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EXPERIMENTAL INOCULATION OF COYOTES WITH *MYCOBACTERIUM BOVIS*: SUSCEPTIBILITY AND SHEDDING

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Abstract

Several wildlife species have tested positive for bovine tuberculosis in Michigan and may potentially transmit the disease to other animals. Coyotes have the highest known prevalence in the endemic area and thus, our objective was to investigate the shedding of *Mycobacterium bovis* by coyotes. Four coyotes were orally inoculated with 1 ml of 1 x 10^5 CFU/ml of *M. bovis*. Oral and nasal swabs, and feces were collected regularly and tested by culture. Fecal samples were also tested by exposing guinea pigs to the coyotes' feces. All animals were necropsied to determine if infection occurred. All swabs, feces and tissues were negative on culture. The dosage of *M. bovis* given to these coyotes was considered biologically relevant, but was insufficient for causing infection. Due to the lack of infection, we still do not know the risk coyotes pose for shedding *M. bovis*.

Introduction

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a contagious bacterial disease that can affect both humans and animals, including domestic and wild. Because of this, human-wildlife-livestock interactions resulting from actual or potential disease transmission has become an area of increasing concern. In 1975 and again in 1994, bTB was discovered in Michigan's white-tailed deer (*Odocoileous virginianus*). Since then, the disease has become endemic in deer in the northeast corner of Michigan's Lower Peninsula as indicated by follow-up surveillance in 1995 and later (Schmitt et al., 2006).
While no additional reservoir host has yet been identified, spillover infections have been identified in at least six other wildlife species. Bovine TB has been found in black bears (*Ursus americanus*), bobcats (*Felis rufus*), coyotes (*Canis latrans*), raccoons (*Procyon lotor)*, red fox (*Vulpes vulpes*), and North American opossums (*Didelphis virginiana*)

(Bruning-Fann, 2001,Witmer, 2006). Of these wildlife species, coyotes are infected with an average prevalence of 33 percent in the endemic area (VerCauteren et. al., 2008). If coyotes shed the infectious organism coupled with the high prevalence, the potential of serving as a transmission host to other animals is also high. The objective of this study was to investigate the susceptibility of coyotes to bTB and the coyotes' potential for shedding the organism.

Materials and Methods

Four captive-raised coyotes from USDA-APHIS-WS-NWRC Logan Field Station, Utah, consisting of two females and two males, eight to nine years old, were used. They were housed at Colorado State University (CSU), Animal Disease Building, Fort Collins, Colorado in individual cages within the same room. The cage size was 3'x6'x6'h and clear acrylic glass separated adjacent cages. They were fed Mazuri Canine Diet (PMI Nutrition International, LLC, P.O. Box 19798, Brentwood, Missouri 63144, USA) and given water ad libitum. Eight guinea pigs (Harlan Sprague Dawley Inc, Indianapolis, IN) were located in an adjacent room to the coyotes. They were housed two to a cage, which had clear polycarbonate sides and flooring and a wire lid meeting Institute for Laboratory Animal Research (ILAR) guidelines. They had food and water ad libitum. Bedding for the guinea pigs was changed every other day. A protocol detailing experimental procedures and animal care was approved by the CSU Institutional Animal Care and Use Committee prior to the experiment.

The coyotes were orally inoculated with 1 ml of 1 x 10^5 CFU/ml of deer-origin *M. bovis* on Day 0 of the study. We received 6 isolates cultured from Michigan white-tailed deer (Tuberculosis Laboratory, Michigan Department of Community Health (MDCH) Lansing, Michigan, USA) which were pooled and grown to reach the counts necessary for the inoculums. Pre-inoculation oral and nasal swabs, and fecal samples were also collected on Day 0. We anesthetized the coyotes with 5:1 mixture of ketamine: xylazine for inoculation and collection of swab samples. Starting on Day 10, fecal samples were collected weekly from the coyote cages for culture and PCR testing. Oral and nasal swabs were collected fortnightly starting on Day 17. Two sets of oral and nasal swabs were collected from each coyote. Oropharyngeal and nasal swabs were pooled separately and placed in 35ml of DNA/RNA free water. The swab and fecal samples were cultured for *M. bovis* at the biosafety level (BSL) 3 labs at CSU using a modified version of the protocol from Whitlock and Rosenberger (1994) to reduce bacterial contamination growth. Positive and negative controls were included at each culture timepoint and plates were checked for growth up to eight weeks.

On Day 24, we started exposing the guinea pigs to the coyote feces. Coyote feces were crumbled on the bedding and replaced every other day after the bedding was cleaned. A pair of guinea pigs only received feces from one coyote for the duration of the study.

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