# Disease Issues Affecting Species Recovery of Pygmy Rabbits

Lisa Harrenstien, DVM, Dipl ACZM, Mitch Finnegan, DVM, Allison Case, DVM, Nina Woodford, DVM, MPH, Dipl ACLAM, Kristin Mansfield, DVM, MPVM, Michael Garner, DVM, Dipl ACVP, and Penny Becker, PhD

**Session: Poster 1**

*Affiliation:* From Oregon Zoo, 4001 SW Canyon Rd, Portland, OR 97221, USA (Harrenstien, Finnegan); Northwest Trek Wildlife Park, 11610 Trek Dr East, Eatonville, WA 98328, USA (Case); the Office of the Campus Veterinarian, Washington State University, PO Box 641165, Pullman, WA 99164, USA (Woodford); Washington Department of Fish and Wildlife, 2315 N Discovery Place, Spokane Valley, WA 99216, USA (Mansfield); Northwest Zoopath, 654 West Main St, Monroe, WA 98272, USA (Garner); and Washington Department of Fish and Wildlife, 1550 Alder St NW, Ephrata, WA 98823, USA (Becker).

*Abstract:* The Columbia Basin pygmy rabbit (*Brachylagus idahoensis*) is critically endangered in the wild and captive propagation efforts have been active since 2001 in an attempt to raise rabbits for restoration of a free-ranging population. Disease issues have had a major impact on the numbers of captive pygmy rabbits available for breeding and/or release to the wild. The most common causes of morbidity and mortality encountered in captivity have included poor neonatal survival due to maternal neglect or neonate-based causes, coccidiosis with *Eimeria* and *Cryptosporidium* species, enteritis or typhlitis/colitis, trauma, poxviral fibromas, and mycobacteriosis. These medical issues have accounted for 152 (80%) of 190 pygmy rabbit deaths in captivity in the past 3 years, seriously impacting the numbers of animals available for release to the wild.

## Introduction

The pygmy rabbit (*Brachylagus idahoensis*) is the smallest rabbit native to North America. It reaches an average adult body weight of 400 g, has a typical lifespan of up to 3 years in captivity, and is the only native North American species of lagomorph that burrows. It is found in the Great Basin area, including portions of the states of Washington, Oregon, Idaho, Montana, Wyoming, Utah, Nevada, and California.[1] Columbia Basin pygmy rabbits (CBPR) comprise a subpopulation of pygmy rabbits, which were geographically and genetically isolated in central Washington from at least 10,000 years ago until the last known wild subpopulation was extirpated by 2004.[1] The Columbia Basin pygmy rabbit was listed as a state endangered species by the Washington Department of Fish and Wildlife (WDFW) in 1993, and has been the subject of an endangered species recovery effort by WDFW and the United States Fish and Wildlife Service (USFWS) since some of the last animals remaining in the wild were taken into captivity for breeding in 2001. A population bottleneck that largely resulted from habitat fragmentation led to low genetic variation in the Columbia Basin subpopulation of pygmy rabbits, and it is believed that cell-mediated immunosuppression has occurred as a result of inbreeding depression.[2] Breeding ("intercrossing") of CBPR with pygmy rabbits from nonendangered populations has occurred in an attempt to improve general hardiness and the CBPR's chance of survival.

## Captive Husbandry

Captive propagation has been a significant part of the effort of the endangered species recovery program. Captive pygmy rabbits are maintained at 3 facilities: Washington State University in Pullman, Washington (approximately

60% of the overall captive population, WSU), Oregon Zoo in Portland, Oregon (30%, OZ), and Northwest Trek Wildlife Park in Eatonville, Washington (10%, NWT). Daily care of pygmy rabbits at captive facilities is accomplished by zookeeper staff and veterinarians at OZ and NWT, and by laboratory animal resources staff and veterinarians at WSU.

Pygmy rabbits require big sagebrush (*Artemisia tridentate tridentata*) as part of their diet, with as much as 99% of their winter diet being the leaves of this plant.[3] Big sagebrush contains high amounts of terpenes, which would be toxic to most other species of herbivores. Other diet items in the wild include a variety of grasses and forbs. Captive diets include ad libitum grass hay (timothy or orchard grass), ad libitum *Artemisia* which is provided for dietary and climbing/hiding purposes, native grasses and forbs (eg, rabbitbrush [*Chrysothamnus* species], wheat grass [*Triticum aestivum*], Nevada blue grass [*Poa nevadensis*]), formulated pellets (16%–20% protein, 30%–38% neutral detergent fiber, approx one-third cup per rabbit), small amounts of fresh green produce (eg, dandelion greens or clover leaves), and supplemental vitamin E (Emcelle Vitamin E TPGS, PMI Nutrition International, St. Louis, MO, USA).

Pygmy rabbits are territorial, solitary animals starting in juvenile life. Reproduction is highly seasonal, occurring February through July. Captive breeding occurs during a 1–6 day period during which a male is temporarily moved into a female's enclosure.[4] Gestation length is approximately 22–24 days and litter size is 2–7 kits. These kits live in a "natal burrow" built by the dam for approximately 14 days until they emerge from the natal burrow. Captive pygmy rabbit juveniles are weaned from their dams at 15–21 days of age, transferred as a litter into a new enclosure, and maintained as a litter group until signs of littermate incompatibility (fur tufts found in the cage indicative of minor aggression) dictate that the rabbits should be housed in separate enclosures.

## Preventative Health Procedures

Juvenile captive pygmy rabbits are anesthetized for examination at approximately 3 months of age, at which time they are given physical examinations, skin biopsies are collected from a pinna for genetic analysis, a transponder is placed in subcutaneous tissue for individual identification, and gender is determined. Adult captive pygmy rabbits are examined annually using manual restraint, during which thoracic auscultation is considered especially important due to the propensity of this species to develop mycobacterial pneumonia (see later section of this manuscript). Reference ranges for blood parameters of healthy adult pygmy rabbits have been generated as part of the preventative medicine program.

General anesthesia of pygmy rabbits has involved chamber induction with 5% isoflurane, followed by maintenance with 3% isoflurane via mask. This anesthetic technique has proven to be relatively easy, safe, and successful. In the early years of the program, pygmy rabbits were premedicated with subcutaneous or intramuscular medetomidine before isoflurane induction because of the possibility that the rabbits would breath-hold and arrest during anesthetic induction. However, pygmy rabbits do not appear to breath-hold during isoflurane administration, and isoflurane induction without medetomidine has been much more straightforward. Systemic analgesics have included butorphanol, buprenorphine, meloxicam or ketoprofen for short-term injectable needs, and meloxicam orally for long-term use.

Pygmy rabbits are medically treated using principles extrapolated from other species, with some drug dosages empirically increased compared to domestic rabbit recommendations due to small body size.

Full necropsy examinations including gross and microscopic evaluation are performed for all juvenile and adult pygmy rabbits that die in captivity, as well as necropsies of one or more kits that are found dead from each litter.

Samples of liver, kidney, lung, cerebrum, and rectum are saved at -80°C for possible retrospective analysis, and each carcass is archived frozen by WDFW. Gonads from wild-caught CBPR have been archived by the San Diego Zoo Institute for Conservation Research in case gamete work (eg, in vitro fertilization) is elected in the future.

Summary reports of veterinary issues regarding pygmy rabbits are generated by Dr. Lisa Harrenstien for the CBPR Species Survival Plan and Lagomorph/Rodent Taxon Advisory Group, managed by the American Zoo and Aquarium Association.

## Morbidity and Mortality Issues

### Poor neonatal survival

Captive neonate kits commonly die before the age of 5 days, with only 40% surviving beyond 14 days, the approximate age of emergence from the natal burrow (M. Illig, unpublished data, March 2011). A pygmy rabbit female's first litter typically does not survive to the age of weaning, especially during the early months of the breeding season (February/March). While many of the dead neonates have ingested milk before death, some show enlarged urinary bladders suggestive of a lack of maternal grooming care. Kits are often found dead or nearly dead at the entrance to a natal burrow where they would normally be nursed. These signs are not consistent within each litter of dead kits and sometimes 1 or 2 kits will survive from a litter. One female pygmy rabbit at WSU was human-raised from birth until the age of 7 weeks when it died of coccidiosis. It is unclear whether dam-based or neonate-based factors are responsible for most neonatal deaths. Postmortem gross and histopathologic examinations of dead neonates typically show evidence of shock or low-grade sepsis, but little else. Nutritional analyses have been unrewarding as to etiology. Deaths of litters before emergence from the natal burrow have been correlated with high fecal glucocorticoids in the lactating dam.[5] Lower fecal glucocorticoids were measured later in the breeding season and from those females housed in enclosures with deeper soil; however, housing without soil had been used previously to reduce the incidence of soil-borne diseases such as mycobacteriosis and coccidiosis. Maternal neglect or exposure of kits to cold ambient temperatures are the strongest theories as to the causes of poor neonatal survival.

### Brachydactyla

Many of the captive pygmy rabbits born between 2002 and 2005 showed congenital shortening of a digit of a front or rear foot, termed "brachydactyla." Of the 81 rabbits radiographed to assess for this condition, 10 (12%) showed lack of mineral density in the affected metacarpal or metatarsal bone, and sometimes the associated phalanges were also affected. Histopathologic exam of demineralized bone did not show abnormalities. This condition was most often seen to involve the third metacarpal or metatarsal. A *Science* article from 1935 describes a similar finding as the result of a single simple recessive mutation in domestic rabbits.[6] Affected pygmy rabbits grew grossly normal nails on these digits and did not appear to behave differently or show morbidity related to the deformity. The condition was not seen in rabbits born after 2005.

### *Eimeria* coccidiosis

From as early as May 2002, coccidiosis has been the cause of death for many captive juvenile pygmy rabbits. Cell-mediated immunity is important for defense against coccidial infection, and it is believed that the cell-mediated immunosuppression seen in CBPR's likely contributes to the high morbidity and mortality. Microscopic exam of pygmy rabbits' coccidial oocysts and necropsy tissues has shown a predominance of *Eimeria brachylagia*, which is a newly-described species different from the 12 *Eimeria* species reported to infect the *Sylvilagus* genus.[7] Due

to the risk of coccidiosis in juveniles, all juveniles up to 3 months old are treated with coccidiostat medication. At Oregon Zoo, ponazuril is used at the dosage of 30 mg/kg orally once per week. Adult females at Oregon Zoo during the breeding season also receive ponazuril 30 mg/kg orally weekly in an attempt to reduce coccidial oocyst contamination of enclosures that intermittently house neonates. All rabbits regardless of age receive ponazuril in the week prior to shipment to another facility and in the week after shipment from another facility, due to presumed stress-induced immunosuppression and higher risk of coccidial morbidity and mortality.

### Cryptosporidiosis

At least 5 juvenile pygmy rabbits in 2008 and 2009 died of cecal cryptosporidiosis at OZ, diagnosed by histopathologic examination. Polymerase chain reaction (PCR) or enzyme-linked immunosorbent assay testing of cecal contents of 4 affected dead pygmy rabbits were all negative for cryptosporidia, but *Cryptosporidium muris* was still strongly suspected as the etiologic agent. Biosecurity measures between enclosures were increased but no preventative medications were administered to remaining rabbits. No cryptosporidiosis cases have been found since 2009.

### Diarrhea, coronavirus, and typhlitis/colitis

Beginning in 2004, necropsies of many adult pygmy rabbits included the finding of no formed feces in the colon or rectum. Diarrhea was a peracute clinical sign, with many of these animals showing no diarrheal feces in their enclosure before death. An intensive investigation led to the suspicion of a coronavirus as the cause; however, the direct association of coronavirus with an animal's death was not possible because several rabbits that tested coronavirus-positive (based on PCR testing of feces) did not show signs of illness. During 2008–2010, 11/39 (28%) adult pygmy rabbit deaths at OZ were attributed to typhlitis and/or colitis, suspected to be related to nonclostridial bacterial overgrowth or an unknown etiology. Diarrhea has been a nonspecific clinical sign in pygmy rabbits, and it is believed that intestinal contents of pygmy rabbits rapidly become more liquid due to acute stress (including the stress experienced during severe illness from an extraintestinal cause). Pygmy rabbits presenting nonacutely with abnormal feces are usually successfully managed with diet changes including fewer carbohydrates, a smaller quantity of pellets, and no fresh greens.

### Trauma

Pygmy rabbits may acquire wounds from cagemate (littermate or breeding partner) aggression, but healing from these wounds is typically rapid and complete as in domestic rabbits. Long bone fractures occur occasionally, related to pygmy rabbits' propensity to climb and jump, and they heal well with antibiotic and analgesic treatment, along with internal or external fixation. Captive pygmy rabbit enclosures are surrounded by solid metal panels or wire mesh to prevent entry by predators (eg, weasels, raptors). Death is rarely a result of trauma in captivity (becoming entrapped in the crook of a sagebrush branch, for example).

### Poxviral skin fibromas

Nasal skin masses were seen on 5 pygmy rabbits at OZ in September 2006. Histopathologic evaluation and virus isolation determined these masses were poorly differentiated sarcomas due to infection with a virus related to the Shope fibroma virus. Myxomavirus was not found in these rabbits. Viral transmission was suspected to have occurred from wild *Sylvilagus* rabbits near the pygmy rabbit enclosures, transported by mosquitoes. Masses regressed in 2–6 weeks without treatment, did not recur, and did not cause mortality. Similar cases were not seen at WSU or NWT.

### Mycobacteriosis

Mycobacteriosis due to *Mycobacterium avium-intracellulare* has been the most common cause of death of adult captive pygmy rabbits, accounting for 29/79 (37%) adult deaths since 2008. This organism is common in soil and water, therefore is frequently encountered by burrowing pygmy rabbits. Suboptimal cell-mediated immunity appears to be the strongest possible etiology for the high morbidity and mortality of *M avium-intracellulare* infections in pygmy rabbits.[2] A variety of antemortem tests have been tried for diagnosis of subclinically infected pygmy rabbits, with the most sensitive and specific being thoracic auscultation to detect pneumonia, whole-body radiographic examination to detect granulomas in lung or bone, and serum analysis to detect a reduced albumin/globulin ratio.[2] Affected animals have been treated with a variety of medications, with variable and limited success. The most effective palliative treatment appears to be the long-term administration of a combination of azithromycin, rifabutin, and ethambutol.

## Release Plans

A trial release of 20 rabbits was done in March 2007 in the sagebrush-steppe ecosystem of central Washington, an area in which Columbia Basin pygmy rabbits had previously been found. None of these released rabbits survived longer than a few months, although one conceived and gave birth in the wild. This was a relatively "hard" release technique, and a "softer" release technique is planned for the next releases in spring and fall 2011. The 2011 releases will very likely include all of the captive-held rabbits, therefore the captive propagation program could end at that time.

*Acknowledgments:* The authors would like to thank the staff, volunteers, and graduate students of the United States Fish and Wildlife Service, Washington Department of Fish and Wildlife, Oregon Zoo, Northwest Trek Wildlife Park, and Washington State University for their many years of dedication to the recovery of wild populations of the Columbia Basin pygmy rabbit.

## References

1. United States Fish and Wildlife Service. 2010 Columbia Basin distinct population segment of the pygmy rabbit (*Brachylagus idahoensis*) 5-year review: summary and evaluation. Available at: http://ecos.fws.gov/docs/five year review/doc3591.pdf. Accessed February 24, 2011.

2. Harrenstien LA, Finnegan MV, Woodford NL, et al. *Mycobacterium avium* in pygmy rabbits (*Brachylagus idahoensis*): 28 cases. *J Zoo Wildl Med*. 2006;37:498–512.

3. Shipley LA, Davila TB, Thines NJ, Elias BA. Nutritional requirements and diet choices of the pygmy rabbit (*Brachylagus idahoensis*): a sagebrush specialist. *J Chem Ecol*. 2006;32:2455–2474.

4. Elias BA, Shipley LA, Sayler RD, Lamson RS. Mating and parental care in captive pygmy rabbits. *J Mamm*. 2006;87:921–928.

5. Scarlata CD. Relationships among stress, reproduction and housing conditions in captive pygmy rabbits (*Brachylagus idahoensis*). PhD thesis, North Carolina State University, Raleigh, NC; 2010.

6. Greene HSN. Hereditary brachydactylia and associated abnormalities in the rabbit. *Science* 1935;81: 405–407.

7. Duszynski DW, Harrenstien L, Couch L, Garner MM. A pathogenic new species of *Eimeria* from the pygmy rabbit, *Brachylagus idahoensis*, in Washington and Oregon, with description of the sporulated oocyst and intestinal endogenous stages. *J Parasitol*. 2005;91:618–623.