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MYCOBACTERIUM AVIUM IN PYGMY RABBITS (*BRACHYLAGUS IDAHOENSIS*): 28 CASES

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Abstract: The Columbia basin subpopulation of pygmy rabbit *Brachylagus idahoensis* was listed as endangered by the United States Fish and Wildlife Service in November 2001, and no pygmy rabbits have been seen in the wild since spring 2002. Captive propagation efforts have attempted to increase population size in preparation for reintroduction of animals into central Washington. Disseminated mycobacteriosis due to *Mycobacterium avium* has been the most common cause of death of adult captive pygmy rabbits. Between June 2002 and September 2004, mycobacteriosis was diagnosed in 28 captive adult pygmy rabbits (representing 29% of the captive population), in contrast to 18 adult pygmy rabbits dying of all other causes in the same time period. Antemortem and postmortem medical records were evaluated retrospectively to describe the clinical course of mycobacteriosis in pygmy rabbits, physical examination findings, and diagnostic test results in the diagnosis of mycobacteriosis in pygmy rabbits. Various treatment protocols, possible risk factors for mortality, and recommendations for prevention of mycobacteriosis were evaluated also. Compromised cell-mediated immunity appears to be the best explanation at this time for the observed high morbidity and mortality from mycobacterial infections in pygmy rabbits.

Key words: *Brachylagus idahoensis*, cell-mediated immunity, mycobacteriosis, *Mycobacterium avium* complex, pygmy rabbit.

INTRODUCTION

The pygmy rabbit (*Brachylagus idahoensis*) is the only species in the genus *Brachylagus* and has been found in isolated populations in southeastern Washington, southern Idaho, southwestern Montana, western Wyoming, western Utah, northern Nevada, northeastern California, and eastern Oregon.[9,14,26] The population in Washington, confined to the basin of the Columbia River, is estimated to have been isolated geographically from other populations of the species for at least 10,000 yr and exhibits less genetic diversity than do other populations.[21,22,42,43] Adult pygmy rabbits weigh approximately 400 g and display several dietary and behavioral traits that distinguish them from cottontails (*Sylvilagus* spp.), jackrabbits (*Lepus* spp.), and Old World rabbits (*Oryctolagus* sp.).

In the last decade, pygmy rabbit populations have declined in Washington and other states where sagebrush habitat has been burned, has been converted to agriculture, or has been cleared from large areas and replaced with bunch grasses that increase livestock forage opportunities. The wild Columbia Basin pygmy rabbit (CBPR) population declined precipitously from about 150 rabbits in 1995 to fewer than 30 in 2001.[16,42] Fearing extinction of the CBPR, the Washington Department of Fish and Wildlife initiated a captive breeding program in cooperation with the Washington State University College of Veterinary Medicine (WSU), Oregon Zoo (OZ), and Northwest Trek Zoological Park (NWT). In December 2000, four wild pygmy rabbits from a nonendangered population in the Lemhi Valley in Idaho were brought to OZ for the purpose of developing pygmy rabbit husbandry protocols. Sixteen endangered CBPR were captured from 2001–2002 to serve as founders for captive breed-

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ing, and in 2002, both WSU and OZ began breeding CBPR for eventual reintroduction. The United States Fish and Wildlife Service listed the CBPR under emergency provisions of the Endangered Species Act on November 30, 2001,[39] with full listing status on March 5, 2003.[40] Presently, there are 19 CBPR, 12 Idaho pygmy rabbits, and 24 intercross pygmy rabbits of CBPR–Idaho origin in captive breeding programs in Washington and Oregon. No CBPR have been observed in wild habitat since spring 2002.

Disseminated infection with *Mycobacterium avium* complex has been the most common cause of death in adult captive pygmy rabbits, affecting those of all origins. In most mammalian species, including rabbits, *M. avium* is not pathogenic unless the immune system of the patient is compromised. Domestic rabbits (*Oryctolagus cuniculus*) have rarely been reported to have naturally-acquired mycobacteriosis, and such cases usually have been associated with heavily-contaminated pastures upon which cattle infected with *M. bovis* or *M. avium paratuberculosis* had been living before the rabbits arrived.[13,17] General causes for immunosuppression may include psychological stress, inbreeding, viral infection, and drug therapy. Depressed cell-mediated immune function has been reported as the cause for increased morbidity and mortality of *M. avium* infections in marsupials, humans infected with HIV, and organ transplant patients.[5,7,15,18,23,25,27,34]

In humans with AIDS, mycobacteria may be acquired via gastrointestinal and respiratory routes through contaminated airborne (coughed or sneezed) particles, feces, urine, genital discharges, milk, feed, or water.[24,41] Sometimes leading to infection, *M. avium* may colonize or may pass through the gastrointestinal tract of avian species and may be transmitted intermittently in feces.[20,38]

This retrospective study was initiated due to an apparent high incidence of mycobacteriosis in the captive pygmy rabbit population. Antemortem and postmortem medical records from 28 cases of mycobacteriosis were evaluated retrospectively in order to describe the clinical course of the disease in pygmy rabbits, to determine the relative value of physical examination and diagnostic tests in the diagnosis of mycobacteriosis in pygmy rabbits, to describe the use of various treatment protocols, and to assess possible risk factors for the observed high mortality. Development of husbandry and medical management recommendations for prevention of mycobacteriosis in pygmy rabbits was a goal of this retrospective study.

MATERIALS AND METHODS

Clinical cases

Twenty-eight cases of fatal disseminated mycobacteriosis due to *M. avium* (29% of the captive adult population) were diagnosed in captive pygmy rabbits between June 2002 and September 2004 at two of the three captive holding facilities. Staff at OZ and/or WSU managed clinical cases. A variety of local and reference laboratories performed diagnostic tests. Antemortem and postmortem medical records from all cases ($n = 28$) were evaluated retrospectively by one author (LH). Postmortem diagnoses from all other captive adult pygmy rabbit mortalities at all captive holding facilities during the same time period ($n = 18$) were summarized for comparison.

Mycobacterial cultures

Antemortem mycobacterial surveillance of the OZ captive pygmy rabbit population was conducted for 13 mo using bimonthly fecal mycobacterial cultures from each rabbit. Mycobacterial culture was performed using standard microbiological techniques. Molecular diagnostic testing such as polymerase chain reaction (PCR) amplification or sequencing of DNA of isolates was performed several weeks into the culture period for final placement of the mycobacterial isolate into one of three categories (*M. tuberculosis* complex, *M. avium* complex, or ''other'').

Gross and histopathologic examinations

Histopathologic examination was necessary for confirmation of diagnosis of disseminated mycobacteriosis. Biopsy and necropsy tissues were collected by several prosectors and were preserved in 10% neutral buffered formalin. Tissues were processed routinely, sectioned at 5 μm, mounted on glass microscope slides, and stained with hematoxylin and eosin. Mycobacteriosis-associated lesions were typically histiocystic, granulomatous, and/or necrotizing. On average, two lesions per case were stained with Fite's acid-fast technique to confirm presence of acid-fast bacilli.

Drug choices

Clinicians typically made initial drug choices before a diagnosis of mycobacteriosis had been confirmed. Several antibiotics, often in combination, were used for treatment in 16 of the 28 pygmy rabbits during the time period of their clinical signs. Azithromycin was administered to 12 rabbits, at 50 mg/kg p.o. q 24 hr (Zithromax 40 mg/ml oral suspension, Pfizer Labs, New York, New York 10017,

USA). Rifampin was administered to nine rabbits, at 40 mg/kg p.o. q 12 hr (160 mg/ml compounded oral suspension, University Compounders Inc., Portland, Oregon 97203, USA). Enrofloxacin was administered to six rabbits, at 15 mg/kg p.o. q 24 hr or 5–21 mg/kg s.c. q 24 hr (Baytril 22.7 mg/ml injectable solution, Bayer Healthcare, Shawnee Mission, Kansas 66201, USA). Ethambutol was administered to four rabbits, at 45 mg/kg p.o. q 12 hr (200 mg/ml compounded oral suspension, University Compounders). Rifabutin was administered to 3 rabbits at 25 mg/kg p.o. q 12 hr (40 mg/ml compounded oral suspension, University Compounders). Trimethoprim-sulfadiazine was administered to two rabbits at 30 mg/kg i.m. q 12–24 hr (480 mg/ml compounded injectable suspension, University Compounders). Dosages for these drugs were extrapolated from published references for domestic rabbits.[2,6,11,36]

Although drug susceptibility testing was not predicted to be clinically relevant for individual case management due to the length of time required for completion of testing (2–3 mo), seven postmortem *M. avium* isolates from pygmy rabbit necropsies were assessed for in vitro minimum inhibitory concentration susceptibility to various medications, including amikacin, azithromycin, ciprofloxacin, clarithromycin, ethambutol, ethionamide, rifabutin, rifampin, and streptomycin.

Drug concentration measurement

Banked sera and/or plasma samples from three clinical cases were assayed for azithromycin, rifampin, rifabutin, and ethambutol concentrations to determine whether therapeutic concentrations of these antimicrobials could be achieved in pygmy rabbits. Samples were collected 2–3 hr after oral administration of the drug, were stored frozen at −80°C, and were shipped frozen overnight to National Jewish Medical and Research Center (Denver, Colorado 80206, USA) for analysis. All assays were performed using validated high-performance liquid chromatography (azithromycin, rifampin, rifabutin) or gas chromatography/mass spectrometry (ethambutol) methods that met the criteria of the College of American Pathologists. Standard curves had the following ranges: azithromycin, 0.05–5 μg/ml; rifampin, 0.50–50 μg/ml; rifabutin, 0.10–2 μg/ml; and ethambutol, 0.20–10 μg/ml.[30,31] Six-point standard curves and at least three quality control samples were assayed with the research samples.

Assessment of mycobacterial antibody production

Mycobacterial antibodies were evaluated retrospectively in healthy and infected pygmy rabbits for determination of important antigenic proteins that could be used in future mycobacterial surveillance. Immunoblot assays were performed according to previously reported procedures, using a whole cell sonicate of a pygmy rabbit *M. avium* strain,[1,44] as well as serum and plasma samples from pygmy rabbits against the whole cell sonicate. Serum and plasma samples included 30 samples from 26 rabbits presumed to be healthy and 23 samples from 11 animals currently infected with mycobacteria.

Assessment of cell-mediated immunity and cytokines

The cell-mediated immunocompetence of pygmy rabbits was evaluated. Heparinized blood samples were collected from 51 captive pygmy rabbits (ages 3–50 mo), as well as from domestic laboratory rabbits (*Oryctolagus cuniculus*, $n = 5$, adult), and from a California riparian brush rabbit (*Sylvilagus bachmani riparius*, $n = 1$, adult). Lymphocytes and plasma from each sample were used for cell-mediated immunity assays (lymphocyte transformation with mitogens, cell–cell interaction of mixed lymphocyte cultures), and cytokine measurement. Lymphocyte transformation studies were performed as previously described for the Matschie's tree kangaroo (*Dendrolagus matschiei*).[25] Mixed lymphocyte cultures to study cell–cell interaction were performed in a similar manner, using allogeneic animals instead of plant mitogens as stimulators. Cytokines were measured in rabbit plasma by immunoaffinity capillary electrophoresis using isolated rabbit cytokine standards in a similar manner to that previously described for human cytokines.[32,33] Numerical data were interpreted to be significantly different if mean values were associated with $P < 0.01$, using Student's *t*-test.

RESULTS

Clinical cases

Mycobacteriosis was diagnosed definitively antemortem ($n = 9$) or postmortem ($n = 19$) in pygmy rabbits with histologic lesions that were positive for acid-fast bacilli and/or histologic lesions from which mycobacteria were cultured.

The gender distribution of mycobacteriosis cases was biased slightly toward females (71%, 20 females of 28 total mycobacteriosis cases), compared with the gender distribution of nonmycobacteriosis captive adult mortalities (56%, 10 females of 18 total nonmycobacteriosis mortalities). Mycobacteriosis was seen in both wild-caught ($n = 10$) and captive-born ($n = 18$) animals. The wild-caught an-

imals that died of mycobacteriosis were in captivity for 12–30 mo before death.

The average age at time of onset of clinical signs, when present, due to mycobacteriosis was 28.5 mo, with the earliest onset of clinical signs observed in a 10.5-mo-old intercross rabbit. Healthy pygmy rabbits have been known to live longer than 4 yr in captivity, regardless of population origin (L. Harrenstien, unpubl. data).

The nonspecific clinical signs of weight loss, lethargy, perineal soiling, and anorexia were those most commonly reported (64%, 50%, 39%, and 32% of cases, respectively), with other signs being evident in less than 20% of cases. Lameness due to osteomyelitis was seen in 14% of the rabbits in this study. Many clinical signs could be associated with pathophysiology typical of mycobacteriosis in other species (e.g., pale mucous membranes associated with anemia of chronic inflammation, dyspnea associated with pneumonia). However a few were unexpected, including lactation in three nonpregnant rabbits (11% of cases).

Ultrasonographic examination of liver and kidney parenchyma was not useful for detection of hepatic and renal granulomas. Radiographic findings of pneumonia, osteomyelitis, or soft tissue masses did not change during the course of disease in three animals that underwent five sets of radiographs over a 2-mo period. If abnormal lung, bone, or a soft tissue mass was localized during physical or radiographic examination, a biopsy was performed and the collected material was cultured or stained for acid-fast organisms. Only 32% of eventually confirmed mycobacteriosis cases (9 of 28 cases, Table 1) showed direct evidence of mycobacterial infection before death, either as acid-fast positive bacilli in a microscopic exam of a lesion aspirate or as positive mycobacterial culture of blood (3 of 4 animals tested), urine (7 of 8 animals tested), feces (3 of 15 animals tested), or an abnormal mass (4 of 9 animals tested). Given that mycobacterial cultures require several weeks to complete, some of these antemortem positive findings were not known until after death.

Diagnostic blood analyses (complete blood count [CBC] and plasma or serum biochemistry panel) were performed for 57% of mycobacteriosis rabbits at some point during their illness, and their data were compared to reference ranges generated from presumed healthy pygmy rabbits at OZ (Table 1). Ninety-one percent of mycobacteriosis rabbits showed abnormal CBC values at some point during illness, with heterophilia being most common. Monocytosis was seen in 44% of the rabbits tested (29% of the samples). Anemia was present in 38% of the rabbits tested (39% of the samples), with relatively equal numbers of anemic rabbits showing normocytic normochromic nonregenerative versus macrocytic hypochromic regenerative red cell indices. Six rabbits had nucleated red blood cells in their peripheral blood smears, despite a normal or increased hematocrit, and one had metamyelocytes in a peripheral blood smear, despite lack of noted postmortem histologic lesions in bone marrow. Approximately one half of the biochemistry panels revealed hypoalbuminemia and/or hyperglobulinemia (decreased albumin/globulin ratio). Hypercholesterolemia and azotemia were noted less frequently. Elevation of alkaline phosphatase concentration, which sometimes occurs in cases of osteolysis, was only noted in one animal with osteomyelitis.

Mycobacteriosis mortalities occurred at OZ and WSU. Adult mortalities at the OZ were typically due to mycobacteriosis (72%, 13 of 18 total adult mortalities) versus other etiologies; adult mortalities at WSU were less frequently due to mycobacteriosis (54%, 15 of 28 total adult mortalities). One mortality due to mycobacteriosis occurred at WSU 2 wk after the animal had been transferred from NWT, and four rabbits strongly suspected to have mycobacteriosis were transferred from WSU to OZ, where they were euthanized.

A greater percentage of mortalities were attributed to mycobacteriosis in captive CBPR than in captive Idaho rabbits, despite a similar age range of these populations (Table 2).

Mycobacterial cultures

Eleven OZ mycobacteriosis mortalities had antemortem fecal mycobacterial cultures performed; only one animal was fecal positive, despite six of the 11 animals showing intestinal granulomas at necropsy. Three animals previously culture-negative generated culture-positive urine or blood samples after 2–10 wk of antibiotic therapy.

Gross and histopathologic examinations

Gross lesions due to mycobacteriosis were typically yellow, moderately firm (slightly firmer than normal fat), and of variable size up to several millimeters. The number and size of lesions seen during gross necropsy did not correlate with the degree of clinical debilitation. For example, 27 rabbits exhibited pulmonary lesions histologically, although just 18 rabbits showed pulmonary lesions in gross postmortem examination and few rabbits had been diagnosed antemortem with pulmonary disease. Mycobacterial lesions were found histologically in liver and kidney in more than 50% of the cases. Overall, 19 tissues were found histologically to

Table 1. Antemortem diagnostic procedures and results reported in medical records of 28 adult pygmy rabbits (*Brachylagus idahoensis*) that died of disseminated mycobacteriosis originating from Columbia Basin, Idaho, or Columbia Basin–Idaho intercross populations, 2001–2004.

Diagnostic test	Rabbits tested[b] *n* (%)	Rabbits positive[c] *n* (%)	Test positive[d] *n* (%)
Radiography	15 (54)	10 (67)	21 of 33 (64)
Osteolysis		6 (40)	14 of 33 (42)
Pneumonia		4 (27)	4 of 33 (12)
Aspirate (lesion) cytology	9 (32)	4 (44)	4 of 9 (44)
Transtracheal lavage acid-fast cytology	1 (4)	0 (0)	0 (0)
Ultrasonography (hepatic/renal)	6 (21)	0 (0)	0 (0)
Complete blood count[a]	16 (57)		
Leukocytosis (>11,443/μl)		11 (69)	16 of 35 (46)
Absolute heterophilia (>5,653/μl)		12 (75)	24 of 35 (69)
Absolute monocytosis (>505/μl)		7 (44)	10 of 35 (29)
Absolute lymphocytosis (>5,164/μl)		4 (25)	4 of 35 (11)
Absolute lymphopenia (<1,332/μl)		3 (19)	5 of 35 (14)
Thrombocytopenia (qualitative)		2 (13)	2 of 34 (6)
Anemia (hematocrit <37%)		6 (38)	13 of 33 (39)
≥one hematologic abnormality		14 (88)	32 of 35 (91)
Serum/plasma biochemistry panel[a]	16 (57)		
Low albumin/globulin ratio (<1.2)	16 (57)	12 (75)	17 of 33 (52)
Hypercholesterolemia (>45 mg/dl)	13 (46)	10 (77)	11 of 18 (61)
Elevated alkaline phosphatase (>146 IU/l)	16 (57)	1 (6)	1 of 33 (3)
Azotemia (elevated blood urea nitrogen >38 mg/dl)	16 (57)	3 (19)	6 of 34 (18)
Elevated creatinine (>1.0 mg/dl)	16 (57)	2 (13)	4 of 34 (12)
≥ one biochemical abnormality		15 (94)	24 of 34 (71)
Fecal mycobacterial culture[e]	15 (54)	3 (20)	3 of 22 (14)
Urine mycobacterial culture[e]	8 (29)	7 (88)	10 of 16 (63)
Blood mycobacterial culture[e]	4 (14)	3 (75)	3 of 10 (30)
Acid-fast bacilli found, or positive mycobacterial culture, in any antemortem sample		9 (32)	
Immunoblot of plasma/serum for *Mycobacterium avium* antibody[e]	7 (25)	5 (71) "positive," 2 (29) "suspect"	16 of 19 (84) "positive," 3 of 19 (16) "suspect"

[a] Hematologic and biochemical reference ranges were established as the mean ± 2 SD from healthy adult captive pygmy rabbits, and sample values were considered abnormal if ≥2 SD above or below the reference population's mean value.

[b] Rabbits tested: *n* = number of rabbits that were evaluated by the listed diagnostic test, % = percentage of rabbits evaluated compared with 28 rabbits with disseminated mycobacteriosis.

[c] Rabbits positive: *n* = number of rabbits in which an abnormal result ever was found; % = percentage of rabbits positive compared with the number of rabbits ever receiving that test.

[d] Test positive: *n* = number of tests in which an abnormal result was found compared with the total number of tests performed; % = percentage of abnormal test results compared with the number of tests performed.

[e] ≤6 mo before illness was first noticed.

contain mycobacterial lesions in the rabbits of this study.

Histologically, mycobacterial lesions in pygmy rabbits included granulomatous inflammation, discrete granulomas, and areas of necrosis. Inflammation was predominantly comprised of macrophages with fewer neutrophils, lymphocytes, and plasma cells, sometimes organized into discrete granulomas oriented around foci of caseous necrosis (Fig. 1). Occasional Langhans-type multinucleate giant cells were present at the margin of necrotic tissue and inflammation in the granulomas. Fite's acid-fast stained sections revealed low to moderate numbers of acid-fast bacilli in the foci of necrosis and sometimes within the cytoplasm of macrophages or multinucleate cells (Fig. 2).

Drug choices

Drug susceptibility results showed similar, but not identical, profiles. Most of the seven isolates

Table 2. Causes of mortality of captive adult pygmy rabbits (*Brachylagus idahoensis*) originating from Columbia Basin (CBPR), Idaho, or Columbia Basin–Idaho intercross populations, 2001–2004, determined by postmortem gross and histopathologic evaluation.

Cause of pygmy rabbit mortality	CBPR *n*[a] (%)[b]	Idaho *n*[a] (%)[b]	Intercross *n*[a] (%)[b]	All *n*[a] (%)[b]
Mycobacteriosis	19 (70)	8 (47)	1 (50)	28 (61)
Enterocolitis, nonparasitic	4 (15)	2 (12)	0	6 (13)
Trauma	2 (7)	3 (18)	1 (50)	6 (13)
Cysticercosis	0	1 (6)	0	1 (2)
Nonmycobacterial abscess	0	1 (6)	0	1 (2)
Neoplasia	1 (4)	0	0	1 (2)
Capture complication	1 (4)	0	0	1 (2)
Unknown	0	2 (12)	0	2 (4)
Mortality totals by population	27	17	2	46

[a] *n* = number of adult animals of the designated population in which the stated cause of mortality was found.
[b] % = percentage of the total number of necropsied adult animals of the designated population that died of that particular cause.

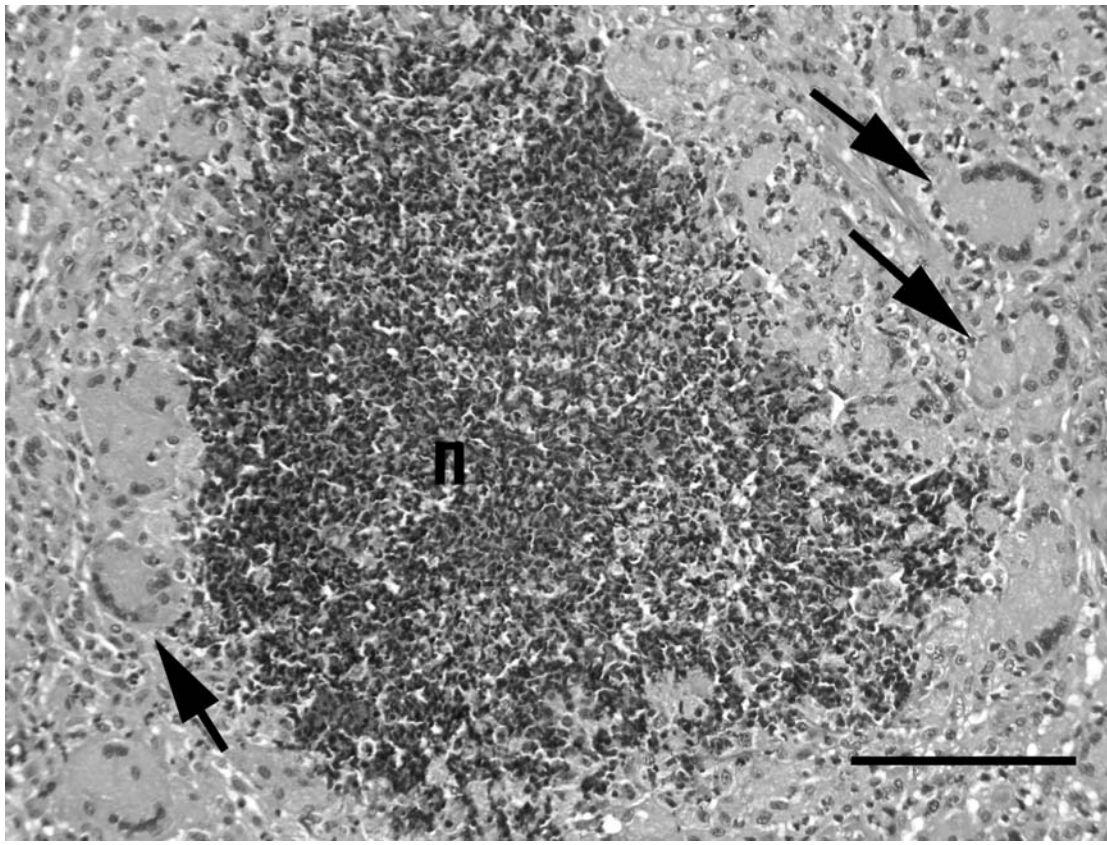

Figure 1. Brain, pygmy rabbit (*Brachylagus idahoensis*) with mycobacteriosis. Note granuloma in neuropil with core of necrosis (n) surrounded by macrophages and Langhans type multinucleate giant cells (arrows). Hematoxylin and eosin stain, bar = 500 μm.

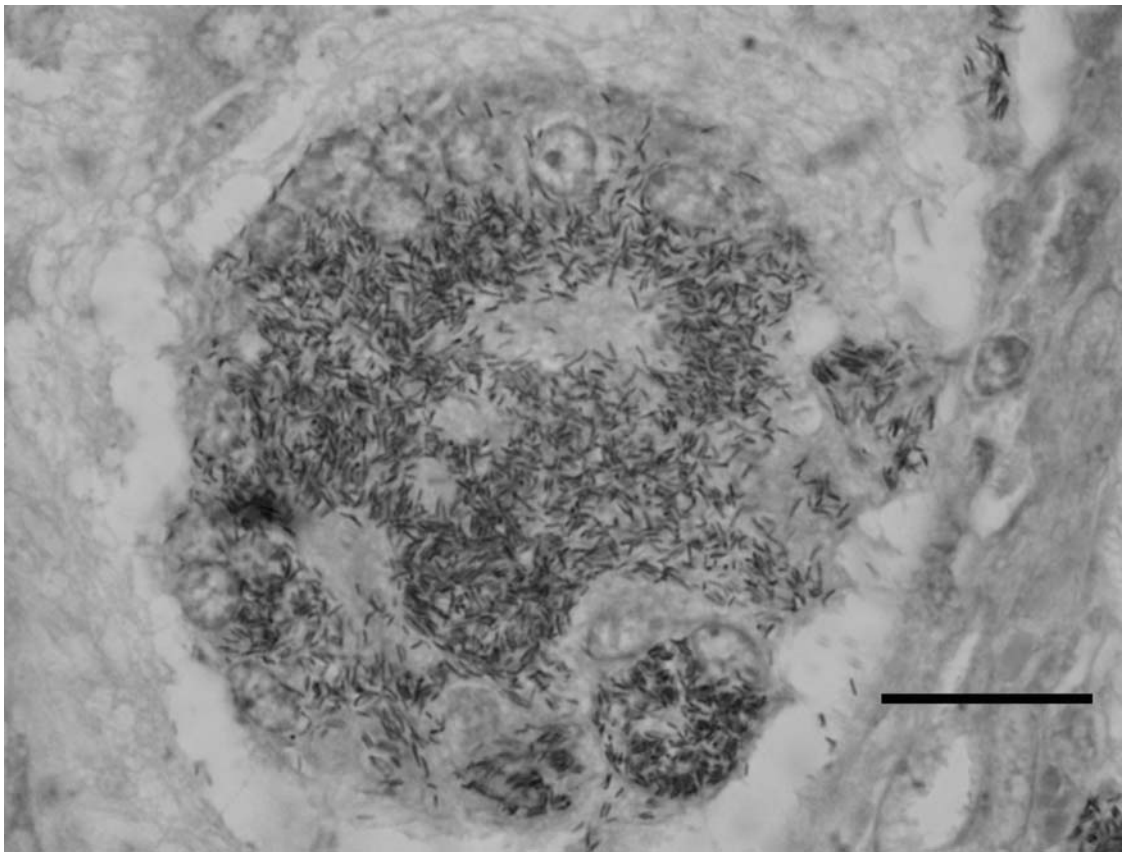

Figure 2. Brain, pygmy rabbit (*Brachylagus idahoensis*) with mycobacteriosis. Note large numbers of bacilli in the cytoplasm of a Langhans-type multinucleate giant cell. Fite's acid fast stain, bar = 60 μm.

tested were susceptible to azithromycin, clarithromycin, ethambutol, and rifabutin in vitro; therefore, these drugs were preferentially used for treatment of later cases. Subjectively, antibiotic treatment appeared to improve or to stabilize the clinical status of an affected rabbit, with weight gain and cessation of lesion growth.

Drug concentration measurement

A preliminary analysis of achieved serum concentrations was performed for azithromycin, rifampin, rifabutin, and ethambutol. Azithromycin and rifabutin serum or plasma concentrations approached human target ranges (0.2–0.7 μg/ml and 0.3–0.9 μg/ml, respectively), with three rifabutin concentrations exceeding those typically sought in humans. Conversely, at the time points measured, rifampin and ethambutol serum or plasma concentrations were well below the human target ranges (8–24 μg/ml and 2–6 μg/ml, respectively).

Assessment of mycobacterial antibody production

Fifty-three archived serum or plasma samples were evaluated by immunoblot to detect immunoglobulin reactive to a *M. avium* whole cell sonicate. In general, pygmy rabbits with active mycobacterial infection showed *M. avium*–specific antibody in immunoblots, and noninfected pygmy rabbits were *M. avium* immunoblot negative. As illustrated in Figure 3, analysis of serum obtained from a *M. avium* infected rabbit showed reactivity to proteins of ~100 kDa and ~42 kDa in *M. avium* whole cell sonicate. Responses were not detected in serum obtained from a noninfected rabbit. Analysis of serial samples from another infected rabbit showed reactivity to proteins of ~100 kDa, ~42 kDa, and ~32–37 kDa in *M. avium* whole cell sonicate. Responses were not detected in serum obtained from this rabbit prior to infection, 2 yr earlier.

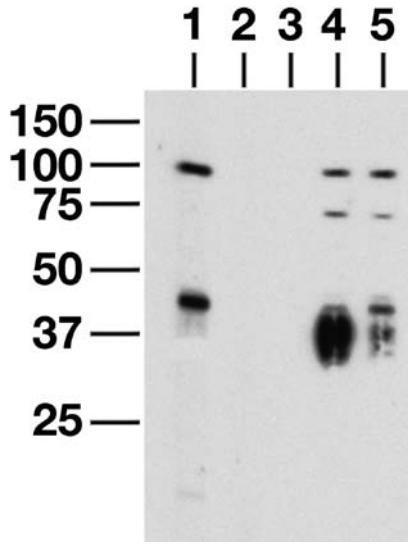

Figure 3. Preparative immunoblot of *Mycobacterium avium* pygmy rabbit isolate probed with three sequential blood samples from a pygmy rabbit (*Brachylagus idahoensis*) with clinical suspicion of mycobacteriosis. Lane 1: a pygmy rabbit diagnosed with *M. avium* infection; lane 2: a pygmy rabbit known to be free of *M. avium* infection; lane 3: clinical suspect, archive sample from 2 yr prior to onset of clinical signs of illness; lane 4: same clinical suspect, at time of onset of clinical signs of illness; lane 5: same clinical suspect, 3 wk after onset of clinical signs of illness. Protein size markers are indicated in kilodaltons in the left margin.

Assessment of cell-mediated immunity and cytokines

In lymphocyte mitogen and antigen stimulation assays (Table 3), the CBPR and Idaho pygmy rabbits responded to both mitogen (phytohemagglutinin, concanavalin A, and pokeweed mitogen) and allogeneic mixed lymphocyte antigens (major histocompatibility complex) to significantly lesser degrees than did either domestic rabbits or intercross pygmy rabbits. None of the pygmy rabbits responded to the bovine derivative of mycobacteria (bacille Calmette-Guérin, BCG) or to the saprophytic mycobacteria, *M. vaccae*. However, the CBPR and Idaho pygmy rabbits, as well as the intercross pygmy rabbits, responded to *M. avium*. This positive response to the *M. avium* antigen did not depend on the clinical status of the individual rabbit.

Pygmy rabbits produced significantly less gamma-interferon and more interleukin-10 than did domestic rabbits (Table 4). They produced lower concentrations of tumor necrosis factor, interleukin-1, and interleukin-6, but more interleukin-2 than domestic rabbits. Specifically, T helper 1 (Th1) cytokines were reduced, whereas T helper 2 (Th2) cytokines were increased, indicating a Th2 polarization of the immune response.

DISCUSSION

Captive pygmy rabbits appear to be extremely susceptible to infection from *M. avium* complex that progresses to fatal disease. The pygmy rabbits of our study acquired mycobacterial infections from unknown sources. We suspect substrate contaminated with *M. avium* to be the source of infection, based on the ubiquitous natural prevalence of the organism. Soil substrate used in captive pygmy rabbit enclosures is not sterilized before use; it is also possible that small birds could gain entry to captive enclosures, despite wire mesh walls and ceilings.

Based on our retrospective analysis, it was not possible to determine with confidence whether rabbits were colonized first in the wild or in captivity, although the long interval between capture and onset of disease (mean, 20 mo) suggests that infection occurred in captivity.

Comparison of disease prevalence between captive facilities is difficult, because pygmy rabbits were transferred frequently between facilities and the course of mycobacteriosis is presumed to be long. At each facility, occasional acid-fast granulomas were noted on routine necropsy screening of a few other captive animals of other species, however, cultures of the tissues were not submitted so additional information regarding a presumed *Mycobacterium* species is not available. Animal keepers, equipment, and exhibits in each instance were not associated with pygmy rabbit housing.

Although clinical signs of mycobacteriosis in pygmy rabbits are nonspecific, the high incidence of mycobacteriosis in the population suggests mycobacteriosis should be considered in any pygmy rabbit with clinical signs of illness. The nonspecific hematologic and biochemistry panel abnormalities of heterophilia, monocytosis, anemia, hypoalbuminemia, hyperglobulinemia, and/or hypercholesterolemia are common findings in clinical cases.

Diagnosis required sampling of masses, tissues, or excreta (blood, urine, or feces) for discovery of acid-fast positive bacilli through cytologic preparation or culture. Urine or blood mycobacterial cultures were more sensitive than fecal mycobacterial culture. Several sites should be sampled, because individual patients may have mycobacterial organisms in only one particular organ system; serial sampling also should be considered, because detectable shedding may wax and wane during the disease course.[38] Variable shedding over time and/or suboptimal sensitivity of initial mycobacterial

Table 3. Lymphocyte stimulation indices of adult pygmy rabbits (*Brachylagus idahoensis*) of Columbia Basin subpopulation origin (CBPR), adult pygmy rabbits of Idaho subpopulation origin (Idaho), adult pygmy rabbits of CBPR–Idaho intercross origin (Intercross), an adult California riparian brush rabbit (*Sylvilagus bachmani riparius*, Control-Brush), and adult domestic rabbits (*Oryctolagus cuniculus*, Control-Domestic). Each data point is presented as a ''stimulation index'' value, which is the ratio of counts per minute in the stimulated culture divided by a parallel unstimulated control culture (mean ± 1 SD). A stimulation index value of >10 for the mitogens and >3 for the antigens is considered positive.

Test	CBPR, $n = 18$	Idaho, $n = 15$	Intercross, $n = 7$	Control–Brush, $n = 1$[a]	Control–Domestic, $n = 5$	Significant differences, $P < 0.01$[b]
Phytohemagglutinin	66.8 ± 4.5	68.4 ± 11.6	96.3 ± 4.7	159.3	173.9 ± 16.8	Domestic > Intercross Domestic > Idaho Domestic > CBPR Intercross > Idaho Intercross > CBPR
Concanavalin A	78.5 ± 4.8	88.3 ± 10.4	126.0 ± 5.4	141.6	143.7 ± 14.4	Domestic > Idaho Domestic > CBPR Intercross > Idaho Intercross > CBPR Idaho > CBPR
Pokeweed mitogen	126.9 ± 4.5	133.5 ± 6.8	155.9 ± 5.7	150.2	162.0 ± 14.3	Domestic > Idaho Domestic > CBPR Intercross > Idaho Intercross > CBPR Idaho > CBPR
Mixed lymphocyte culture	41.2 ± 6.5	50.5 ± 10.4	86.0 ± 4.9	88.5	120.6 ± 28.6	Domestic > Idaho Domestic > CBPR Intercross > Idaho Intercross > CBPR Idaho > CBPR
Bacille Calmette-Guérin	1.7 ± 0.4	1.3 ± 0.3	1.1 ± 0.4	1.3	1.6 ± 0.2	Intercross < CBPR Idaho < CBPR
Mycobacterium vaccae	2.2 ± 0.5	1.7 ± 0.4	0.9 ± 0.5	0.5	1.0 ± 0.3	Domestic < Idaho Domestic < CBPR Intercross < Idaho Intercross < CBPR Idaho < CBPR
Mycobacterium avium	10.8 ± 2.0	9.5 ± 2.3	2.2 ± 0.4	0.2	0.5 ± 0.2	Domestic < Intercross Domestic < Idaho Domestic < CBPR Intercross < Idaho Intercross < CBPR

[a] Values for the California riparian brush rabbit were not statistically evaluated because the sample size was one.

[b] Values were compared between groups using Student's *t*-test at the significance threshold of $P < 0.01$.

Table 4. Cytokine profiles of adult pygmy rabbits (*Brachylagus idahoensis*) of Columbia Basin subpopulation origin (CBPR), adult pygmy rabbits of Idaho subpopulation origin (Idaho), adult pygmy rabbits of CBPR–Idaho intercross origin (Intercross), an adult California riparian brush rabbit (*Sylvilagus bachmani riparius*, Control–Brush), and adult domestic rabbits (*Oryctolagus cuniculus*, Control–Domestic).

Test	CBPR, $n = 18$	Idaho, $n = 15$	Intercross, $n = 7$	Control–Brush, $n = 1$[a]	Control–Domestic, $n = 5$	Significant differences, $P < 0.01$[b]
Complement CH50 (pg/ml)	101.8 ± 3.3	106.3 ± 4.2	111.1 ± 2.3	121.5	117.0 ± 12.2	Intercross > Idaho Intercross > CBPR Idaho > CBPR
Interleukin-1 (pg/ml)	2.7 ± 0.3	24.0 ± 11.0	23.2 ± 3.9	21.5	19.5 ± 4.0	Domestic > CBPR Intercross > CBPR Idaho > CBPR
Interleukin-2 (pg/ml)	46.2 ± 5.3	30.8 ± 5.9	23.1 ± 4.8	24.3	22.1 ± 5.1	Domestic < CBPR Intercross < Idaho Intercross < CBPR Idaho < CBPR
Interleukin-6 (pg/ml)	4.1 ± 0.5	20.3 ± 10.9	5.7 ± 1.7	14.6	14.3 ± 2.6	Domestic > Intercross Domestic > CBPR Intercross < Idaho Idaho > CBPR
Interleukin-10 (pg/ml)	12.7 ± 2.0	7.1 ± 2.9	5.5 ± 0.8	8.5	6.2 ± 1.1	Domestic < CBPR Intercross < CBPR Idaho < CBPR
Gamma-interferon (pg/ml)	2.8 ± 0.5	15.2 ± 6.9	9.4 ± 1.8	16.2	12.3 ± 1.7	Domestic > CBPR Intercross < Idaho Intercross < CBPR Idaho > CBPR
Tumor necrosis factor-α (pg/ml)	8.1 ± 0.6	39.9 ± 20.2	22.7 ± 4.0	23.8	23.3 ± 4.2	Domestic < Idaho Domestic > CBPR Intercross < Idaho Intercross > CBPR Idaho > CBPR

[a] Values for the California riparian brush rabbit were not statistically evaluated, because the sample was 1.

[b] Values were compared between groups using Student's *t*-test at the significance threshold of $P < 0.01$, except for the California riparian brush rabbit's values, which were not statistically compared due to a sample size of 1.

cultures could be responsible for the conversion of some rabbits from culture-negative to culture-positive status during the course of antibiotic therapy. The long interval between sample collection and generation of results (up to 6 wk in some cases) interfered with prompt diagnosis of mycobacteriosis in pygmy rabbits; PCR technology should be used more extensively to reduce diagnostic turnaround time.[19]

One rabbit of this series grew *M. avium scrofulaceum* in repeated cultures of urine and blood; culture isolates were initially PCR-negative for *M. tuberculosis* and *M. avium* and further testing clarified the species identity. This finding underscores the importance of full speciation of acid-fast bacilli in cultures from patients.

Intradermal tuberculin testing was considered as a possible surveillance tool for pygmy rabbits, but was not investigated. If employed, exposure of pygmy rabbits to *M. avium* antigens via intradermal testing may have sensitized them and caused later antibody assays to be invalid.[7]

After initial thoracic, abdominal, and skeletal radiographs were carefully evaluated, further radiographic evaluation did not appear necessary, because the presence and character of radiographic lesions did not change over the 1–3 mo course of disease. Given the prevalence of bone colonization, it is possible that advanced diagnostics such as scintigraphy may detect early osteomyelitis cases.[37]

Captive facilities have adopted postmortem sampling protocols that require histopathology and mycobacterial testing of all dead adult animals. Recently, a case of mycobacterial infection without granuloma formation was found in a wild-caught male of Idaho population origin that had been in captivity at OZ and WSU for 17 mo. The rabbit was euthanized due to chronic weight loss; postmortem tissue cultures were positive for *M. avium*, but histologic evidence of granuloma formation was not seen. If more extensive antemortem and postmortem sampling had been performed or if a longer time interval before euthanasia had transpired, granulomas may have been found. In fact, immunoblot data from serum collected at time of euthanasia from this animal showed a high antibody response to *M. avium*. Nonfatal mycobacterial infection has been reported in marsupials.[7]

Given the high morbidity and mortality associated with *M. avium* in pygmy rabbits, pharmaceutic treatment should be considered as soon as acid-fast bacilli are seen or a positive mycobacterial culture is obtained. *M. avium* is not a public health concern except for immunocompromised persons; therefore, treatment decisions remain in the hands of veterinarians and facility managers, related to individual case circumstances and to determination of treatment benefits and risks. Because captive reproduction is critical to the CBPR endangered species recovery project, current recommendations include treatment and attempted cure and/or control of *M. avium*–infected breeding animals. Concerns were raised initially regarding the tolerance of these shy animals for twice-daily manual restraint for administration of medication. Body weights, fecal character, and mentation appeared unchanged throughout the course of therapy in several animals that were monitored closely for adverse effects. Pygmy rabbits that were transferred from soil enclosures to standard laboratory metal rabbit hutches for disease treatment appeared to adapt to the provided artificial burrow systems. As of this writing, one *M. avium* culture-positive rabbit has received more than 6 mo of continuous azithromycin-ethambutol-rifabutin treatment without visible adverse effects.

Tree kangaroos housed without soil substrate do not appear to develop disseminated mycobacterial infections, in contrast to the many *M. avium* cases noted in tree kangaroos with soil access (M. Bush, pers. comm.). Housing of captive pygmy rabbits without soil substrate is being investigated as a husbandry measure for mycobacteriosis prevention.

Lymphocyte stimulation and cytokine assays were helpful in explaining the high morbidity and mortality associated with mycobacterial infection in pygmy rabbits. Cell-mediated immune responses are necessary for clearance of mycobacterial infection in healthy animals. Mononuclear cell responses to plant mitogens and major histocompatibility complex antigens give an assessment of total cell-mediated immune response, because these agents stimulate a large fraction of mononuclear cells. The control rabbits responded more vigorously to mitogens and major histocompatibility complex antigens than did the CBPR and Idaho pygmy rabbits, as shown by higher stimulation indices. The reduced responses of CBPR and Idaho pygmy rabbits may be due to multiple factors such as environment, genetics, and external stressors. In contrast, the lymphocyte responses of the intercross pygmy rabbits to mitogens were similar to those of the domestic rabbits, suggesting a possible ''hybrid vigor'' benefit of better cell-mediated immunity in these intercrossed animals. As individuals of the intercross pygmy rabbit population become older, it is theorized that they will show a lower incidence of fatal mycobacteriosis than do CBPR or Idaho pygmy rabbits.

All of the pygmy rabbits responded to the *M. avium* antigen, but not the BCG or *M. vaccae*. The

uniform response to *M. avium* suggests that exposed pygmy rabbits could mount some degree of response to the presented antigen. However, their response did not lead to effective control and/or killing of the *M. avium* organism, a situation often observed in species with impaired immune systems.[8,25,27,34] For this reason, mycobacterial vaccination of pygmy rabbits is not predicted to be of clinical benefit for disease prevention.

Under the influence of cytokines, T helper cells can differentiate either into Th1 cells, which induce a cytotoxic, cellular immune response, or into Th2 cells, which induce a humoral response. Therefore, the Th1/Th2 differentiation state of T helper cells can be assessed by differential cytokine production. Compared with domestic rabbits, the pygmy rabbits demonstrated lower expression of interleukin-1, interleukin-6, and gamma-interferon and elevated expression of interleukin-10. These data imply that they are mounting a Th2, or humoral, type of immune response. Immunoblot and/or enzyme-linked immunosorbent assay (ELISA) testing of serum or plasma for antibody relies upon presence of a humoral response to *M. avium* exposure. Specific antibody responses to *M. avium* were detected by immunoblot in all of the tested pygmy rabbits of this study, and preliminary ELISA values show strong correlation with immunoblot data (Paustian, unpubl. data). Assuming mycobacterial exposure results in high mortality in pygmy rabbits, antibody testing may be a valuable means of early detection of those rabbits considered candidates for mycobacterial treatment.

A Th1 response, or cytotoxic cellular response, is required to clear mycobacterial infections. Th1 responses are inhibited by high amounts of Th2 cytokines. The pygmy rabbit Th1/Th2 response was imbalanced. Gamma-interferon concentrations were reduced, and therefore, macrophage activation was decreased. Under such circumstances, low numbers of killer T cells also would be expected. Humans with clinical mycobacteriosis typically have low gamma-interferon production.[46] A Th2 polarization is found in some strains of mice, associated with multiple genetic loci that control susceptibility to intracellular infections.[4,12,35] It is likely that the same types of multigeneic differences induce a Th2 response in pygmy rabbits leading to disseminated infection, rather than a protective Th1 response.

Humans with *M. avium* infection typically are treated with a three-drug combination consisting of a macrolide (azithromycin or clarithromycin), ethambutol, and rifabutin (or rifampin).[10] The use of drug susceptibility testing to guide the treatment of mycobacteriosis in humans currently is subject to clinical debate. Human peak serum values of these drugs are seen at 2–3 hr after oral administration, therefore, pygmy rabbits were sampled during this period; peak values, however, may have been achieved at different times in the pygmy rabbits. Domestic rabbits receiving the same drug dosages as the pygmy rabbits of our study have shown peak serum values that correspond well with recommended human peak serum concentrations, but peak concentrations in domestic rabbits were seen at times other than 2–3 hr after administration: azithromycin at approximately 1 hr postadministration, rifabutin at 4 hr postadministration, and ethambutol at 0.5 hr postadministration.[2,11,36] Due to sparse drug concentration sampling performed in this study, definitive conclusions regarding the bioavailability and potential clinical utility of the drugs tested cannot be drawn. Based on available data from this report, azithromycin (50 mg/kg p.o. q 24 hr) and rifabutin (25 mg/kg p.o. q 12 hr) appear to be viable options for the treatment of pygmy rabbits, but the dosing of rifampin and ethambutol require additional study before each can be recommended with confidence.[28,29] Preliminary evidence suggests that ethambutol may be a useful adjunct therapy at 45 mg/kg p.o. q 12 hr as part of triple combination therapy. Additional testing is required to fully describe the pharmacokinetics of these drugs in pygmy rabbits.

Several adjunct therapies have been investigated for *M. avium* disease in other species and may show promise in the future for pygmy rabbits as well, pending investigation of appropriate administration logistics. Synthetic gamma-interferon-1-beta (Actimmune®, Intermune, Inc., Brisbane, California 94005 USA) is often recommended as adjunct therapy for humans with *M. avium*–associated disease. Although Th1 response depression in humans is due typically to retroviral infection, rather than inherent cell-mediated immunity depression as suspected in pygmy rabbits, it is possible that pygmy rabbits with mycobacteriosis could benefit from gamma-interferon therapy. Granulocyte-macrophage colony-stimulating factor has been found to be synergistic with azithromycin in the treatment of murine *M. avium* infections and may be synergistic with azithromycin in pygmy rabbits, as well.[3] Other adjunct therapies may be warranted, including vitamin E and/or nonsteroidal anti-inflammatory medications for relief of tissue inflammation and discomfort. Mycobacterial colonization may occur more readily in traumatized or inflamed tissue than in healthy tissue,[45] therefore, reduction of tissue inflammation might reduce the number of possible foci of granuloma formation and make systemic

mycobacterial clearance more effective. Once- or twice-weekly azithromycin administration is recommended for at-risk human patients (HIV-infected, with fewer than 50 CD4 T-lymphocytes per μl of peripheral blood),[10] and prophylactic therapy is under investigation for pygmy rabbits at our facilities, as well.

CONCLUSIONS

The pygmy rabbits examined in this study exhibited partially ineffective cell-mediated immunity, which we believe predisposed them to morbidity and mortality from *M. avium* complex infection. Physical examination, acid-fast staining of cytologic preparations of directed samples (aspirates or impression smears of masses), thoracic and skeletal radiography, hematologic and serum chemistry analysis, and serum/plasma antibody assays appear to be useful tools for presumptive diagnosis of mycobacteriosis in pygmy rabbits, allowing early medical treatment and a potential cure. Mycobacterial culture of blood, urine, feces, and/or directed samples is a specific technique for diagnosis, but the low sensitivity and long time required for growth of mycobacteria make culture less valuable for initial clinical assessments and more valuable for long-term confirmation of diagnosis. Pygmy rabbits with *M. avium* infection should be treated with azithromycin (50 mg/kg p.o. q 24 hr) and rifabutin (25 mg/kg p.o. q 12 hr). Failure to eliminate mycobacterial infections in these pygmy rabbits likely was related to advanced infection status at diagnosis and/or poor drug selection in some of the cases. Mycobacteriosis prevention in pygmy rabbits should include nonsoil housing or use of presterilized soil in enclosures, exclusion of carrier birds from enclosures, and improvement of cell-mediated immunocompetence of the population, which might be achieved through intercross breeding of animals of CBPR and Idaho subpopulation origins.

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LITERATURE CITED

1. Bannantine, J., and J. R. Stabel. 2000. HspX is present within *Mycobacterium paratuberculosis*-infected macrophages and is recognized by sera from some infected cattle. Vet. Microbiol. 76: 343–358.

2. Battaglia, R., E. Pianezzola, G. Salgarollo, G. Zini, and M. Strolin-Benedetti. 1990. Absorption, disposition and preliminary metabolic pathway of 14C-rifabutin in animals and man. J. Antimicrob. Chemother. 26: 813–822.

3. Bermudez, L. E., J. Martinelli, M. Petrofsky, P. Kolonosky, and L. S. Young. 1994. Recombinant granulocyte-macrophage colony-stimulating factor enhances the effects of antibiotics against *Mycobacterium avium* complex infection in the beige mouse model. J. Infect. Dis. 169: 575–580.

4. Bix, M., and R. M. Locksley. 1998. Independent and epigenetic regulation of the IL-4 alleles in CD4+ T cells. Science 281: 1352–1354.

5. Buddle, B. M., and L. J. Young. 2000. Immunobiology of mycobacterial infections in marsupials. Dev. Compar. Immunol. 24: 517–529.

6. Burns, D. L., R. S. Wallace, and J. A. Teare. 1994. Successful treatment of mycobacterial osteomyelitis in a Matschie's tree kangaroo (*Dendrolagus matschiei*). J. Zoo Wildl. Med. 25: 274–280.

7. Bush, M., and R. J. Montali. 1998. Medical management of tree kangaroos. *In:* Fowler, M. E., and R. E. Miller (eds.). Zoo and Wild Animal Medicine, 4th ed. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 337–343.

8. Bush, M., R. J. Montali, S. Murray, S. Holland, and T. M. Phillips. 1995. The diagnosis, treatment and prevention of tuberculosis in captive Matschie's tree kangaroos (*Dendrolagus matschiei*). Proc. Am. Assoc. Zoo Vet., Wildl. Dis. Assoc., Am. Assoc. Wildl. Vet. 1995: 312–314 (Abstr.).

9. Campbell III, T. M., T. W. Clark, and C. R. Groves. 1982. First record of pygmy rabbits (*Brachylagus idahoensis*) in Wyoming. Great Basin Nat. 42: 100.

10. Centers for Disease Control. 2002. Guidelines for preventing opportunistic infections among HIV-infected persons, 2002. Morb. Mortal. Wkly. Rep. 51(RR-8): 10–11. Available at: http://www.cdc.gov/mmwr/PDF/rr/rr5108.pdf. Accessed on 25 October 2004.

11. Chen, M. M., C. S. Lee, and J. H. Perrin. 1984. Absorption and disposition of ethambutol in rabbits. J. Pharm. Sci. 73: 1053–1055.

12. Coffman, R. L., and A. M. Beebe. 1998. Genetic control of the T cell response to *Leishmania major* infection. Adv. Exp. Med. Biol. 452: 61–66.

13. Daniels, M. J., M. R. Hutchings, P. M. Beard, D.

Henderson, A. Greig, K. Stevenson, and J. M. Sharp. 2003. Do non-ruminant wildlife pose a risk of paratuberculosis to domestic livestock and vice versa in Scotland? J. Wildl. Dis. 39: 10–15.

14. Green, J. S., and J. T. Flinders. 1980. *Brachylagus idahoensis*. Mamm. Species 125: 1–4.

15. Griffin, A., A. L. Newton, L. R. Aronson, D. C. Brown, and R. S. Hess. 2003. Disseminated *Mycobacterium avium* complex infection following renal transplantation in a cat. J. Am. Vet. Med. Assoc. 222: 1097–1101.

16. Hays, D. W. 2001. Washington Pygmy Rabbit: Emergency Action Plan for Species Survival. Washington Department of Fish and Wildlife, Wildlife Program, Olympia, Washington.

17. Hines, M. E., J. M. Kreeger, and A. J. Herron. 1995. Mycobacterial infections of animals: pathology and pathogenesis. Lab. Anim. Sci. 45: 334–351.

18. Holland, S. M. 2001. Immune deficiency presenting as mycobacterial infection. Clin. Rev. Allergy Immunol. 20: 121–137.

19. Iralu, J. V., V. K. Sritharan, W. S. Pieciak, D. F. Wirth, J. H. Maguire, and R. H. Barker Jr. 1993. Diagnosis of *Mycobacterium avium* bacteremia by polymerase chain reaction. J. Clin. Microbiol. 31: 1811–1814.

20. Isaza, R. 2003. Tuberculosis in all taxa. *In:* Fowler, M. E., and R. E. Miller (eds.). Zoo and Wild Animal Medicine, 5th ed. Elsevier Science, St. Louis, Missouri. Pp. 689–696.

21. Lyman, R. L. 1991. Late quaternary biogeography of the pygmy rabbit (*Brachylagus idahoensis*) in eastern Washington. J. Mammal. 72: 110–117.

22. Lyman, R. L. 2004. Biogeographic and conservation implications of late quaternary pygmy rabbits (*Brachylagus idahoensis*) in eastern Washington. West. North Am. Nat. 64: 1–6.

23. Mann, P. C., R. J. Montali, and M. Bush. 1982. Mycobacterial osteomyelitis in captive marsupials. J. Am. Vet. Med. Assoc. 181: 1331–1333.

24. Mazurek, G. H., D. P. Chin, S. Hartman, V. Reddy, C. R. Horsburgh Jr., T. A Green, D. M. Yajko, P. C. Hopewell, A. L. Reingold, and J. T. Crawford. 1997. Genetic similarity among *Mycobacterium avium* isolates from blood, stool, and sputum of persons with AIDS. J. Infect. Dis. 176: 976–983.

25. Montali, R. J., M. Bush, R. Cromie, S. M. Holland, J. N. Maslow, M. Worley, F. G. Witebsky, and T. M. Phillips. 1998. Primary *Mycobacterium avium* complex infections correlate with lowered cellular immune reactivity in Matschie's tree kangaroos (*Dendrolagus matschiei*). J. Infect. Dis. 178: 1719–1725.

26. Nowak, R. M. 1991. Order Lagomorpha. *In:* R. M. Nowak (ed.). 1991. Walker's Mammals of the World, 5th ed., vol. 1. Johns Hopkins University Press, Baltimore, Maryland. Pp. 539–560.

27. Ohkusu, K., L. E. Bermudez, K. A. Nash, R. R. MacGregor, and C. B. Inderlied. 2004. Differential virulence of *Mycobacterium avium* strains isolated from HIV-infected patients with disseminated *M. avium* complex disease. J. Infect. Dis. 190: 1347–1354.

28. Peloquin, C. A. 1997. *Mycobacterium avium* complex infection: pharmacokinetic and pharmacodynamic considerations that may improve clinical outcomes. Clin. Pharmacokinetics 32: 132–44.

29. Peloquin, C. A. 2002. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs 62: 2169–2183.

30. Peloquin, C. A., A. E. Bulpitt, G. S. Jaresko, R. W. Jelliffe, J. M. Childs, and D. E. Nix. 1999. Pharmacokinetics of ethambutol under fasting conditions, with food, and with antacids. Antimicrob. Agents Chemother. 43: 568–572.

31. Peloquin, C. A., R. Namdar, M. D. Singleton, and D. E. Nix. 1999. Pharmacokinetics of rifampin under fasting conditions, with food, and with antacids. Chest 115: 12–18.

32. Phillips, T. M. 2001. Analysis of single-cell cultures by immunoaffinity capillary electrophoresis with laser-induced fluorescence detection. Luminescence 16: 145–152.

33. Phillips, T. M., and B. F. Dickens. 1998. Analysis of recombinant cytokines in human body fluids by immunoaffinity capillary electrophoresis. Electrophoresis 19: 2991–2996.

34. Raymond, J. T., L. Tell, M. Bush, D. K. Nichols, F. Y. Schulman, and R. J. Montali. 2000. Subcutaneous atypical mycobacteriosis in captive tiger quolls (*Dasyurus maculatus*). Vet. Pathol. 37: 137–142.

35. Reiner, S. L., and R. M. Locksley. 1995. The regulation of immunity to *Leishmania major*. Ann. Rev. Immunol. 13: 151–177.

36. Shirtliff, M. E., J. T. Mader, and J. Calhoun. 1999. Oral rifampin plus azithromycin or clarithromycin to treat osteomyelitis in rabbits. Clin. Orthop. Related Res. 359: 229–236.

37. Southwood, L. L., C. E. Kawcak, C. W. McIlwraith, D. D. Frisbie, and P. F. Steyn. 2003. Use of scintigraphy for assessment of fracture healing and early diagnosis of osteomyelitis following fracture repair in rabbits. Am. J. Vet. Res. 64: 736–745.

38. Tell, L. A., J. Foley, M. A. Needham, and R. L. Walker. 2003. Diagnosis of avian mycobacteriosis: comparison of culture, acid-fast stains, and polymerase chain reaction for the identification of *Mycobacterium avium* in experimentally inoculated Japanese quail (*Coturnix coturnix japonica*). Avian Dis. 47: 444–452.

39. United States Fish and Wildlife Service. 2001. Emergency rule to list the Columbia basin distinct population segment of the pygmy rabbit (*Brachylagus idahoensis*) as endangered. 66 FR 59734. Office of the Federal Register. Washington, D.C.

40. United States Fish and Wildlife Service. 2003. Final Rule to List the Columbia Basin Distinct Population Segment of the Pygmy Rabbit (*Brachylagus idahoensis*) as endangered. 68 FR 10388. Office of the Federal Register. Washington, D.C.

41. Von Reyn, C. F., J. N. Maslow, T. W. Barber, J. O. Falkinham III,and R. D. Arbeit. 1994. Persistent colonization of potable water as a source of *Mycobacterium avium* infection in AIDS. Lancet 343: 1137–1141.

42. Warheit, K. I. 2001. Genetic Diversity and Population Differentiation of Pygmy Rabbits (*Brachylagus ida-*

hoensis). Washington Department of Fish and Wildlife, Olympia, Washington.

43. Warheit, K. I. 2004. Preliminary assessment of rangewide population differentiation and geographic structure in pygmy rabbits: microsatellite and cytochrome b analysis. First Pygmy Rabbit Workshop (Washington Department of Fish and Wildlife) 2004: 2. (Abstr.)

44. Waters, W. R., J. M. Miller, M. V. Palmer, J. R. Stabel, D. E. Jones, K. A. Koistinen, E. M. Steadham, M. J. Hamilton, W. C. Davis, and J. P. Bannantine. 2003. Early induction of a humoral and cellular immune response during experimental *Mycobacterium avium* subsp. *paratuberculosis* infection of calves. Infect. Immunol. 71: 5130–5138.

45. Zenone, T., A. Boibieux, S. Tigaud, J. F. Fredenucci, V. Vincent, C. Chidiac, and D. Peyramond. 1999. Non-tuberculous mycobacterial tenosynovitis: a review. Scand. J. Infect. Dis. 31: 221–228.

46. Zhang, M., Y. Lin, D. V. Iyer, J. Gong, J. S. Abrams, and P. F. Barnes. 1995. T-cell cytokine responses in human infection with *Mycobacterium tuberculosis*. Infect. Immun. 63: 3231–3234.

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