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Infectious disease and red wolf conservation: assessment of disease occurrence and associated risks

Kristin E. Brzeski,* Rebecca B. Harrison, William T. Waddell, Karen N. Wolf, David R. Rabon, Jr., and Sabrina S. Taylor

School of Renewable Natural Resources, Louisiana State University Agricultural Center and Louisiana State University, RNR 227, Baton Rouge, LA 70803, USA (KEB, SST)
U.S. Fish & Wildlife Service, Red Wolf Recovery Program, P.O. Box 1969, Manteo, NC 27954, USA (RBH, DRR)
Point Defiance Zoo and Aquarium, 5400 North Pearl Street, Tacoma, WA 98407, USA (WTW, KNW)

Present address of DRR: Endangered Wolf Center, P.O. Box 760, Eureka, MO 63025, USA

* *Correspondent: kbrzes1@lsu.edu*

Infectious diseases pose a significant threat to global biodiversity and may contribute to extinction. As such, establishing baseline disease prevalence in vulnerable species where disease could affect persistence is important to conservation. We assessed potential disease threats to endangered red wolves (*Canis rufus*) by evaluating regional (southeastern United States) disease occurrences in mammals and parasite prevalence in red wolves and sympatric coyotes (*Canis latrans*) in North Carolina. Common viral pathogens in the southeast region, such as canine distemper and canine parvovirus, and numerous widespread endoparasites could pose a threat to the red wolf population. The most prevalent parasites in red wolves and sympatric coyotes were heartworm (*Dirofilaria immitis*), hookworm (*Ancylostoma caninum*), and *Ehrlichia* spp.; several red wolves and coyotes were also positive for bacteria causing Lyme disease (*Borrelia burgdorferi*). Coyotes had a more species-rich parasite community than red wolves, suggesting they could harbor more parasites and act as a disease reservoir. Species identity and sex did not significantly affect parasite loads, but young canids were less likely to have heartworm and more likely to have high levels of endoparasites. Continued disease monitoring is important for red wolf recovery because low levels of genetic variability may compromise the wolves' abilities to combat novel pathogens from closely related species, such as domestic dogs and coyotes.

Key words: *Canis latrans*, *Canis rufus*, disease management, endangered species, endoparasites, helminths, species richness

Wildlife disease epizootics, or epidemics, are becoming an urgent issue for the conservation and management of threatened and endangered species (Daszak 2000; Smith et al. 2009). For instance, disease outbreaks have contributed to several near extinctions and population crashes (see references in Woodroffe 1999; de Castro and Bolker 2004), directly and indirectly threatening wildlife populations by killing hosts faster than replacement, an outcome that makes small populations vulnerable to stochastic extinction (Woodroffe 1999). Generalist pathogens may pose the greatest risk to threatened wild populations because they can remain at high prevalence in numerous host species, lowering a pathogen's density threshold for transmission in small populations, which themselves are not dense enough for disease transmission (Lyles and Dobson 1993; Woodroffe 1999). The threat of infectious disease and pathogen-mediated population declines is compounded in threatened and endangered populations because they are small and often lack the genetic variability necessary to combat virulent pathogens (Spielman et al. 2004), making disease monitoring a necessary component of conservation programs.

Threatened and endangered populations can be especially vulnerable to disease that is transmitted by common, wide ranging species (Murray et al. 1999). For example, the catastrophic canine distemper virus (CDV) epizootic in wild endangered black-footed ferrets (*Mustela nigripes*) was likely transmitted by sympatric coyotes or badgers (*Taxidea taxus*—Williams et al. 1988). Similarly, dense populations of domestic dogs (*Canis lupus familiaris*) act as rabies vectors for endangered Ethiopian wolves (*Canis simensis*—Randall et al. 2004). Generalist viral pathogens like CDV or rabies are most often responsible for disease-driven population declines, but other pathogenic groups, such as bacteria, helminths, arthropods, or protozoa, can also be detrimental for small populations (Pedersen et al. 2007). Although such pathogens

are generally not lethal on their own, co-infections combined with stressful situations could reduce individual fitness and negatively affect population growth, as well as reduce juvenile survival (Forrester 1971). Inbreeding and reduced genetic variation can also interact with sublethal parasites to decrease fitness, as observed in an inbred population of Soay sheep (*Ovis aries*), where individuals with low genetic variation had more gastrointestinal parasites and lower survival rates during harsh winters than more genetically diverse sheep (Coltman et al. 1999).

Among mammals, carnivores are particularly susceptible to disease, with the highest number of species threatened by pathogens found in the canid family (Pedersen et al. 2007). Canid social behavior may explain their heightened susceptibility to pathogens as they commonly lick each other, smell and eat feces, and smell urine that may be infectious (Woodroffe et al. 2004). Other disease risk factors for wild canids include their close genetic relatedness to domestic dogs, which are globally distributed and harbor diseases easily transmissible to wild canids, their trophic position, which can expose canids to infected prey (Woodroffe et al. 2004), and their low population size. These various risk factors emphasize how disease can contribute to population declines and local extinction in canids, the best documented examples of which include: rabies in African wild dogs (*Lycaon pictus*—Gascoyne et al. 1993), gray wolves (*Canis lupus*—Chapman 1978; Ballard and Krausman 1997), and Ethiopian wolves (*C. simensis*—Sillero-Zubiri et al. 1996; Randall et al. 2004); canine parvovirus (CPV) and CDV in gray wolves (Johnson et al. 1994; Mech and Goyal 1995); and sarcoptic mange (caused by the mite *Sarcoptes scabiei*) in arctic foxes (*Vulpes lagopus semenovi*—Goltsman et al. 1996; Ploshnitsa et al. 2011). In the United States, the red wolf (*Canis rufus*), one of the most endangered canids in the world, is emblematic of the need to evaluate and incorporate disease in canid species management.

Historically, red wolves were abundant throughout the eastern and southeastern United States, but populations were decimated in the 20th century due to habitat loss, intense predator control programs, hybridization, and disease, and the species was declared extinct in the wild by 1980 (Phillips and Parker 1988; Hinton et al. 2013). In the 1970s, the last remnant red wolves were trapped from southwestern Louisiana and southeastern Texas to start a captive breeding program. Two populations of red wolves were reintroduced, one in northeastern North Carolina (1987) and one in the Great Smoky Mountains National Park, Tennessee (1991). In 1998, Tennessee restoration efforts were discontinued due to poor pup survival associated with malnutrition and possibly parasites and CPV infections (Henry 1998). As a result, the northeastern North Carolina population, with 90–110 individuals, represents the only wild red wolf population (United States Fish & Wildlife Service 2014).

Red wolf viability had already been critically affected by disease in the remnant Louisiana-Texas population and the Smoky Mountain site, and contemporary wild red wolves in North Carolina could be vulnerable as well. North Carolina red wolves may be at risk for disease-driven declines because they persist in one small population, are inbred (Brzeski et al. 2014), and co-occur with high population density species, such as domestic dogs and coyotes (*Canis latrans*), that can be infected with the same pathogens and act as pathogen reservoirs (Eads 1948; Almberg et al. 2009). Coyotes are of particular concern because they hybridize and interact with red wolves, and although hybridization is effectively controlled by management (Stoskopf et al. 2005; Gese et al. 2015), their frequent interaction could increase disease transmission to red wolves. Additionally, coyotes may expose red wolves to new diseases that they carry into the recovery area from surrounding regions (Hinton et al. 2012) and from elsewhere in the southeast where coyotes have been moved by humans (Hill et al. 1987).

Disease risk in the red wolf recovery area may be offset because wolves and sympatric coyotes are both opportunistically given an 8-way dog vaccination (CDV, CPV2, Adenovirus Types 1 and 2, parainfluenza, 2-Leptospirosis, and corona virus, supplied from Boehringer Ingelheim Vetmedica, Inc., St. Joseph, Missouri), rabies vaccination (Merial Limited, Duluth, Georgia), and flea/tick prevention when they are captured during seasonal trapping efforts. Yet, vaccines may not adequately protect red wolves because they are administered opportunistically, only a small fraction of the coyote population is vaccinated, and the efficacy of domestic dog vaccines for wild species is uncertain (Harrenstien et al. 1997; Acton et al. 2000; Acton 2008). For instance, initial vaccines are administered to wolves around 9–12 months of age, leaving younger pups exposed to infection after losing maternal antibodies around 5 months of age (Johnson et al. 1994). Another possible threat is the emergence of new vaccine-resistant viral strains, a scenario observed in Africa when a virulent new bio-type of CDV was responsible for mortality among Serengeti lions (*Panthera leo*—Roelke-Parker et al. 1996).

Potential vulnerability of red wolves to disease highlights the critical need for a systematic, focused, and informed disease monitoring and prevention plan. Evaluating pathogen loads and diversity in red wolves and sympatric coyotes, and the factors that influence disease infection are needed to inform any disease prevention plan in the recovery area. The first steps for assessing disease risk factors include an evaluation of past red wolf disease and disease occurrence in the region surrounding the North Carolina population to identify potential threats already present on the landscape. Additionally, collecting contemporary disease data on both red wolves and sympatric coyotes will establish baseline parasite prevalence and diversity and reveal differences and similarities between the species' pathogens. To accomplish these goals, we 1) reviewed past disease occurrences in wild and captive red wolves, 2) reviewed wildlife disease literature from the southeastern United States to evaluate broadly the regional disease occurrence in mammals, and 3) collected contemporary parasite data from wild red wolves and sympatric coyotes to examine current baseline infection patterns.

Materials and Methods

Assessment of red wolf and regional parasite literature.—We reviewed existing literature on disease prevalence and risk in

wild and captive red wolves by searching Web of Science for articles containing the words ["canis rufus" AND (_disease_ OR _parasit*_ OR _pathogen_)]. Additionally, we checked citations of pertinent red wolf papers to ensure that we did not miss information. We also reviewed the Red Wolf Recovery Program's records, which provide information on causes of death and necropsy results. To review literature related to infectious disease in southeastern United States wildlife populations and identify potential regional disease threats to red wolves, we searched for articles containing the words ["United States" AND south* AND (_disease_ OR _parasit*_ OR _pathogen_)] and surveyed the following journals for relevant studies: *Journal of Zoo and Wildlife Medicine*, *Journal of Wildlife Disease*, *Journal of Veterinary Medicine, American Journal of Veterinary Research, Journal of Parasitology, American Midland Naturalist*, and *Southeastern Naturalist*. We only examined articles evaluating terrestrial mammal pathogens since they are the most likely source of infections for red wolves. We also searched the Global Mammal Parasite Database, www.mammalparasites.org (Nunn and Altizer 2005) by region.

Parasite prevalence in the contemporary red wolf and coyote population.—Red wolves and coyotes were trapped during the winter every year for routine management by United States Fish & Wildlife Service biologists. Canids were captured with padded leg hold traps and physically restrained for processing, during which they were weighed, aged, measured, sampled for blood, and fitted with telemetry radio-collars. We evaluated several aspects of parasite prevalence in red wolves and coyotes during this process in 2013 and 2014; we used the term parasite to include microparasites (i.e., bacteria) and macroparasites (i.e., helminths, arthropods, protozoans).

Endoparasites, which can reduce a host's physical condition and survival (Eira et al. 2006), were measured through several analyses. We collected fresh fecal samples during processing and sent them to the University of Tennessee's Veterinary Medical Center diagnostic laboratory (Knoxville, Tennessee) for sugar and zinc fecal floats to assess species prevalence and individual infection levels. Infection levels were based on the number of eggs, cysts, or oocysts detected on fecal slides surveyed at 10× magnification across 12 transects, where none = no eggs, cysts, or oocysts detected; low = 1–12 eggs, cysts, or oocysts; intermediate ≥ 12, but eggs, cysts, or oocysts not present on every transect; heavy ≥ eggs, cysts, or oocysts on every transect. We tested for canine heartworm (*Dirofilaria immitis*) infections with SNAP Heartworm RT Tests (IDEXX Laboratories, Westbrook, Maine) in 2013 and SNAP 4Dx Tests (IDEXX Laboratories) in 2014. We tested for CPV in 2013 with SNAP Parvo Tests (IDEXX Laboratories), but as no active infections were detected, we did not test for CPV in 2014. We also tested for tick-borne illnesses with SNAP 4DX Tests, which provide a negative or positive for bacteria causing Lyme disease (*Borrelia burgdorferi*), and for *Ehrlichia* spp. (*E. canis* or *E. ewingii*), and *Anaplasma* spp. (*A. platys* or *A. phagocytophilum*).

We evaluated ectoparasite infestations for each canid by inspecting the neck, ears, perianal area, and axillae. We removed ectoparasites by hand or with a flea comb, storing them in 70% ethanol; combs were sterilized between canids. Ectoparasites were grouped by order and counted to establish an ectoparasite load for each captured canid; loads were defined as few (< 5), intermediate (5–100), and heavy (> 100). All research on live canids followed the guidelines of the American Society of Mammalogists (Sikes et al. 2011) and was approved by the Louisiana State University AgCenter Animal Care and Use Committee (protocol # A2013-16).

Statistical methods.—We compared endoparasite communities (including heartworm) in red wolves and coyotes with rarefaction estimates of species richness using the program EstimateS version 9.1.0 (Colwell 2013). The sample-based, rarefaction method estimates the expected number of parasite species represented among red wolves and coyotes, given the observed samples to generate predicted estimates of parasite richness. We also extrapolated the rarefaction curve to a sample size of 50 canid individuals to evaluate how endoparasite species richness varied between red wolves and coyotes with equal and larger sample sizes. We based significant differences between red wolf and coyote rarefaction estimates on nonoverlapping 95% *CI*s generated through bootstrapping routines in EstimateS, which is a conservative estimate of significance (Colwell et al. 2012).

We assessed factors influencing parasite infections with generalized linear mixed effect models (GLMMs) using the R package lme4 (Bates and Maechler 2010) and with cumulative link mixed models (CLMMs) using the R package ordinal (Christensen 2012). Explanatory variables for each model included age class, sex, species, and year collected with a random effect of region captured (coyotes) or pack (wolves). We included random effects to control for nonindependence between individuals from the same pack or trapping region. We defined age classes as pup (less than 12 months old), juvenile (greater than 12 months but under 2 years), and adult (greater than 2 years); we determined age by date of birth for wolves and based estimated ages on tooth wear (Gier 1975) and sexual maturity for coyotes. We ran 12 a priori candidate model sets, including a null and global model (Supporting Information S1–8), separately for each of the following response variables: endoparasite counts (tally of infectious species, weighted by infection level), heartworm presence, ectoparasite loads, and any other pathogenic parasite (either individual endoparasite species or tick-borne bacteria) with an observed infection rate above 10%. We evaluated the probability of specific endoparasite species, heartworm, and tick-borne bacteria using GLMMs with a logit-link function and binomial error distribution; models with ectoparasite loads were evaluated using CLMMs with a log-link function. We assessed endoparasite counts using GLMMs with a log-link function and Poisson distribution. All models were ranked with AIC_c and AIC_c weight (w_i—Burnham and Anderson 2002) and validated by examining residuals and fitted values as suggested by Zuur et al. (2009). We averaged models within $\Delta 2$ AIC_c of the top model using the natural-average method (Burnham and Anderson 2002) in R package MuMIn (Bartoń 2009); we also used analysis of variance to evaluate if additional variables significantly improved model

fit. Given that adult heartworm prevalence was high, we evaluated if adult red wolves were more likely to have heartworm than adult coyotes with Fisher's exact test; we were unable to test this with GLMMs given small sample sizes (adult red wolves tested for heartworm = 13, adult coyotes tested for heartworm = 10).

Results

Red wolf literature.—The last free ranging red wolves in the historic Louisiana and Texas populations had high infection rates of hookworm (Riley and McBride 1972; Carley 1975; Custer and Pence 1981a), heartworm (*D. immitis*—Riley and McBride 1972; Carley 1975; Custer and Pence 1981b), and sarcoptic mange (*S. scabiei*—Riley and McBride 1972; Carley 1975; Pence et al. 1981). All 3 parasites were considered limiting factors to red wolf survival and may have affected morbidity and mortality significantly (Riley and McBride 1972; Carley 1975; Custer and Pence 1981b). Hookworm infections were especially high in pups and juveniles and may have been a leading cause of juvenile mortality (Custer and Pence 1981a). The severity of heartworm infections increased with age (Custer and Pence 1981b), resulting in pathological responses such as enlarged and deformed hearts, and increasing stress-induced mortality that healthy wolves would likely have survived (Riley and McBride 1972; Carley 1975). Sarcoptic mange was the most serious ectoparasite; infections were so numerous that by the 1970s, 90% of observed red wolves were at least partially devoid of hair (Riley and McBride 1972). Other detected parasites included tapeworm (*Taenia* sp.), demodectic mange mites (*Demodex* sp.), spiny headed worms (class Archiacanthocephala), flatworms (*Heterobilharzia americana*), several species of ticks (*Amblyomma* sp., *Ixodes scapularis*), and 1 louse (*Trichodectes canis*—Riley and McBride 1972; Custer and Pence 1981a; Pence et al. 1981).

Heartworm, endoparasite, and ectoparasite prevalence were evaluated in several of the first reintroduced wild wolves in North Carolina, as well as in captive wolves housed at the initial North Carolina release site (Phillips and Scheck 1991). No captive red wolves had heartworm, and only 1 of 7 tested wild wolves was heartworm positive. Wild adult wolves, however, had been regularly treated with ivermectin, a heartworm prophylactic, prior to release. Captive red wolves had fewer endoparasites (48% infected) than wild wolves (67% infected), but both were infected with several different intestinal parasites including hookworms (both wild and captive wolves), ascarids (more common in captive wolves and only found in pups), whipworms (wild only), and tape-worms (both wild and captive wolves—Phillips and Scheck 1991). Phillips and Scheck (1991) suggested that hookworm was the only parasite occurring at high enough frequencies to be of concern to red wolf health. Three tick species, American dog tick (*Dermacentor variabilis*), lone star tick (*Amblyomma americanum*), and black legged tick (*I. scapularis*), were detected on wild and captive wolves (Phillips and Scheck 1991). Since reintroductions, several tick related illnesses have been detected in wild wolves. Tick paralysis may have occurred in a female red wolf from North Carolina and was positively observed in 1 male, who recovered fully once ticks were removed (Beyer and Grossman 1997). Several red wolves housed at the Great Smoky Mountains National Park were serologically positive for the bacteria causing Lyme disease (*B. burgdorferi*); one positive wolf also exhibited *B. burgdorferi* clinical symptoms, including decreased appetite, weight loss, and carpal lesions (Penrose et al. 2000).

Acton (2008) evaluated CPV2 and CDV prevalence in northeastern North Carolina carnivores, including red wolves, and assessed vaccine efficacy. Based on samples collected from 2000 to 2006, red wolves and coyotes were naturally exposed to both CPV2 and CDV, but North Carolina canid titers were lower than those for other wild canid populations (Acton 2008). CDV vaccines appeared to elicit 100% seroconversion, or the development of detectable vaccine antibodies, but CPV2 vaccines did not reliably elicit seroconversion (Acton 2008). This is similar to results reported by Harrenstien et al. (1997), where red wolf response to CPV2 vaccines was minimal. Based on seroprevalence, poor vaccine efficacy, and neonatal antibody assays, Acton (2008) suggested that CPV2 may contribute to juvenile mortality in wild red wolves. A recent study by Anderson et al. (2014) found 100% and 96.9% of captive wolves had positive CPV and CDV vaccine titers, respectively, 3 years after vaccination, but this was after a full juvenile vaccination series and a 1 year booster, which wild canids usually do not receive. Seroconversion for canine adenovirus was sporadic (Anderson et al. 2014).

Several additional studies document rare medical conditions in captive red wolves, such as bilateral idiopathic dry eye, pyometra, and paten ductus venosus (Day et al. 1992; Neiffer et al. 1999; Kearns et al. 2000; Crissey et al. 2001; Larsen et al. 2002; Acton et al. 2006; Anderson and Wolf 2013). A comprehensive necropsy survey in the captive breeding program documented several causes of death, including neonatal parasitism, cardiovascular and gastrointestinal problems, and possibly one CPV mortality, but chronic infectious diseases did not appear to be a widespread problem (Acton et al. 2000).

Records from the Red Wolf Recovery Program indicated that mange contributed to the death of 18 red wolves in the wild North Carolina population since 1993, and in 46 additional documented cases of mange, wolves were treated and released; both sarcoptic and demodectic mange were identified. Heartworms were regularly reported and have been confirmed as the cause of mortality for 9 wolves. One wolf died due to complications with heartworm treatment; Red Wolf Recovery Program biologists no longer attempt to treat heartworm infections in wild wolves. One wolf died due to CPV.

Disease review in southeastern United States.—We reviewed 185 references that reported wildlife pathogens in the southeastern United States. The most reported, and probably the most tested, viral pathogens were CPV, CDV, rabies, canine adenovirus, and equine encephalitis virus, all of which are pathogenic in canids (Supporting Information S9). Endoparasites, which include organisms such as Cestodes, Nematodes, Protozoa, and

Trematodes, were the most commonly evaluated parasite and were widespread across different host species throughout the southeastern states (Supporting Information S10). Given their prevalence and pathology, several endoparasite species (currently absent in red wolves) may be of particular concern: *Babasia* spp., causing lethargy and neurologic problems (Birkenheuer 2014); *Hepatozoon* spp., causing fever, lameness, lethargy, and skeletal lesions (Vincent-Johnson 2014); *Toxocara* spp., which was detected in 1 North Carolina coyote and can cause lethargy and intestinal distress; *Toxoplasma gondii*, causing organ lesions (Lappin 2014); and, *Trypanosoma cruzi*, causing lethargy, loss of appetite, and sudden death (Barr et al. 2014; Supporting Information S10; see also Supporting Information S11 for disease occurrence in North American canids).

There were several tick-borne bacterial pathogens with high incidence rates in the Southeast including *Ehrlichia* spp., *Borrelia burgdorferi* (bacteria causing Lyme disease) and *Leptospira* spp. (bacteria causing Leptospirosis; Supporting Information S12). *Leptospira* spp., although included in the administered 8-way vaccine and never detected in red wolves, may be a future concern given it is epizootic in domestic dogs and causes symptoms such as fever, lethargy, reluctance to move, anorexia, and respiratory difficulty (Sykes 2014).

Contemporary red wolf and coyote parasite prevalence.—During the winters of 2013 and 2014, 37 red wolves, 51 coyotes, and 3 hybrids (included with coyotes in our analyses) were trapped and examined. One red wolf and 1 coyote were captured in both years; we only analyzed data from their first complete sampling. Fecal parasites were analyzed for 49 individuals, 69 were tested for heartworm, 56 were tested for tick-borne pathogens, and 91 canids were evaluated for ectoparasite loads.

Coyotes harbored more endoparasite species than did red wolves based on rarefaction curves but 95% *CI*s overlapped between the species (Fig. 1). The species accumulation curves showed that parasite richness of red wolves appeared to plateau while coyotes were projected to accumulate more parasites (Fig. 1).

All individuals sampled had at least one endoparasite. Of the 20 different fecal pathogen species detected, 6 are considered nonpathogenic to canids or were possibly incidental ingestions, e.g., mites (Table 1). The most prevalent fecal pathogens, with detection rates over 10%, included *Ancylostoma* spp., *Uncinaria stenocephala*, *Capillaria* spp., *Cystoisospora ohioensis*, *Spirometra*, *Sarcocystis* spp., and *Taeniid* type eggs (*Taenia* spp. and *Echinococcus* spp. eggs are indistinguishable and can only be categorized by egg type). *Ancylostoma* spp. was the most common endoparasite and was detected in 94% of individuals. GLMM model results suggest young canids had more endoparasites (Fig. 2; Supporting Information S1). Year of sampling was also within the top $\Delta 2$ AIC_c models but *CI*s overlapped zero (Table 2). Most GLMM models with individual endoparasite species either encompassed the null model within the top $\Delta 2$ AIC_c models or did not converge (Supporting Information S2–4), except for *U. stenocephala* models, where canids captured in 2014 were less likely to have *U. stenocephala* infections (Table 2; Supporting Information S5).

Heartworm prevalence was high with a 45% infection rate (Fig. 3), and based on the top GLMM model, age class significantly influenced probability of infection where adults were more likely to have heartworm (Table 2; Supporting Information S6). Year of collection was included within the top $\Delta 2$ AIC_c models, but *CI*s overlapped 0 (Table 2). Species and sex did not affect the probability of heartworm infection significantly. Adult red wolves appeared to be more susceptible to heartworm than adult coyotes ($P = 0.02$; Fisher's exact test).

The occurrence of tick-borne diseases varied. Five canids tested positive for Lyme disease: 2 adult male red wolves and 3 coyotes (2 juveniles, 1 pup). One adult male red wolf that tested positive for Lyme disease was in poor condition when trapped and showed symptoms of mange. Due to health concerns, he was tested at a local vet where he was found positive for Lyme disease, *Ehrlichia* spp., and Rocky Mountain spotted fever (*Rickettsia rickettsii*—A. B. Beyer, U.S. Fish & Wildlife Service, April 2013). This same male was recaptured in 2014 in poor condition and was found to be positive for Rocky Mountain

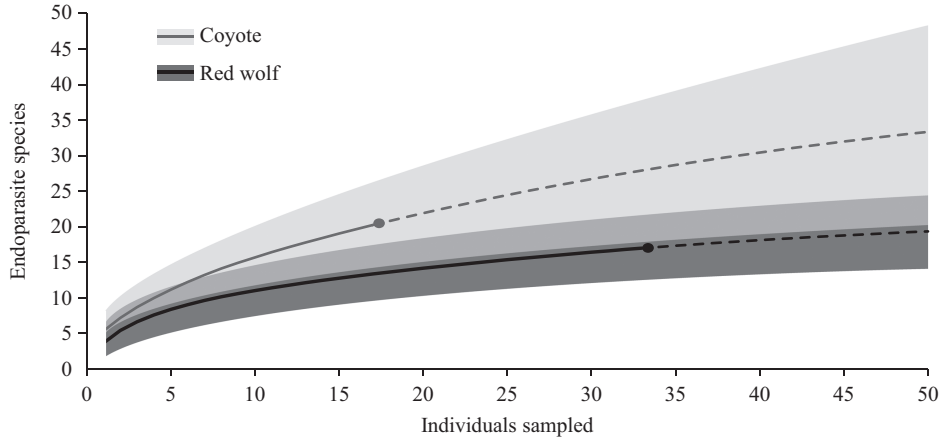

Fig. 1.—Estimated number of endoparasites in endangered red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*) in northeastern North Carolina based on rarefaction (solid lines) and extrapolation (hashed lines). The shaded regions denote 95% confidence limits. Sample sizes, indicated by the solid circles, varied by species (red wolf = 33, coyote = 17).

Table 1.—Endoparasites detected in endangered wild red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*) in northeastern North Carolina 2013 and 2014.

Parasite	Red wolf (n = 33)	Coyote (n = 17)	Prevalence (%)
Helminths			
Ancylostoma spp. *(canimum)*[a,b]	31	16	94
Capillaria spp.[a]	2	6	16
Eucoleus aerophilus	1	0	2
Eucoleus boehmi	1	1	4
Filaroides osleri	0	1	2
Hymenolepis diminuta	2	2	8
Physaloptera spp.[a]	0	1	2
Spirometra	6	2	16
Taeniid type eggs[b,c]	5	1	12
Toxocara canis	0	1	2
Trichuris vulpis[a,b]	1	3	8
Uncinaria stenocephala	11	5	32
Protozoa			
Cystoisospora canis	1	1	4
Cystoisospora ohioensis	6	1	14
Neospora/ Hammondia	1	1	4
Sarcocystis spp.	24	12	72
Arthropoda			
Demodex spp.	1	3	8
Louse spp.[d]	0	1	2
Mite spp.[d]	11	5	32
Coccidia			
Eimeria spp.	2	1	6

[a] Endoparasite species previously detected in remnant Louisiana and Texas red wolf population.
[b] Endoparasite species previously detected in current North Carolina red wolf population.
[c] *Taenia* spp. and *Echinococcus* spp. eggs are indistinguishable and can only be categorized by egg type.
[d] Mite and louse species may be incidental and nonpathogenic.

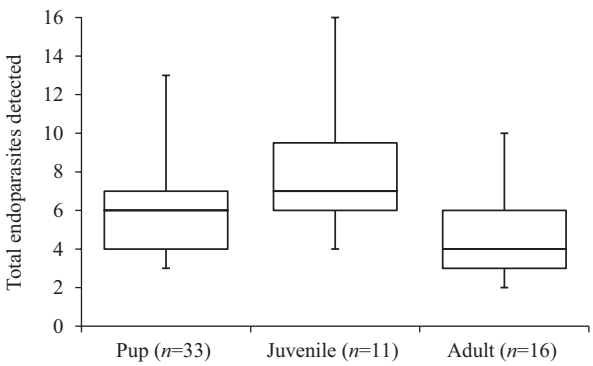

Fig. 2.—Box-and-whisker plot comparing total endoparasites detected in different age classes of endangered wild red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*) in northeastern North Carolina. The bottom of the box is the 25th percentile, the top is the 75th, the middle line represents the median value, and whiskers extend to the highest and lowest observation in each age class. Pups (under 12 months) and juveniles (between 12 and 24 months) were more likely than adults (over 24 months) to have higher endoparasite loads.

spotted fever but not Lyme disease; he was held and re-treated. All of the positive Lyme disease canids were trapped in 2013 except for the one positive coyote pup, which was trapped in 2014 (Table 3). There were no conclusive *Anaplasma* spp.-positive canids, although one male coyote trapped in 2013 had a faint, inconclusive positive SNAP test result. *Ehrlichia* spp. were common, with a 45% infection rate. The top GLMM model indicated older canids were more likely to have *Ehrlichia* spp. infections (Fig. 4). Sex, species ID, and year had little influence on the probability of infection but the null model was within Δ2 AIC_c of the top model (Table 2; Supporting Information S7).

The most common ectoparasites were ticks and biting lice. Individuals were more likely to have higher ectoparasite loads in 2014 than 2013 (Fig. 5), but age class and sex had no effect (Table 2; Supporting Information S8). Species ID was within the top Δ2 AIC_c models where red wolves were more likely to have higher parasite loads than coyotes, but *CI*s overlapped 0 (Table 2).

Discussion

We assessed past red wolf disease occurrence, regional disease threats (Supporting Information S9–13), and collected baseline parasite data on endangered red wolves (Tables 1 and 3) to inform a monitoring plan aimed at preventing disease-mediated population declines in red wolves. Our results highlight several possible pathogen threats to contemporary wild red wolves: (i) coyotes, which may act as a source or reservoir for disease, and (ii) several regional diseases that are prevalent on the landscape and could be detrimental to the small red wolf population.

Coyotes may be a disease threat because their endoparasite community has greater species richness than red wolves and it is projected to increase with more intensive sampling (Fig. 1). Interactions between coyotes and red wolves may facilitate disease transmission between the species, leading to the introduction of new pathogens to the red wolf population. This could affect long-term population recovery because small, endangered populations like red wolves are likely to be immunologically naïve and lack the genetic variation necessary to combat new diseases (Spielman et al. 2004). Interestingly, coyotes and red wolves did not significantly differ in their probability of infection in any of the parasites we evaluated (Table 2), with the exception that adult red wolves were more susceptible to heartworm than coyotes and twice as many coyotes tested positive for the bacteria causing Lyme disease. Perhaps differences in red wolf and coyote diet, foraging behavior, or habitat preference cause differential exposure to the heartworm and Lyme disease vectors: mosquitoes and *Ixodes* ticks, respectively. Long-term temporal data would help determine with more certainty if coyotes act as a disease reservoir and inform the dynamics of disease transmission between the species.

Mange was identified as an important parasite to monitor in the red wolf recovery area and the southeastern region. Mange had caused mortalities in coyotes and foxes regionally (Supporting Information S10) and has already impacted red wolves, killing at least 18 wolves in the North Carolina population.

Table 2.—Parameter estimates (β), adjusted *SE*, and 95% confidence intervals (*CI*) of variables in the final averaged models evaluating infection probability of total endoparasites detected, *Uncinaria stenocephala* (a type of hookworm), heartworm (*Dirofilaria immitis*), *Ehrlichia* spp., and ectoparasite loads (few, intermediate, and heavy), in endangered wild red wolf (*Canis rufus*) and sympatric coyotes (*Canis latrans*); 95% confidence limits not overlapping 0 are in bold.

Dependent variable	Explanatory variable	β	*SE*	*Z*-score	*P*-value	*CI* (2.5%)	*CI* (97.5%)
Endoparasite totals	**Age class (adult)**	**1.58**	**0.14**	**11.39**	**0.00**	**1.31**	**1.85**
	Age class (juvenile)	**0.54**	**0.17**	**3.19**	**0.00**	**0.21**	**0.87**
	Age class (pup)	**0.33**	**0.16**	**2.01**	**0.04**	**0.01**	**0.65**
	Year (2014)	–0.12	0.14	0.86	0.39	–0.40	0.16
U. stenocephala	**Year (2014)**	**–2.37**	**0.81**	**2.94**	**0.00**	**–3.95**	**–0.79**
	Age class (adult)	0.86	0.81	1.07	0.29	–0.72	2.45
	Age class (juvenile)	–0.24	0.65	0.36	0.72	–3.12	0.87
	Age class (pup)	–0.33	0.75	0.43	0.66	–3.29	0.20
	Sex (M)	–0.15	0.47	0.32	0.75	–2.33	0.80
	Species (Red wolf)	–0.17	0.54	0.32	0.75	–2.68	0.94
Heartworm	**Age class (adult)**	**2.19**	**0.78**	**2.81**	**0.00**	**0.66**	**3.71**
	Age class (juvenile)	**–2.75**	**0.85**	**3.23**	**0.00**	**–4.43**	**–1.08**
	Age class (pup)	**–3.51**	**0.93**	**3.79**	**0.00**	**–5.33**	**–1.69**
	Year (2014)	–1.42	0.73	1.94	0.05	–2.85	0.01
Ehrlichia spp.	Age class (adult)	0.61	0.61	1.00	0.32	–0.58	1.79
	Age class (juvenile)	–0.58	0.79	0.73	0.46	–2.14	0.97
	Age class (pup)	**–1.86**	**0.77**	**2.42**	**0.02**	**–3.37**	**–0.36**
	Year (2014)	–0.56	0.65	0.87	0.39	–1.83	0.71
	Species (Red wolf)	0.54	0.77	0.70	0.48	–0.97	2.06
Ectoparasite load	**Year (2014)**	**2.27**	**0.76**	**2.98**	**0.00**	**0.8**	**3.8**
	Species (Red wolf)	0.72	0.65	1.10	0.27	–0.6	2.0

[a] Null model within Δ2 AIC_c of the top model.

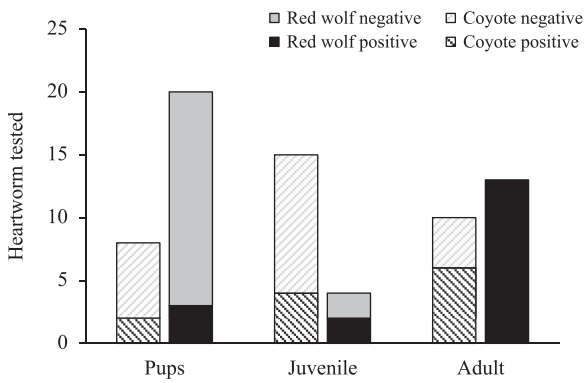

Fig. 3.—Heartworm (*Dirofilaria immitis*) prevalence among endangered wild red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*) in northeastern North Carolina. Adults (older than 2 years) were more likely than pups (under 12 months) or juveniles (between 12 and 24 months) to be heartworm positive; adult red wolves may also be more susceptible than adult coyotes to heartworm.

Mange epizootics likely do not have long-term demographic effects for common species like coyotes or foxes but can be devastating to small populations such as red wolves because the loss of just a few individuals can reduce population growth (Pence and Ueckermann 2002) or even lead to local extinction (Henriksen et al. 1993; Ploshnitsa et al. 2011). Treatment of mange is difficult in wild animals because it requires capturing and administering ivermectin to both infected individuals and those they contacted (Bornstein et al. 2001) but would be warranted for red wolves if a mange epizootic occurred since there have been 46 cases of mange infections successfully treated in wild red wolves.

The most virulent regional disease threats detected were viral infections such as CPV, CDV, and rabies (Dobson and Foufopoulos 2001; Pedersen et al. 2007; Smith et al. 2009), which were widespread in southeastern wildlife populations (Supporting Information S9; see also Supporting Information S13 for disease-driven declines in threatened species). Although currently these viruses do not appear to be epizootic within the southeast region (although see Dyer et al. 2013; Supporting Information S9), the red wolf population may be at risk. Wild red wolves and sympatric coyotes have been exposed to both CPV and CDV in North Carolina (Acton 2008), where at least one red wolf death was attributed to CPV. Red wolves can mount a positive serological response to CPV and CDV vaccines, but the efficacy of opportunistic vaccinations in the wild population is not well established (Acton 2008; Anderson et al. 2014).

Another consideration is the long-term effects of prophylactic vaccination and medical treatments. Vaccines and other interventions such as ivermectin for mange could have negative evolutionary consequences in wild populations because selection pressures for immunity may be weakened with continued treatment. Opportunistic vaccines and treatments that do not provide life-long immunity could also result in multiple individuals becoming susceptible to disease simultaneously, increasing the risk of an epizootic (Woodroffe 1999). The potential drawbacks of vaccines and medical intervention need to be considered by managers and the risk of infection found sufficient to justify intensive prevention efforts. For red wolves, the very real risk of extinction due to their extremely small population size outweighs the potential negative effects of intervention, especially for virulent viral pathogens such as rabies and treatable conditions like mange. As the red wolf population

Table 3.—Number of tick-borne pathogens (Lyme disease, *Anaplasma* spp., and *Ehrlichia* spp.) detected in endangered wild red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*) in northeastern North Carolina, 2013 and 2014. Age classes were defined as pups (under 12 months), juveniles (between 12 and 24 months), and adults (over 24 months). Minus and plus signs indicate the number of negative and positive infections, respectively, detected with SNAP 4DX Tests.

Age	Red wolves						Coyotes					
	Lyme		*Anaplasma*		*Ehrlichia*		Lyme		*Anaplasma*		*Ehrlichia*	
	−	+	−	+	−	+	−	+	−	+	−	+
Pups	17	0	17	0	14	3	4	1	5	1[a]	3	2
Juvenile	4	0	4	0	2	2	10	2	11	0	7	5
Adult	9	2	10	0	2	8	10	0	10	0	5	5
Total	30	2	31	0	18	13	24	3	26	1[a]	15	12

[a] Inconclusive.

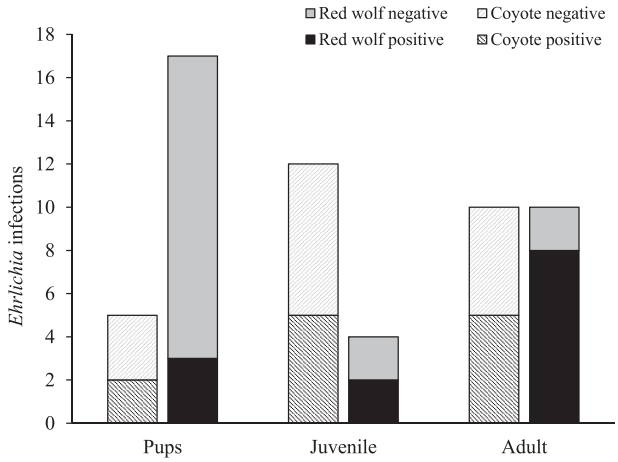

Fig. 4.—*Ehrlichia* spp. prevalence among endangered wild red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*) in northeastern North Carolina. Marginal evidence suggests adults (older than 2 years) were more likely than pups (under 12 months) or juveniles (between 12 and 24 months) to be *Ehrilichia* spp.-positive.

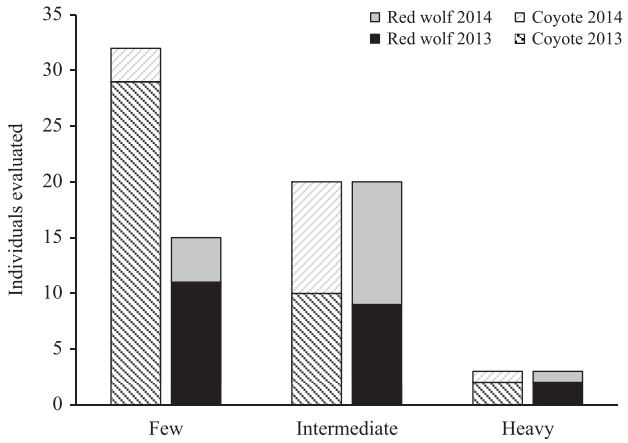

Fig. 5.—Ectoparasite loads detected on endangered wild red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*) in northeastern North Carolina. The likelihood of having heavier ectoparasite loads was greater in 2014 than 2013.

increases and additional wild population are established, vaccinations and intensive treatment may no longer be necessary.

The most prevalent parasites detected in red wolves during our 2013–2014 sampling were hookworm (*Ancylostoma* spp.) and heartworm (*D. immitis*), both of which were widespread throughout southeastern wildlife as well; positive infection rates were 94% and 45%, respectively. Hookworm increased pup mortality in the remnant Louisiana and Texas population (Custer and Pence 1981a) and remains a management concern due to its current prevalence rate and high loads in young wolves (Fig. 2). High heartworm prevalence may be a more immediate threat because heartworm infections have caused the death of at least nine red wolves and adults may be especially susceptible to them (Fig. 3). Compounding the negative effects of hookworm and heartworm is that wild red wolves are inbred (Brzeski et al. 2014), which may cause them to suffer more from co-infections or stressful conditions than an outbred population, like coyotes (Coltman et al. 1999; Spielman et al. 2004). Management efforts, such as cross fostering captive born pups into wild litters, can help mitigate the deleterious effects of inbreeding (Brzeski et al. 2014), but continued monitoring of endoparasites and more rigorous demographic modeling of the impact of heartworm related deaths will be useful for future disease prevention.

The detection of tick-borne diseases is an additional risk factor for red wolves and wildlife in general because the expansion of vector-borne diseases have been associated with climate change (Sutherst 1998; Patz et al. 2008). For instance, climate and landscape changes have facilitated the spread of the bacteria causing Lyme disease, *B. burgdorferi*, and based on climate models, Lyme disease is expected to continue to expand northward (Ostfeld et al. 1996; Ogden et al. 2006). The presence of Lyme disease, *Ehrlichia* spp., and Rocky Mountain spotted fever in red wolves and coyotes serves as a benchmark for detecting the emergence of additional vector-borne pathogens in North Carolina.

Currently, disease may not be the primary threat to red wolf recovery given that there were no major disease outbreaks or frequent red wolf mortality events directly caused by disease. This may be due in part to vaccines and medical interventions, or wild red wolves may not have been exposed to extremely virulent pathogens. But the prevalence rate of parasites in the red wolf and sympatric coyote populations as well as several regional trends reveal substantial concerns. In a critically endangered population such as wild red wolves, every wolf is important for species persistence and pathogens that reduce

fitness, result in occasional deaths, or even moderately affect population growth could contribute to extinction (Woodroffe 1999). To mitigate disease-driven declines, endangered species programs such as the Red Wolf Recovery Program must incorporate disease monitoring and prevention plans to ensure long-term recovery, the first steps of which we presented here.

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Supporting Information

The Supporting Information documents are linked to this manuscript and are available at Journal of Mammalogy online (jmammal.oxfordjournals.org). The materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supporting data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Supporting Information S1.—Corrected delta Akaike information criteria (ΔAIC_c), and AIC_c weights (*wi*) for all generalized linear mixed effect models evaluating endoparasite loads in endangered red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*). Data was collected in the winters of 2013 and 2014 in northeastern North Carolina.

Supporting Information S2.—Corrected delta Akaike information criteria (ΔAIC_c), and AIC_c weights (w_i) for all generalized linear mixed effect models evaluating *Spirometra* prevalence in endangered red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*).

Supporting Information S3.—Corrected delta Akaike information criteria (ΔAIC_c), and AIC_c weights (w_i) for all generalized linear mixed effect models evaluating *Sarcocystis* spp .prevalence in endangered red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*).

Supporting Information S4.—Corrected delta Akaike information criteria (ΔAIC_c), and AIC_c weights (w_i) for all generalized linear mixed effect models evaluating *Taeniid* type eggs (*Taenia* spp. and *Echinoccus* spp. eggs are indistinguishable and can only be categorized by egg type) prevalence in endangered red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*).

Supporting Information S5.—Corrected delta Akaike information criteria (ΔAIC_c), and AIC_c weights (w_i) for all generalized linear mixed effect models evaluating *Uncinaria stenocephala* prevalence in endangered red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*

Supporting Information S6.—Corrected delta Akaike information criteria (ΔAIC_c), and AIC_c weights (w_i) for all generalized linear mixed effect models evaluating heartworm (*Dirofilaria immitis*) prevalence in endangered red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*).

Supporting Information S7..—Corrected delta Akaike information criteria (ΔAIC_c), and AIC_c weights (w_i) for all generalized linear mixed effect models evaluating *Ehrlichia* spp. prevalence in endangered red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*).

Supporting Information S8.—Corrected delta Akaike information criteria (ΔAIC_c), and AIC_c weights (w_i) for all cumulative link mixed models evaluating ectoparasites loads in endangered red wolves (*Canis rufus*) and sympatric coyotes (*Canis latrans*).

Supporting Information S9.—Viral pathogens detected in southeastern wildlife populations based on articles containing the words [“United States” AND south* AND (_disease_ OR _parasit*_ OR _pathogen_)] and keyword searches in the following journals: *Journal of Zoo and Wildlife Medicine*, *Journal of Wildlife Disease*, *Journal of Veterinary Medicine*, *American Journal of Veterinary Research*, *Journal of Parasitology, American Midland Naturalist*, and *Southeastern Naturalist*.

Supporting Information S10.—Endoparasites detected in southeastern wildlife populations based on articles containing the words [“United States” AND south* AND (_disease_ OR _parasit*_ OR _pathogen_)] and keyword searches in the following journals: *Journal of Zoo and Wildlife Medicine*, *Journal of Wildlife Disease*, *Journal of Veterinary Medicine*, *American Journal of Veterinary Research*, *Journal of Parasitology*, *American Midland Naturalist*, and *Southeastern Naturalist*.

Supporting Information S11.—Detected disease occurrence in wild North American canids, including bacteria, endoparasites, fungal, and virus infections.

Supporting Information S12.—Bacterial pathogens detected in southeastern wildlife populations based on articles containing the words [“United States” AND south* AND (_disease_ OR _parasit*_ OR _pathogen_)] and keyword searches in the following journals: *Journal of Zoo and Wildlife Medicine*, *Journal of Wildlife Disease*, *Journal of Veterinary Medicine*, *American Journal of Veterinary Research*, *Journal of Parasitology*, *American Midland Naturalist*, and *Southeastern Naturalist*.

Supporting Information S13.—Published accounts of suspected or documented disease mediated population declines in threatened species.

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