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Ectoparasites in Black-footed Ferrets (*Mustela nigripes*) from the Largest Reintroduced Population of the Conata Basin, South Dakota, USA

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ABSTRACT: The black-footed ferret, *Mustela nigripes*, is an endangered carnivore endemic to the grasslands of North America. We present the first investigation of ectoparasites associated with black-footed ferrets since reintroduction. We sampled more than 200 individuals from one of the largest and most successful reintroduced populations located in the Conata Basin of South Dakota, USA. We compared our findings with ectoparasite assemblages of sympatric carnivores and historic ferret records. We collected more than 1,000 ectoparasites consisting mainly of three flea and tick species, two of which were known historically from South Dakota. Despite our extensive sampling efforts, we did not detect any lice. This is notable because a putative host-specific louse, *Neotrichodectes* sp., was presumed to have gone extinct when black-footed ferrets were extirpated from the wild. The ectoparasite assemblage on black-footed ferrets comprised only generalist parasites, particularly those found on their prey such as prairie dogs (*Cynomys* sp.). *Oropsylla hirsuta* was the most abundant ectoparasite, representing 57% of all ectoparasites detected; a flea vector important in the persistence and transmission of plague. Black-footed ferrets like other endangered species undergo repeated parasite removal and vaccination efforts to facilitate population recovery, which may have unintentionally contributed to their depauperate ectoparasite community.

Key words: Coextinction, diversity, *Oropsylla hirsuta*, parasite, plague, prairie dog, recovery.

Despite 40 yr of legal protection, the black-footed ferret remains one of the most threatened carnivores in North America (Clark 1987). Black-footed ferrets (*Mustela nigripes*) were once found from the Great Plains of Canada to Mexico, but their populations declined precipitously throughout the 20th century from the conversion of grasslands to agriculture, epidemics of canine distemper and plague, and local attempts to exterminate prairie dogs (*Cynomys* spp.), their primary prey (Lockhart et al. 2006; Biggins et al. 2011). When black-footed ferrets numbered just 18 individuals, all were brought into captivity for breeding. Once reproduction was achieved, reintroductions began, and newly established wild populations became a source to restore ferrets to their previous geographic distribution. Today, wild black-footed ferrets can be found at 18 reintroduction sites and are overall viewed as a conservation success story, although captive efforts continue (Jachowski and Lockhart 2009).

Because black-footed ferrets remain a threatened species, investigating their parasite assemblage is useful to assess potential disease risks and the likelihood of coextinction events. We describe the extant assemblage of ectoparasites from a large sample of black-footed ferrets. We also compare the ectoparasites of ferrets to those reported from other North American carnivores and determine whether ectoparasites known in association with ferrets from historic records still persist today.

We captured black-footed ferrets during annual spotlight surveys from 8:00 PM to 5:00 AM from August to December in 2006–10 in Conata Basin/Badlands National Park, one of the most successful reintroduction sites (Lockhart et al. 2006). Once ferrets were located, we placed unbaited, live-cage traps (Black-footed ferret trap, Prairie Wildlife Research,

TABLE 1. Ectoparasite species collected from black-footed ferrets (*Mustela nigripes*) in Conata Basin, South Dakota, 2006–10. Prevalence (95% CI) calculated as the proportion of infected individuals of the total number of individuals examined across sites throughout the duration of the study (n=227).

Ectoparasite species	Total count	Prevalence (95% CI)
Ticks		
Ixodes sculptus	189	0.37 (0.30–0.43)
Ixodes kingi	271	0.45 (0.38–0.51)
Fleas		
Oropsylla hirsuta[a]	612	0.43 (0.37–0.50)
Pulex irritans[a]	4	0.02 (0.01–0.05)
Epitedia wenmanni[a]	3	0.01 (0.00–0.03)
Oropsylla tuberculata[a]	2	0.01 (0.00–0.03)
Peromyscopsylla hesperomys[a]	2	0.01 (0.00–0.03)

[a] Also reportedly found in prairie dog burrows or on prairie dogs (Salkeld and Stapp 2008).

Wellington, Colorado, USA) over the prairie dog entrance holes. While black-footed ferrets were anesthetized with isoflurane, we systematically searched, moving from anterior to posterior then dorsal to ventral, for fleas and ticks for approximately 1 min. All methods were approved under US Fish and Wildlife Permit TE064682-0. Ectoparasites were frozen or immediately stored in 95% ethanol for subsequent identification. We included specimens identified using morphometric keys (e.g., Keirans and Clifford 1978) and that were compared with museum vouchers in our analyses. We estimated total parasite richness using the Chao1 richness estimator and constructed the smoothed species accumulation curve to determine whether our sampling effort was sufficient (Colwell 2006). For each species of parasite encountered, we calculated prevalence and associated 95% confidence intervals as the number of hosts infested divided by the total number of individuals examined (Rozsa et al. 2000).

We captured and sampled 587 individual black-footed ferrets throughout our study areas: a sample size that represents over half of the estimated total wild population (Jachowski and Lockhart 2009). However, we focus on data only from black-footed ferrets that occupied areas without insecticide application (n=227; Table 1) because these actions are known to reduce flea populations (Biggins et al. 2010). Nevertheless, three ectoparasites were found on rare occasions at dusted locations only: *Stenoponia americana*, *Amaradix euphorbia*, and *Aetheca wagneri*. Additionally, specimens only identified to the genus level and those indistinguishable through morphologic examination, such as mites and immature ticks, were excluded (22% of ectoparasites). Of the remaining 1,083 samples, we found seven flea and tick species, but only three species in regular occurrence. We detected no lice, despite claims of a putative host-specific louse, *Neotrichodectes* sp., presumed to have gone extinct when black-footed ferrets were extirpated from the wild (Gompper and Williams 1998). We report the first known records of fleas, *Epitedia wenmanni* and *Peromyscopsylla hesperomys* in associations with black-footed ferrets.

We confirmed *Ixodes kingi* and *Oropsylla hirsuta* from black-footed ferrets as reported from historic records by Boddicker (1968). *Oropsylla hirsuta* was the most abundant ectoparasite species, a flea implicated in the transmission of plague, accounting for 57% of all parasites encountered. The same two tick species we found on ferrets today, *I. kingi* and *Ixodes* *sculptus*, were also reported in museum records dating back nearly 70 yr, when ferrets was widespread and abundant (U.S. National Tick Collection database, Institute of Arthropodology and Parasitology, Georgia Southern University, Statesville, Georgia). Chao1 species richness estimator (Chao1=10.33) suggested no additional ectoparasite species were missing, given our extensive sampling effort.

Overall, 64% of captured black-footed ferrets had ectoparasites, but prevalence varied among ectoparasite species (Table 1). The diversity of ectoparasites found on a given host individual ranged from one to four species, with 28% (41 of 146) of infested ferrets having at least one ectoparasite species. Hosts with more parasite individuals (i.e., greater load) were also likely to have more parasite species. Nonspecialist parasites found on black-footed ferrets could have colonized post-reintroduction either from their prey or from interactions with other carnivores. All flea species we found on black-footed ferrets have also been found in association with prairie dog burrows or on prairie dogs (Salkeld and Stapp 2008). However, several fleas commonly found on other carnivores in South Dakota were not detected on black-footed ferrets, including *Foxella ignota*, *Euhoplopsyllys affinis*, and *Ctenocephalides felis* (Easton 1982).

Black-footed ferrets, in many respects, were once thought to be the "flagship" host for parasite conservation (Gompper and Williams 1998). However, the ectoparasite assemblage on the recovered population in our study was depauperate and comprised only generalist parasites, particularly those found on the ferrets' prey, such as prairie dogs and *Peromyscus* spp. mice (e.g., Friggens et al. 2010). Because of its size and early origin, the population we sampled seemed the most likely to be the one to host any residual specialists. The absence of specialist parasites is surprising because most North American carnivore species harbor at least one specialist parasite (Harris and Dunn 2010). In most cases, when specialist parasites are present in communities, they are most prevalent on hosts that are abundant and widespread (Hughes and Page 2007). Results from our study indicate that current ectoparasites of black-footed ferrets are not at risk of coextinction (Colwell et al. 2012) because none were solely dependent upon ferrets in South Dakota. Nevertheless, we recommend continued sampling of ectoparasites from black-footed ferrets at other recovery sites and across seasons to determine whether specialist parasites of black-footed ferrets may exist elsewhere.

Our results indicate that black-footed ferrets in South Dakota may continue to succumb to plague because of the high prevalence of the flea vector, *O. hirsuta*. An important next step would be to determine the proportion of fleas harboring *Yersina pestis* and assess fitness consequences of parasite loads for ferrets. Our finding suggests that using the Conata Basin population for translocations is unlikely to introduce novel ectoparasites into new areas but could increase the distribution of plague due to the persistence of the vector, if not actively managed. As such, ectoparasite removal or deltamethrin application in prairie dog burrows to lessen parasite loads of vectors such as *O. hirsuta* may be necessary to promote the full recovery of black-footed ferrets (Biggins et al. 2010).

Tick specimens are stored at the Centers for Disease Control and Prevention, Fort Collins, Colorado, and the Essig Museum of Entomology at the University of California, Berkeley. All flea specimens are now being examined for plague at the University of South Dakota, Vermillion, South Dakota. We extend great appreciation to L. Beati, L. Durden, G. Dietrich, J. Montenieri, E. Mize, and C. Apperson for assistance with ectoparasite identification. We thank N. Haddad, S.A.C. Nelson, and M. Moir for insightful comments on the manuscript. We thank D. Biggins for guidance during project implementation and the many partners and volunteers that assisted with ferret captures. N.C.H was supported by a graduate fellowship through the Department of Forestry and Environmental Resources at North Carolina State University, the Eastern Region and Pacific Northwest Research Station of the US Forest Service, and a Grants-in-aid award from Sigma Xi. We acknowledge additional support from the US Fish and Wildlife Service (Permit: TE064682-0).

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