern Illinois University, Carbondale, USA.

Hardin, J. W., W. D. Klimstra, and N. J. Silvy. 1984. Florida Keys. Pages 381-390 *in* L.K. Halls, ed. White-tailed deer: ecology and management. Stackpole Books; Harrisburg, Pennsylvania.

Haverson, P. M., R. R. Lopez, N. J. Silvy, and P. A. Frank. 2004. Source-sink dynamics of Florida Key deer on Big Pine Key, Florida. Journal of Wildlife Management 68: 909-915

Hoffmeister, J. E., and H. G. Muller. 1968. Geology and origin of the Florida Keys. Geological Society of America Bulletin 79: 1487-1502.

Hole, N. H. 1958. Johne's disease. Advances in Veterinary Science 4: 341-387.

Jacobson, B. N. 1974. Effect of drinking-water on habitat utilization by Key deer. Thesis. Department of Zoology, Southern Illinois University at Carbondale.

Jessup, D. A., B. Abbas, D. Behymer, and P. Gogan. 1981. Paratuberculosis in tule elk in California. Journal of the American Veterinary Medical Association. 179: 1252-1254.

Jessup, D. A. and E. S. Williams. 1999. Paratuberculosis in free-ranging wildlife in North America. Zoo and Wild Animal Medicine, 4th edition. W. B. Saunders, Philadelphia, PA p.616-620

Jorgensen, J. B. 1977. Survival of *Mycobacterium paratuberculosis* in slurry. Nordisk Veterinaer Medicin 29: 267-270.

Klimstra, W. D., J. W. Hardin, N.J. Silvy, B. N. Jacobson, and V. A. Terpening. 1974. Key deer investigation final report: December 1967-June 1973. U.S. Fish and Wildlife Service, Big Pine Key, Florida, USA.

Klimstra, W. D., J. W. Hardin, and N. J. Silvy. 1978. Endangered Key deer. Pg 15-17 in J.N. Layne, ed., Rare and endangered biota of Florida. Vol. 1. Mammals. University Presses of Florida, Gainesville.

Klimstra, W. D., J. W. Hardin, M. P. Carpenter, and S. Jenkasky. 1980. Key deer recovery plan. U.S. Fish and Wildlife Service.

Klimstra, W. D., N. J. Silvy, and J. W. Hardin. 1982. The Key deer: Its status and prospects for the future. Proceedings of the Nongame and Endangered Wildlife Symposium. Georgia Department of Natural Resources. Technical Bulletin WL5:137-141.

Klimstra, W. D. and A. L. Dooley. 1990. Foods of the Key deer. Florida Scientist 53: 264-273

Klimstra, W. D., M. J. Folk, and R. W. Ellis. 1991. Skull size of two insular and one mainland subspecies of *Odocoileus virginianus* from the southeast. Transactions Illinois State Academy of Science 84: 185-191.

Larsen, A. B. and K. E. Kopecky. 1970. *Mycobacterium paratuberculosis* in reproductive organs and semen of bulls. American Journal of Veterinary Research 31: 255-257.

Larsen, A. B., R. S. Merkal and T. H. Vardaman. 1956. Survival time of *Mycobacterium paratuberculosis.* American Journal of Veterinary Research 17: 549-551.

Lawrence, W. E. 1956. Congenital infection with *Mycobacterium johnei* in cattle. Veterinary Record 68: 312-314.

Lopez, R. R. 2001. Population ecology of Florida Key deer. Dissertation, Texas A&M University, College Station, Texas, USA.

Lopez, R. R., M. E. P. Vieira, N. J. Silvy, P. A. Frank, S. W. Whisenant, and D. A. Jones. 2003. Survival, mortality, and life expectancy of Florida Key deer. Journal of Wildlife Management 67: 34-45.

Lovell, R., M. Levi and J. Francis. 1944. Studies on the survival of Johne's bacilli. Journal of Comparative Pathology 54: 120-129.

Maffei, M. D., W. D. Klimstra, and T. J. Wilmers. 1988. Cranial and mandibular characteristics of Key deer (*Odocoileus virginianus clavium*) Journal of Mammalogy 69: 403-407.

Merkal, R. S., A. B. Larsen and G. D. Booth. 1975. Analysis of the effects of inapparent bovine paratuberculosis. American Journal of Veterinary Research 36: 837-838.

Merkal, R. S., J. M. Miller, A. M. Hintz and J. H. Bryner. 1982. Intrauterine inoculation of *Mycobacterium paratuberculosis* into guinea pigs and cattle. American Journal of Veterinary Research 43: 676-678.

Palmer, M. V., W. C. Stoffregen, J. G. Carpenter, and J. R. Stabel. 2005. Isolation of *Mycobacterium avium* subsp *paratuberculosis* (Map) from feral cats on a dairy farm with Map-infected cattle. Journal of Wildlife Diseases 41: 629-635.

Pearson, J. K. L. and T. G. McCelland. 1955. Uterine infection and congenital Johne's disease in cattle. Veterinary Record 67: 615-616.

Pedersen, K., E. J. B. Manning, and J. L. Corn. 2008. Distribution of *Mycobacterium avium* subspecies *paratuberculosis* in the Lower Florida Keys. Journal of Wildlife Diseases 44: 578-584.

Peterson, M. N., R. R. Lopez, P. A. Frank, B. A. Porter, and N. J. Silvy. 2004. Key deer fawn response to urbanization: is sustainable development possible? Wildlife Society Bulletin 32:493-499

Pickup, R. W., G. Rhodes, T. J. Bull, S. Arnott, K. Sidi-Boumedine, M. Hurley, and J. Hermon-Taylor. 2006. *Mycobacterium avium* subsp. *paratuberculosis* in lake catchments, in river water abstracted for domestic use, and in effluent from domestic sewage treatment works: diverse opportunities for environmental cycling and human exposure. Applied and Environmental Microbiology 72: 4067-4077.

Quist, C. F., V. F. Nettles, E. J. B. Manning, D. G. Hall, J. K. Gaydos, T. J. Wilmers, and R. R. Lopez. 2002. Paratuberculosis in Key deer (*Odocoileus virginianus clavium*). Journal of Wildlife Diseases 38: 729-737.

Richards, W. D. and C. O. Thoen. 1977. Effect of freezing on the viability of *Mycobacterium paratuberculosis* in bovine feces. Journal of Clinical Microbiology 6: 392-395.

Silvy, N. J. 1975. Population density, movements, and habitat utilization of Key deer (*Odocoileus virginianus clavium*). Dissertation. Department of Zoology, Southern Illinois University at Carbondale.

Tunkl, B. and Z. Aleraj. 1965. The recovery of *M. johnei* in the semen of bull from one station for artificial insemination. Veterinarski glasnik 19: 845-849.

U.S. Fish & Wildlife Service. 2012. Key deer (Odocoileus virginianus clavium): Species Profile. http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?spcode=A003

White, C. I., R. J. Birtles, P. Wigley, and P. H. Jones. 2010. *Mycobacterium avium* subspecies *paratuberculosis* in free-living amoebae isolated from fields not used for grazing. Veterinary Record 166: 401-402.

Whittington, R., D. J. Marshall, P. J. Nicholls, I. B. Marsh, and L. A. Reddacliff. 2004. Survival and dormancy of *Mycobacterium avium* subsp. *paratuberculosis* in the environment. Applied and Environmental Microbiology 70: 2989-3004.

Williams, E. S. 1983. Experimental infection of some North American wild ruminants and domestic sheep with *Mycobacterium paratuberculosis*: Clinical and bacteriological findings. Journal of Wildlife Diseases 19: 185-191.

Williams, E. S. 2001. Paratuberculosis and other mycobacterial diseases. Infectious diseases of wild mammals. 3rd Edition. E. S. Williams and I. K. Bakers (eds.). Iowa State University Press, Ames, Iowa, pp. 361-371.

CHAPTER 2

POTENTIAL CONTAMINATION OF ENDANGERED FLORIDA KEY DEER HABITAT WITH *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*[1]

[1]Murray, Heidi L., Michael J. Yabsley, M. Kevin Keel, Elizabeth J. B. Manning, Thomas J. Wilmers, and Joseph L. Corn. To be submitted to Journal of Wildlife Diseases.

Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease, a progressive wasting disease of domestic animals that has also been found in wild ruminants. Johne's disease was first reported in the endangered Key deer (*Odocoileus virginianus clavium*) in 1996 on Big Pine Key, Florida. By 2008, eight additional MAP-positive Key deer had been identified on Big Pine Key and the nearby Newfound Harbor Keys. Our study was conducted to determine the current distribution of MAP in Key deer and to determine if natural or man-made freshwater sources on Big Pine Key and the Newfound Harbor Keys are contaminated with MAP. Between November 2009 and July 2011, a total of 249 fecal samples, tissue and fecal samples from 43 necropsied Key deer, and 675 environmental samples (soil, sediment, and water) were collected and cultured for MAP. MAP was isolated from three of 43 (7%) sampled Key deer, all from Little Palm Island. Pooled tissue samples from the ileum, cecum, and ileocecal lymph node from two deer were MAP-positive; feces from one was also culture positive. A third deer was determined MAP-positive with PCR. However, all fecal and environmental samples tested were negative for MAP. Results of this study indicate that Johne's disease was restricted to Key deer from Little Palm Island during this sampling period and that MAP transmission to Key deer through environmental contamination was not occurring or occurred at a very low rate. Negative results for environmental samples and an apparent decreased range of MAP on Big Pine Key and the Newfound Harbor Keys are both positive signs that current management plans may be working to decrease the incidence of MAP infection in Key deer.

INDEX WORDS: *Mycobacterium avium* subspecies *paratuberculosis*, Johne's disease, Key deer, *Odocoileus virginianus clavium*, environmental contamination

Introduction

The Key deer (*Odocoileus virginianus clavium*) is a subspecies of the white-tailed deer that is endemic to approximately 22 islands in the Lower Florida Keys and is currently listed as endangered (U.S. Fish & Wildlife Service, 2012). Approximately 65-75% of the population resides on Big Pine and No Name Keys (Lopez, 2001). While the population size has increased over the past five decades from 25-80 deer to approximately 800, the amount of habitat has not increased (Gallant and Soliz, 2009). Ecological consequences associated with high population densities, including disease, have become a concern for Key deer management.

Johne's disease was first reported in Florida Key deer in 1996 at a private residence on Long Beach Road on Big Pine Key (Quist et al., 2002). From 1998 to 2008, eight additional infected Key deer were detected on Big Pine Key and the nearby Newfound Harbor Keys (Pederson et al., 2008). Since 1996, MAP has been found in tissue and/or fecal samples south of U.S. 1 on Big Pine Key and the Newfound Harbor Keys (Big Munson Island, Little Palm Island, and Hopkins Island). Importantly, MAP has not been found north of U.S. 1 on Big Pine Key where the majority of the remaining Key deer population currently resides (Pederson et al., 2008).

Johne's disease is a progressive wasting disease found mainly in domestic ruminants (White et al., 2010). Transmission usually occurs through the fecal-oral route and clinical signs can take years to develop. The disease is associated with intestinal lesions, chronic diarrhea, and weight loss, eventually leading to emaciation and death (Williams, 2001). In North America, Johne's disease is rare among free-ranging ruminants but has been reported from Rocky Mountain bighorn sheep (*Ovis canadensis*) and a Rocky Mountain goat (*Oreamnos americanus*) in Colorado (Jessup and Williams, 1999, Williams, et al., 1979), tule elk (*Cervus elaphus* *nannodes*) (Manning et al., 2003), Key deer, and exotic fallow deer (*Dama dama*) (Marco et al., 2002) and axis deer (*Axis axis*) (Riemann et al., 1979).

Previous studies have detected MAP in Key deer fecal samples and tissues (Pederson et al. 2008), but no surveys have been conducted to determine if environmental contamination is occurring in Key deer habitat. Studies conducted at farms and ranches with MAP-infected domestic animals indicate that soil, water, and food and water containers can become contaminated with MAP (Florou et al. 2008, Pickup et al. 2006).

This study was conducted to determine if natural freshwater sources, artificial water sources, and artificial feeding sites are contaminated with MAP and thus part of the transmission cycle. We hypothesized that MAP is present in the environment south of U.S. 1 on Big Pine Key and on the Newfound Harbor Keys in Monroe County, Florida. Our specific objectives were to (1) identify freshwater sources, artificial water sources, and artificial feeding sites used by Key deer within the known distribution of MAP-infected Key deer, (2) determine if MAP is present in identified freshwater sources, artificial water sources, and/or artificial feeding sites, and (3) determine if MAP has spread from the Newfound Harbor Keys and Long Beach Road on Big Pine Key to the main population of Key deer on Big Pine Key north of U.S. 1.

Methods

Study Area

This study was conducted in the Lower Florida Keys, the southernmost section of the continuous chain of islands extending southwest off the southern tip of the Florida peninsula. U.S. Highway 1 connects the various Keys, running from Florida City on the mainland to Key West. Based on orientation, surface limestones, and a seven-mile wide channel, the Lower Keys are naturally separated from the Upper and Middle Keys. The Lower Florida Keys are subtropical and have flora that is Caribbean in origin but fauna mainly from North America (Folk 1991). Samples were collected on Big Pine Key and the Newfound Harbor Keys, including Little Palm Island and Big Munson Island, in Monroe County, Florida (Figure 1).

Environmental Samples

We identified 22 natural and artificial watering sites south of U.S. 1 along Long Beach Road on Big Pine Key and on Big Munson Island and Little Palm Island. These sites were sampled during five time periods between July 2010 and June 2011 (Figure 1): July -September 2010, October - December 2010, January - February 2011, March - April 2011, and May - June 2011. Sampling sites for the natural freshwater sources were along Long Beach Road and on Big Munson Island where MAP has been reported previously in Key deer and from Key deer feces (Quist et al., 2002, Pederson et al., 2008). Fifteen natural freshwater sites (Figure 1) (Sites 2, 3, 4, 42.1, 42.2, 44.1, 44.2, 45, 49, 50, 51, 52.1, 52.2, 78, and 98, Table 1) were sampled one or more times. The artificial freshwater and artificial feeding sites sampled included a pond, bathtub, barrel, bird bath, lawn fountain, and trough on Long Beach Road and a garden fountain on Little Palm Island (Figure 1, Table 1).

Natural water sources were selected based on (1) proximity to where MAP was detected in Key deer tissues and fecal samples in previous studies, (2) salinity level at the surface of the water, and (3) evidence of current Key deer use of the water source. Water sources closest to Little Palm Island were given the highest priority since Key deer tissues and fecal pellets previously collected from this island had the highest percentage of MAP-positive samples (Pederson et al., 2008). Various environmental factors (e.g., evaporation, rain events, tides, moon phase, and mosquito ditches), caused surface water salinity levels to change frequently. Water sites with low salinity levels and evidence of recent Key deer activity took precedence.

Sites were sampled with salinity levels as high as 32 ppt (the low range of ocean water). Key deer have been observed drinking from natural water sites with salinity as high as 14 ppt but their salt tolerance is not fully understood (Klimstra et al., 1974; Folk, 1991). Key deer activity was indicated by presence or absence of fresh tracks leading up to the water source, fresh fecal pellets present in the area, and/or seeing Key deer drinking from the site.

As the sampling periods progressed, some of the water sources from the initial set of sites dried up (sites 2, 3, 4, 44.1, 44.2, 45, 49, 50, 51, 78, and 98) or were found to be inappropriate for sampling (no deer activity observed and a salinity of 34 ppt at the pond on Long Beach Road). These sites were replaced in our sampling by natural water sources found further from Little Palm Island (sites 42.1, 42.2, 52.1, and 52.2) and artificial water sources were sampled more frequently (Table 1). The sites from the initial set of sampling sites that dried up were last sampled in January 2011 during the third sampling period. These sites dried up during February and lacked water and evidence of Key deer activity in March-April during the fourth sampling period.

At each natural and artificial water source, a total of nine environmental samples was collected (3 water, 3 sediment, and 3 soil). At each natural and artificial water source, the water sample was collected first by skimming a 50-ml tube over the surface of the water (the tube was never completely submerged). Next, a substrate sample was collected by submerging a 50-ml tube which was skimmed at the bottom of the water source over the surface substrate (silt, mud, sand, leaf litter, and/or algae depending on the site) to collect bacteria that may have settled to the bottom. Approximately 30g of soil was collected within 15cm of the water source. Water, sediment, and soil samples were placed on ice packs and shipped overnight to the Johne’s Testing Center for MAP culture.

Fecal Samples

Key deer fecal samples were collected from the ground intermittently between August 2010 and July 2011. Each sample included approximately 30g of pellets and consisted of the freshest pellets in the group. Fecal pellets were determined to be fresh (dropped within 48 hours) if they were dark brown and pliable. Light brown and dry or partially dry pellets were not collected. Fecal pellets were collected south of U.S. 1 on Big Pine Key, Big Munson Island, and Little Palm Island to determine if MAP was still present in the Key deer herd. Fecal pellets were also collected between U.S. 1 and Watson Boulevard on Big Pine Key to determine if MAP had spread north of U.S. 1. In Pederson et al. (2008), they found 21 positive Key deer fecal pellets out of 500 samples within the study area. Based on these findings, we expected approximately ten positives out of the allocated 249 fecal samples. Samples were placed in Whirl-Paks and shipped overnight on ice packs to the Johne's Testing Center for MAP culture.

Key Deer Necropsies

Key deer killed by cars or injured by cars and subsequently euthanized by National Key Deer Refuge (NKDR) staff were necropsied. Only adult deer were selected for MAP sampling since the bacterium is slow-growing and difficult to detect in fawns or yearlings (Chiodini et al., 1984). Two samples, a pooled tissue sample and sample of feces from the colon were collected. If gross lesions or abnormalities were noted, additional samples were collected for diagnostic purposes. The pooled tissue sample was comprised of the ileocecal lymph node, a 3x3cm section of the cecum, and a 5cm section from the ileum. The tissue and fecal samples were placed in Whirl-Paks and shipped overnight with ice packs to the Johne's Testing Center for MAP culture. Additional tissues collected for diagnostic purposes were fixed in 10% buffered formalin or were kept on ice packs and shipped overnight to the Southeastern Cooperative Wildlife Disease Study (SCWDS; Athens, GA) for evaluation.

Laboratory Methods

MAP culture and isolate identification was conducted by the Johne's Information Center (Madison, Wisconsin, USA). Isolation of mycobacteria, for both Key deer tissue and fecal samples and environmental samples, was performed using the radiometric method of detection (Collins et al., 1990). Samples were processed by taking 0.8-1.0g of the solid, placing it in a decontamination mixture, and incubating overnight at 37C to remove fast-growing bacteria and fungi. Liquid samples were centrifuged prior to decontamination to create a solid. After decontamination, samples were placed in BACTEC incubation bottles (Bectin Dickerson, Sparks, Maryland) and monitored weekly for ^{14}C release. Positives were confirmed with multiplex PCR (Johne's Testing Center, Madison, WI). Histopathology was conducted at the Southeastern Cooperative Wildlife Disease Study (Athens, Georgia, USA).

Results

Environmental and Fecal Samples

A total of 675 environmental samples was collected and cultured for MAP (Table 1). All 675 samples were negative for MAP but one sample, from natural water site 44.1, was positive for *Mycobacterium intracellulare*. All 249 fecal samples collected for MAP culture north of U.S. 1 on Big Pine Key (n=59), south of U.S. 1 on Big Pine Key (n=147), Big Munson Island (n=21), and Little Palm Island (n=22) were negative (Figure 2).

Key deer Necropsies

We necropsied and tested 43 Key deer for MAP (Figure 3); three were confirmed positive for MAP. One deer was an adult, lactating female that was found dead on Little Palm Island in

July 2010. At necropsy, lesions were observed on her lungs and liver. Fresh samples of lung and liver were sent to SCWDS where *Arcanobacterium pyogenes* and *Staphylococcus* sp. were cultured from the liver. MAP was isolated from the pooled tissue sample; the fecal sample was negative for MAP. The second positive deer was a 1.5-year-old female from Little Palm Island. This deer was emaciated and easily approached by refuge staff prior to being euthanized in June 2011. The intestines were unusually dark and the ileocecal lymph node was enlarged (approximately 2.5cm in diameter). When cultured, both the fecal and pooled tissue samples were positive for MAP. The third positive deer was removed from Little Palm Island by NKDR staff in December 2010 and euthanized due to poor condition. This deer was frozen and sent to SCWDS for necropsy in July 2011. The presence of MAP was confirmed by PCR.

Discussion

The current study confirmed that MAP is still present in the Key deer population in the Lower Florida Keys. However, in contrast to previous reports of MAP from Key deer south of U.S. 1 on Big Pine Key (Long Beach Road) and on the Newfound Harbor Keys (Quist et al., 2002, Pederson et al., 2008), we only found MAP-positive Key deer on the most distal Newfound Harbor Key, Little Palm Island. All environmental and fecal samples collected during this study were negative for MAP. These data suggest that environmental contamination with MAP is insignificant or MAP does not survive in the environmental conditions present.

The three MAP-positive deer were from Little Palm Island which currently has a small (10-20) population of Key deer it shares with Big Munson Island (personal observation). Surprisingly, 22 fecal samples from Little Palm Island were negative for MAP. It is possible that fecal samples collected were not from any of the three positive deer or that MAP-positive deer were not shedding at the time of sampling. In clinical cases, cattle shed over 10^{10} organisms/g of feces (Stabel, 1998) and naturally infected white-tailed deer can shed 10^8 organisms/g of feces (Palmer et al., 2007). We know that shedding is intermittent (Stabel, 1998) and only one of the two Key deer that were necropsied and cultured for MAP had a positive fecal sample. This intermittent shedding of lower numbers of bacteria by infected deer may explain the lack of positive fecal samples.

In contrast to previous studies, all samples collected on Big Pine Key (n=206) were negative for MAP, even those collected south of U.S. 1 (n=147). Continued sampling and monitoring are needed to determine if MAP remains at a low prevalence south of U.S. 1 and if it has spread north of U.S. 1. Importantly, previous telemetry studies have shown that Key deer, males in particular, travel from the Newfound Harbor Keys, including Little Palm Island, to Big Pine Key (personal observation). This movement represents a risk of potential spread of MAP from Little Palm Island to Big Pine Key where a majority of the Key deer population resides. If MAP were to spread into the Big Pine Key population north of U.S. 1, higher herd density will make it difficult to manage. The main population of Key deer on Big Pine Key, north of U.S. 1, has already reached carrying capacity as suggested by increased abomassal parasite counts (Nettles et al., 2002). A study on tule elk (*Cervus elaphus nannodes*) in California found that despite high prevalence of MAP in the herd, individuals maintained good nutritional condition (Manning et al., 2003). At the time of the study, the herd had been infected with MAP for two decades with low MAP-related mortality. It was suggested that as more calves are born to infected dams and as the environment becomes more heavily burdened from shedding, the presence of the bacterium may be more deleterious. Additionally, clinical disease may increase as the population reaches carrying capacity and stressors of reduced forage and increased disease transmission occur (Manning et al., 2003).

All environmental samples were negative for MAP. This was in contrast with previous studies where environmental sampling at MAP-positive farms and ranches detected the bacterium in puddles, troughs, streams, ponds, water tanks, and hose and pipe biofilms (Pickup et al., 2006). These negative results are likely due to an absence or low incidence of infected Key deer shedding MAP into the environment. Particularly since all fecal samples and necropsies from Big Pine Key were negative for MAP. MAP is rare in wild ruminants in North America and white-tailed deer specifically do not appear to act as a reservoir for the bacterium (Davidson et al., 2004). Additionally, the prevalence of MAP in Key deer is much lower than among cattle on MAP-positive farms (Stabel, 1998, Palmer et al., 2007). Finally, the Florida Keys are a harsh environment, even for an environmentally persistent bacterium such as MAP. The air is saturated with salt and the temperatures are tropical near year round. In addition to occasional stochastic events, such as hurricanes and tropical storms, high tides during the summer months flush some natural water holes daily, especially those connected to mosquito ditches, thereby potentially removing or diluting bacteria at the site (Folk et al., 1991). Treatment of artificial water sources by private landowners is unlikely to decrease the presence of the bacterium. MAP is resistant to chlorine and disinfection is believed to select for MAP growth (Pierce, 2009).

Johne's disease is just one of several threats to the Key deer, which was listed as federally endangered in 1967 due to over-hunting and human disturbance to habitat. The Key deer remains listed due to high human-related mortality and continued habitat loss (Lopez et al., 2003). Currently, vehicle strikes are the primary cause of mortality but habitat degradation and illegal feeding are also important management issues for Key deer. If not properly managed, Johne's disease could become an additional factor impacting the Key deer population (Nettles et al., 2002). This is the first study to sample the environment for the detection of MAP within the Key deer range. Negative results for environmental samples and an apparent decreased range of MAP are both positive signs that current management efforts to decrease illegal feeding and watering are working to decrease the impact of Johne's disease on Key deer (Steve Berger, personal communication, July 2010). Continued monitoring is needed to assess the prevalence and extent of MAP in the Key deer range. In the current study, only three positive deer were detected on an isolated island. Potential management options include continued monitoring of clinically ill Key deer and development of management actions to control MAP.

Acknowledgements

Funding for this project was provided through Grant Agreement 401819G546 with the U.S. Fish and Wildlife Service. We thank personnel at the NKDR for their assistance with field studies, and at the Johne's Information Center for sample diagnostics.

Literature Cited

Chiodini, R. J., and H. J. Van Kruiningen. 1984. Eastern white-tailed deer as a reservoir of ruminant paratuberculosis. Journal of American Veterinary Medical Association 182: 168-169.

Collins, M. T., K. B. Kenefick, D. C. Sockett, R. S. Lambrecht, J. McDonald, and J. B. Jorgensen. 1990. Enhanced radiometric detection of Mycobacterium paratuberculosis by using filter-concentrated bovine fecal specimens. Journal of Clinical Microbiology 28: 2514-2519.

Davidson, W. R., E. J. B. Manning, and V. F. Nettles. 2004. Culture and serologic survey for *Mycobacterium avium* subsp. *paratuberculosis* infection among southeastern white-tailed deer (*Odocoileus virginianus*). Journal of Wildlife Diseases 40: 301-306.

Florou, M., L. Leontides, P. Kostoulas, C. Billinis, M. Sofia, I. Kyriazakis, and F. Lykotrafitis. 2008. Isolation of *Mycobacterium avium* subspecies *paratuberculosis* from non-ruminant wildlife living in the sheds and on the pastures of Greek sheep and goats. Epidemiology and Infection 136: 644-652.

Folk, M. L. 1991. Habitat of the Key deer. Dissertation, Southern Illinois University, Carbondale, Illinois, USA.

Galant, D.L. and L. Soliz. 2009. Population ecology of Florida Key deer on outer keys. Final Report to U.S. Fish and Wildlife Service, Big Pine Key, Florida, USA.

Jessup, D. A. and E. S. Williams. 1999. Paratuberculosis in free-ranging wildlife in North America. Zoo and Wild Animal Medicine, 4th edition. W. B. Saunders, Philadelphia, PA p.616-620

Johne's Testing Center, School of Veterinary Medicine, 2015 Linden Drive, Madison, Wisconsin, 53706-1102. http://www.johnes.org

Lopez, R. R. 2001. Population ecology of Florida Key deer. Dissertation, Texas A&M University, College Station, Texas, USA.

Lopez, R. R., M. E. P. Vieira, N. J. Silvy, P. A. Frank, S. W. Whisenant, and D. A. Jones. 2003. Survival, mortality, and life expectancy of Florida Key deer. Journal of Wildlife Management 67: 34-45.

Manning, E. J. B., T. E. Kucera, N. B. Gates, L. M. Woods, and M. Fallon-McKnight. 2003. Testing for *Mycobacterium avium* subsp. *paratuberculosis* infection in asymptomatic free-ranging tule elk from an infected herd. Journal of Wildlife Diseases 39: 323-328.

Marco, I., M. Ruiz, R. Juste, J. M. Garrido, and S. Lavin. 2002. Paratuberculosis in free-ranging fallow deer in Spain. Journal of Wildlife Diseases 38: 629-632.

Nettles, V. F., C. F. Quist, R. R. Lopez, T. J. Wilmers, P. Frank, W. Roberts, S. Chitwood and W. R. Davidson. 2002. Morbidity and mortality factors in Key deer (*Odocoileus virginianus clavium).* Journal of Wildlife Diseases 38: 685-692.

Palmer, M. V., J. R. Stabel, W. R. Waters, J. P. Bannantine, and J. M. Miller. 2007. Experimental infection of white-tailed deer (*Odocoileus virginianus*) with *Mycobacterium avium* subsp. *paratuberculosis*. Journal of Wildlife Diseases 43: 597-608.

Pedersen, K., E. J. B. Manning, and J. L. Corn. 2008. Distribution of *Mycobacterium avium* subspecies *paratuberculosis* in the Lower Florida Keys. Journal of Wildlife Diseases 44: 578-584.

Pickup, R. W., G. Rhodes, T. J. Bull, S. Arnott, K. Sidi-Boumedine, M. Hurley, and J. Hermon-Taylor. 2006. *Mycobacterium avium* subsp. *paratuberculosis* in lake catchments, in river water abstracted for domestic use, and in effluent from domestic sewage treatment works: diverse opportunities for environmental cycling and human exposure. Applied and Environmental Microbiology 72: 4067-4077.

Pierce, E. S. 2009. Possible transmission of *Mycobacterium avium* subspecies *paratuberculosis* through potable water: lessons from an urban cluster of Crohn’s disease. Gut Pathogens 1: 17.

Quist, C. F., V. F. Nettles, E. J. B. Manning, D. G. Hall, J. K. Gaydos, T. J. Wilmers, and R. R. Lopez. 2002. Paratuberculosis in Key deer (*Odocoileus virginianus clavium*). Journal of Wildlife Diseases 38: 729-737.

Riemann, H., M. R. Zaman, and R. Ruppaner. 1979. Paratuberculosis in cattle and free-living exotic deer. Journal of the American Veterinary Medical Association 174: 841-843.

Stabel, J. R. 1998. Johne's disease: A hidden threat. Journal of Dairy Science 81: 283-288.

White, C. I., R. J. Birtles, P. Wigley, and P. H. Jones. 2010. *Mycobacterium avium* subspecies *paratuberculosis* in free-living amoebae isolated from fields not used for grazing. Veterinary Record 166: 401-402.

Williams, E. S. 2001. Paratuberculosis and other mycobacterial diseases. Infectious diseases of wild mammals. 3rd Edition. E. S. Williams and I. K. Bakers (eds.). Iowa State University Press, Ames, Iowa, pp. 361-371.

Williams, E. S., T. R. Spraker, and G. S. Schoonveld. 1979. Paratuberculosis (Johne's disease) in bighorn sheep and a Rocky Mountain goat in Colorado. Journal of Wildlife Diseases 15: 221-227.

Table 1.1: Number of times that environmental samples (water, sediment, and soil) were collected for MAP culture from each natural and artificial water site by sampling period on Big Pine Key, Big Munson Island, and Little Palm Island July 2010-June 2011.

		Sampling Period					
		1	2	3	4	5	
Site	Site Type	July-Sept 2010	Oct-Dec 2010	Jan-Feb 2011	March-April 2011	May-June 2011	Total
2	Natural	1	1	1	2	0	5
3	Natural	1	1	1	0	0	3
4	Natural	1	1	1	0	0	3
42.1	Natural	0	0	0	2	0	2
42.2	Natural	0	0	0	2	0	2
44.1	Natural	1	1	1	1	1	5
44.2	Natural	1	1	1	0	1	4
45	Natural	1	1	1	0	0	3
49	Natural	1	1	1	0	0	3
50	Natural	1	1	1	0	0	3
51	Natural	1	1	1	0	0	3
52.1	Natural	0	0	0	2	0	2
52.2	Natural	0	0	0	2	0	2
78	Natural	1	1	1	0	0	3
98	Natural	1	1	1	0	0	3
Total Natural		11	11	11	11	2	46
Pond	Artificial	1	0	0	0	0	1
Bathtub	Artificial	1	1	2	4	2	10
Bird Bath	Artificial	1	1	1	0	0	3
Barrel	Artificial	0	0	0	0	4	4
LPI Fountain	Artificial	1	1	0	1	0	3
BPK Fountain	Artificial	0	0	0	0	4	4
Trough	Artificial	0	0	0	0	4	4
Total Artificial		4	3	3	5	14	29

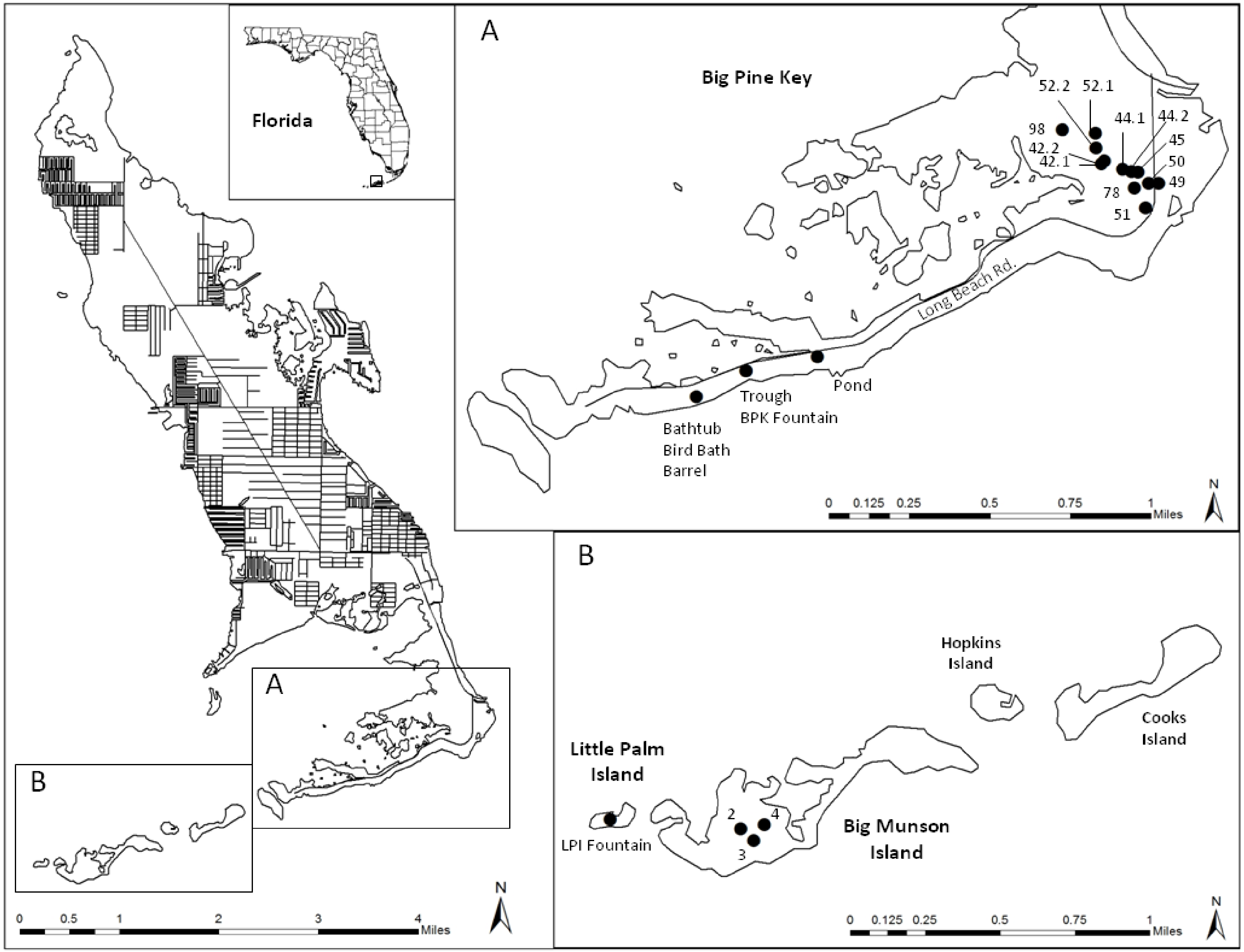

Figure 1.1: Natural and artificial freshwater sites (●) sampled for MAP on Florida's Big Pine Key (A) and Big Munson and Little Palm Islands (B) between July 2010 and June 2011.

Figure 1.2: Sites where Key deer fecal samples (●) were collected for MAP culture from southern Big Pine Key, Big Munson Island, and Little Palm Island, Florida between August 2010 and July 2011. Points do not reflect relative number of samples.

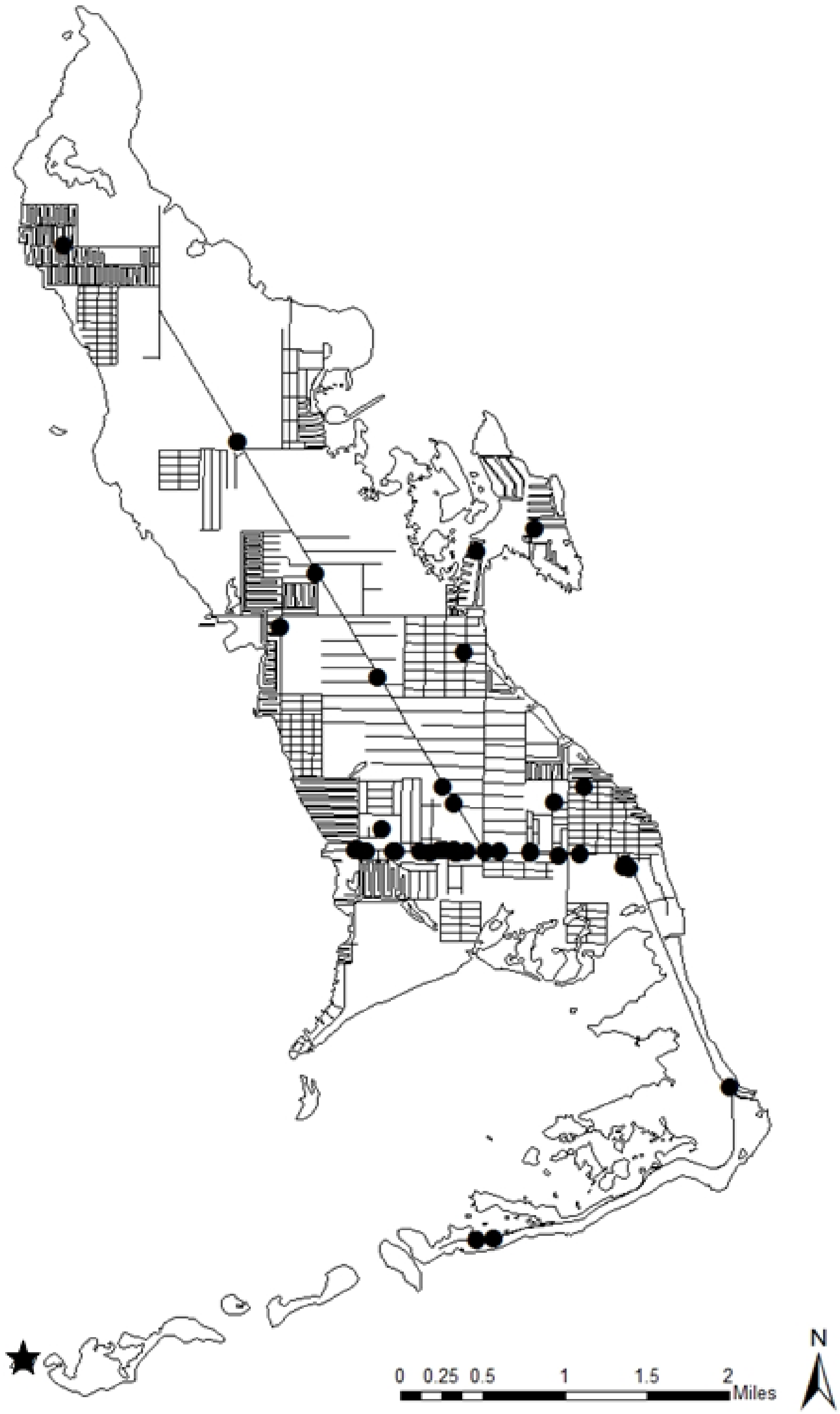

Figure 1.3: Sites where Key deer were found dead or terminally injured that were collected for necropsy and MAP culture on Big Pine Key and Little Palm Island, Florida between November 2009 and June 2011. ●, negative samples and ★, positive samples.

CHAPTER 3

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease, a progressive wasting disease of domestic animals that has also been found in wild ruminants. Johne's disease was first reported in the endangered Key deer (*Odocoileus virginianus clavium*) in 1996 on Big Pine Key, Florida. By 2008, eight additional MAP-positive Key deer had been identified on Big Pine Key and the nearby Newfound Harbor Keys. My study was conducted to determine the current distribution of MAP in Key deer and to determine if natural or man-made freshwater sources on Big Pine Key and the Newfound Harbor Keys are contaminated with MAP. Between November 2009 and July 2011, I collected a total of 249 fecal samples, tissue and fecal samples from 43 necropsied Key deer, and 675 environmental samples (soil, sediment, and water) and cultured them for MAP. MAP was isolated from three of 43 (7%) sampled Key deer, all from Little Palm Island. Pooled tissue samples from the ileum, cecum, and ileocecal lymph node from two deer were MAP-positive; feces from one was also culture positive. A third deer was confirmed MAP-positive with PCR. However, all fecal and environmental samples tested were negative for MAP. Results of this study indicate that Johne's disease was restricted to Key deer from Little Palm Island during this sampling period and that MAP transmission to Key deer through environmental contamination was not occurring or occurred at a very low rate. Negative results for environmental samples and an apparent decreased range of MAP on Big Pine Key and the Newfound Harbor Keys are both positive signs that current management plans may be working to decrease the incidence of MAP infection in Key deer.

Johne's disease is just one of several threats to the Key deer, which was listed as federally endangered in 1967 due to over-hunting and human disturbance to habitat. The Key deer remains listed due to high human-related mortality and continued habitat loss (Lopez et al., 2003). Currently, vehicle strikes are the primary cause of mortality but habitat degradation and illegal feeding are also important management issues for Key deer. If not properly managed, Johne's disease could become an additional factor impacting the Key deer population (Nettles et al., 2002). This is the first study to sample the environment for the detection of MAP within the Key deer range. Negative results for environmental samples and an apparent decreased range of MAP are both positive signs that current management efforts to decrease illegal feeding and watering are helping to decrease the impact of Johne's disease on Key deer. Continued monitoring is needed to assess the prevalence and extent of MAP in the Key deer range. In the current study, only three positive deer were detected on an isolated island. Potential management options include continued monitoring of clinically ill Key deer and development of management actions to control MAP.

Literature Cited

Lopez, R. R., M. E. P. Vieira, N. J. Silvy, P. A. Frank, S. W. Whisenant, and D. A. Jones. 2003. Survival, mortality, and life expectancy of Florida Key deer. Journal of Wildlife Management 67: 34-45.

Nettles, V. F., C. F. Quist, R. R. Lopez, T. J. Wilmers, P. Frank, W. Roberts, S. Chitwood and W. R. Davidson. 2002. Morbidity and mortality factors in Key deer (*Odocoileus virginianus clavium).* Journal of Wildlife Diseases 38: 685-692.