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Diseases and Parasites in Wolves of the Riding Mountain National Park Region, Manitoba, Canada

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ABSTRACT: We examined wolf *(Canis lupus)* blood and fecal samples from the Riding Mountain National Park (RMNP) region of Manitoba, Canada. In 601 fecal samples collected during two study periods in RMNP and the Duck Mountain Provincial Park and Forest (DMPPF) we found gastrointestinal helminth eggs from *Alaria* sp. (15.5%), *Capillaria* sp. (1.0%), taeniid tapeworms (30.8%), *Toxascaris* sp. (1.7%), *Toxocara* sp. (0.2%), *Trichuris* sp. (2.2%), and *Moniezia* sp. (0.5%). In addition, we found *Demodex* sp. (0.2%) and the protozoal cysts/oocysts of *Sarcocystis* sp. (37.3%), *Cryptosporidium* sp. (1.2%), coccidia (*Isospora* sp. or *Eimeria* sp.) (1.7%), and *Giardia* sp. (29.5%). No fecal shedding of canine parvovirus (CPV, $n=387$) was detected. All 18 blood samples collected in RMNP showed CPV exposure and eight of 18 blood samples indicated canine distemper virus (CDV) exposure. One wolf died from CDV. Our results are consistent with previous findings on pathogens affecting wolves and with high *Giardia* sp. prevalence in wolves inhabiting agricultural regions.

Key words: Canine distemper virus (CDV), canine parvovirus (CPV), *Canis lupus*, disease, *Giardia* sp., parasitism.

Infectious diseases are increasingly associated with human-modified ecological transition zones (Despommier et al., 2007) and are of growing concern for isolated wilderness reserves (Aguirre et al., 1995). Disease epidemics can severely reduce and isolate populations within protected areas (León-Vizcaíno et al., 1999), where disease transmission from domestic species is believed to have affected wild species (e.g., Foreyt and Jessup, 1982). Gray wolf (*Canis lupus*) populations in southwestern Manitoba, Canada have become isolated following extensive agricultural development during the past 60 yr. Wolves in Riding Mountain National Park (RMNP) are surrounded by farmland and have distinct mitochondrial DNA haplotypes indicative of an isolated population (Stronen et al., 2010 and references therein). The isolation of RMNP wolves is of conservation concern and warrants an assessment of disease, which could be playing an important role in the regulation of the RMNP wolf population (Carbyn, 1982). Canine distemper virus (CDV), bovine tuberculosis (*Mycobacterium bovis*; Carbyn, 1982), and sarcoptic mange caused by the mite *Sarcoptes scabiei* have been documented in RMNP wolves and local veterinarians have recorded cases of CDV and canine parvovirus (CPV) in dogs (*C. l. familiaris*) around RMNP (RMNP, unpubl. data).

Winter tracking surveys since 1976 have indicated a RMNP population of 30–80 wolves (Sallows, 2007). Ungulate prey abundance and biomass suggest the Park should be able to support at least 100 wolves (Keith, 1983; Fuller, 1989; Fuller and Murray, 1998), although high human-caused mortality outside the Park also might be limiting the RMNP population (Carbyn, 1980). The number of wolf packs in RMNP varies but is approximately 12 (RMNP survey data). Previous work on disease in the region is nearly 20 yr old and the increased isolation of RMNP, concurrent with low density of wolves in a

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protected area supported a new review of disease in the population. We quantified gastrointestinal parasite burdens from 601 wolf fecal samples to assess whether RMNP wolves have higher parasite burdens and a higher number of parasite taxa than wolves outside the Park, where isolation is not a concern. Furthermore, we surveyed infectious diseases known or suspected to affect RMNP-region wolves.

Our study area encompasses the Riding Mountain National Park (RMNP, 50°46′N, 99°59′W) region in southwestern Manitoba, Canada, where removal of forest cover to the RMNP edge has resulted in a wilderness "island" within an agricultural region (Carbyn, 1980). The region includes Riding Mountain Biosphere Reserve (15,000 km^2) and Duck Mountain Provincial Park (1,424 km^2) and Forest (3,760 km^2, DMPPF), about 30 km north of RMNP. In both areas, elk *(Cervus elaphus)*, moose *(Alces alces)*, beaver (*Castor canadensis*), and white-tailed deer *(Odocoileus virginianus)* are abundant.

We obtained gastrointestinal parasite egg/oocyst counts from RMNP and DMPPF wolf fecal samples collected from 2001–2003 (Sallows, 2007) and 2003–2005 (Stronen, 2009). Samples were collected throughout RMNP and DMPPF, primarily during September to March, on nonplowed roads, trails, and off-road. Disease and parasite analyses were done in collaboration with the Canadian Cooperative Wildlife Health Centre (CCWHC) at the University of Saskatchewan.

We measured 4 g of each fecal sample to count parasite eggs/oocysts and used a modified Wisconsin technique (Salb et al., 2008). Parasites within a genus often are difficult to differentiate at the egg/oocyst stage and thus are reported only to this level of classification. For *Giardia* sp. and *Cryptosporidium* sp., we used immunoflorescent detection and the Cyst-a-Glo™ Comprehensive Kit (Waterborne Inc., New Orleans, Louisiana, USA). We processed these samples as above, then smeared a thin layer of fecal slurry on a slide, allowed it to dry and added Cyst-a-Glo™ antibody reagent. Slides were incubated at room temperature for at least 40 min in a humid chamber. The slides were rinsed, dried, and examined for *Giardia* cysts and *Cryptosporidium* oocysts. *Giardia* sp. and *Cryptosporidium* sp. were divided into four prevalence categories: 1+ (1–50 oocysts on a slide), 2+ (51–250 oocysts), 3+ (251–1,000 oocysts), and 4+ (>1,000 oocysts). For other parasites, we report the number of eggs per gram of feces. For *Sarcocystis* sp., the number of sarcocysts was at times noted as too numerous to count (TNTC). We used the nonparametric Kruskal-Wallis test in SPSS 9.0 (SPSS, Inc., Chicago, Illinois, USA) to test for significant differences in the number of parasite taxa per sample, and parasite burdens of *Alaria* sp. and taeniids (the prevalent taxa for which all results had been coded numerically), among groups.

Presence of CPV was based on fecal samples inoculated into tissue cultures using feline kidney cells. Cultured cells were tested for viral infection by monitoring for cytopathic effect and by staining for viral antigen using fluorochrome-labeled antibodies. Blood samples were collected from the cephalic vein of 18 RMNP wolves captured by net-gun from helicopter for radio-collaring during 2003–2005, to examine health status and presence of antibodies to CDV and CPV. Canine distemper virus and CPV status were determined using standard diagnostic assays at Prairie Diagnostic Services, Saskatoon, Saskatchewan. A virus neutralization test (Appel and Robson, 1973) was used to detect antibodies to CDV using the Snyder Hill strain of CDV grown in Vero cells. Positive titers were considered as >1:100. A hemagglutination inhibition test (Carmichael et al., 1980) was used to detect antibodies to CPV. A CPV isolate was used for the hemagglutinating antigen with swine red blood cells. Positive titers were considered as ≥1:20.

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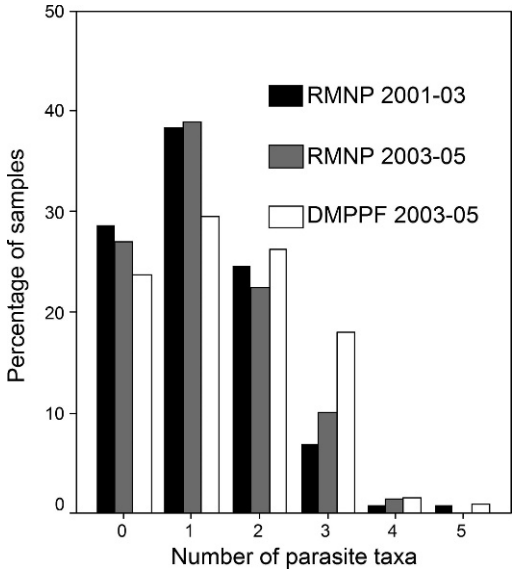

FIGURE 1. Number of parasite taxa found in wolf fecal samples from Riding Mountain National Park 2001–2003 ($n=320$) and 2003–2005 ($n=159$), and Duck Mountain Provincial Park and Forest 2003–2005 ($n=122$) in Manitoba, Canada.

The number of parasite taxa found in fecal samples from RMNP 2001–2003 ($n=320$), RMNP 2003–2005 ($n=159$), and DMPPF 2003–2005 ($n=122$) ranged from none to five (Fig. 1). We found a statistically significant difference in the number of parasite taxa among groups ($H=7.594$, $P=0.022$), with a mean rank of 288.88 for RMNP 2001–2003, 297.56 for RMNP 2003–2005, and 337.27 for DMPPF. We found no significant difference in parasite burden of *Alaria* sp. ($H=2.624$, $P=0.269$) or taeniids ($H=0.013$, $P=0.994$) among groups. The occurrence, prevalence, total count, and mean intensities of parasite taxa are outlined in Table 1.

We found antibodies to CPV in 18/18 blood samples and all were classified as exposed (titers ranging from >1:20, <1:480 to 1:20,480). Similarly, 8/18 blood samples showed exposure to CDV (titers ranging from 1:108 to 1:972). For three wolf packs, one captured wolf was classified as exposed to CDV, whereas other captured wolves were not (titer 1:108 vs. 1:6; titer 1:324 vs. <1:6; and titer 1:972 vs. two individuals with titer <1:6). One male wolf died from CDV in April 2005 (diagnosis based on histopathology and immunochemistry; CCWHC, unpubl. data). When captured on 1 February 2005, he was categorized as an adult and neither he nor the female adult captured January 29 in the same pack were classified as exposed to CDV (both had titers <1:6) based on the blood samples collected. No fecal shedding of CPV was detected.

Our results showed that RMNP and DMPPF wolves carried the same parasite taxa. We found no evidence that the isolated RMNP population had higher parasite burdens or a higher number of parasite taxa. Hence, present levels of isolation might play a negligible role or has yet to have any effect. Wolves from the two areas feed on the same prey species, and telemetry (RMNP monitoring data) and genetic data (P. Paquet, unpubl. data) show that elk move between the two areas. Many of the parasites we identified involve ungulates in their life cycle and ungulates might therefore ameliorate some expected effects of isolation for the RMNP wolf population. Duck Mountain Provincial Park and Forest receives less protection from human disturbance than RMNP and permits activities such as cottage development, trapping, and hunting. These activities likely augment the possibility of parasite transmission via dogs and waste, which could explain why we detected a higher number of parasite taxa per sample in DMPPF wolves.

Wolves are considered definitive hosts and frequent carriers of parasites such as *Alaria* sp. (Byman et al., 1977), *Sarcocystis* sp. (Dubey and Odening, 2001), taeniid tapeworms (Bush et al., 2001), *Toxocara* sp., and *Toxascaris* sp. (Craig and Craig, 2005). This appears consistent with their relatively high prevalence in both RMNP and DMPPF. Several of the parasites we found were relatively uncommon and are believed to be ingested accidentally by wolves through consumption of prey,

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TABLE 1. Occurrence (O), percent prevalence (P), total count (C), and mean intensities (I) of parasites in wolf fecal samples from Riding Mountain National Park (RMNP) and Duck Mountain Provincial Park and Forest (DMPPF) in southwestern Manitoba, Canada, 2001–2005.

	RMNP 2001–2003 (n=320)				RMNP 2003–2005 (n=159)				DMPPF 2003–2005 (n=122)			
Parasite	O	P	C	I	O	P	C[a]	I	O	P	C [a]	I
Helminths												
Alaria sp.	31	9.7	945	30.5	27	17.0	2,570	95.2	35	28.7	1,620	46.3
Capillaria sp.	6	1.9	20,248	3,374.7	—	—	—	—	—	—	—	—
Coccidia	10	3.1	2,063	206.3	—	—	—	—	—	—	—	—
Moniezia sp.	3	0.9	655	218.3	—	—	—	—	—	—	—	—
Taeniidae	108	33.8	216,625	2,005.8	50	31.4	25,710	514.2	27	22.1	17,950	664.8
Toxascaris sp.	3	0.9	685	228.3	4	2.5	270	67.5	3	2.5	210	70
Toxocara sp.	1	0.3	18	18.0	—	—	—	—	—	—	—	—
Trichuris sp.	11	3.4	428	38.9	2	1.3	7.5	3.8	—	—	—	—
Protozoa												
Cryptosporidium sp.	1	0.3	800	800	1	0.6	1+	N/A	5	4.1	1+ (n=2) 2+ (n=2) 4+ (n=1)	N/A
Giardia sp.	70	21.9	25,181	359.7	50	31.4	1+ (n=25) 2+ (n=12) 3+ (n=7) 4+ (n=5)	N/A	57	46.7	1+ (n=29) 2+ (n=19) 3+ (n=6) 4+ (n=3)	N/A
Sarcocystis sp.	120	37.5	1,376,913	11,474.3	58	36.5	TNTC	N/A	46	37.7	TNTC	N/A
Arthropods												
Demodex sp.	1	0.3	3	3	—	—	—	—	—	—	—	—

[a] 1+ = (1–50 cysts present), 2+ = (51–250 cysts), 3+ = (251–1,000 cysts) and 4+ = (>1,000 cysts), TNTC = too numerous to count.

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including *Capillaria* sp., coccidia (*Eimeria* or *Isospora* sp.), and *Moniezia* sp. (Bush et al., 2001). Three Minnesota wolf pups nonetheless appear to have died from *Isospora* sp. infection, possibly contracted from dog feces (Mech and Kurtz, 1999). *Giardia* sp. was prevalent throughout our study area, whereas *Cryptosporidium* sp. was rare. Both *Giardia* sp. and *Cryptosporidium* sp. were common in coyotes on the Canadian prairies (Thompson et al., 2009). *Giardia* sp. and *C. parvum* also were prevalent in wolves within a region of human-managed forests and agricultural land in Poland (Kloch et al., 2005), where the occurrence of *Giardia* sp. (45.5%) was similar to that of DMPPF (46.7%).

We believe our studies of the parasite and disease status of RMNP-region wolves represent a valuable baseline for future monitoring. Although infection by parasites did not appear to cause overt disease, parasite presence might cause subclinical problems, and continued monitoring could be a helpful indicator of health or health decline for this disease-susceptible population.

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LITERATURE CITED

AGUIRRE, A. A., E. E. STARKEY, AND D. E. HANSEN. 1995. Wildlife disease in national park ecosystems. Wildlife Society Bulletin 23: 415–419.

APPEL, M., AND D. S. ROBSON. 1973. A microneutralization test for canine distemper virus. American Journal of Veterinary Research 34: 1459–1463.

BUSH, A. O., J. C. FERNANDEZ, G. W. ESCH, AND J. R. SEED. 2001. Parasitism: The diversity and ecology of animal parasites. Cambridge University Press, Cambridge, UK, 566 pp.

BYMAN, D., V. VAN BALLENBERGHE, AND A. W. ERICKSON. 1977. Parasites of wolves, *Canis lupus*, in northeastern Minnesota, as indicated by analysis of fecal samples. Canadian Journal of Zoology 55: 376–380.

CARBYN, L. N. 1980. Ecology and management of wolves in Riding Mountain National Park, Manitoba. Final Report, Large Mammal System Studies, Report No. 10, September 1975–March 1979, Canadian Wildlife Service, Edmonton, Alberta, Canada, 167 pp, plus appendices.

———. 1982. Incidence of disease and its potential role in the population dynamics of wolves in Riding Mountain National Park, Manitoba. *In* Wolves of the world: Perspectives of behaviour, ecology and conservation, F. H. Harrington and P. C. Paquet (eds.). Noyes Publications, Park Ridge, New Jersey, pp. 106–115.

CARMICHAEL, L. E., J. C. JOUBERT, AND R. V. POLLOCK. 1980. Hemagglutination by canine parvovirus: Serologic studies and diagnostic applications. American Journal of Veterinary Research 41: 784–791.

CRAIG, H. L., AND P. S. CRAIG. 2005. Helminth parasites of wolves (*Canis lupus*): A species list and an analysis of published prevalence studies in Nearctic and Palearctic populations. Journal of Helminthology 79: 95–103.

DESPOMMIER, D., B. R. ELLIS, AND B. A. WILCOX. 2007. The role of ecotones in emerging infectious diseases. EcoHealth 3: 281–289.

DUBEY, J. P., AND K. ODENING. 2001. Toxoplasmosis and related infections. *In* Infectious Diseases of Wild Mammals, E. S. Williams and I. K. Barker (eds.). 3rd Edition, Iowa State University Press, Ames, Iowa, USA, pp. 478–519.

FOREYT, W. J., AND D. A. JESSUP. 1982. Fatal pneumonia of bighorn sheep following association with domestic sheep. Journal of Wildlife Diseases 18: 163–168.

FULLER, T. K. 1989. Population dynamics of wolves in north-central Minnesota. Wildlife Monographs 105: 1–41.

———, AND D. L. MURRAY. 1998. Biological and logistical explanations of variation in wolf population density. Animal Conservation 1: 153–157.

KEITH, L. B. 1983. Population dynamics of wolves. *In* Wolves in Canada and Alaska: Their status, biology, and management, L. N. Carbyn (ed.), Canadian Wildlife Service, Ottawa, Ontario, Canada, Report Series 45, pp. 66–77.

KLOCH, A., M. BEDNARSKA, AND A. BAJER. 2005. Intestinal macro- and microparasites of wolves (*Canis lupus* L.) from north-eastern Poland recovered by coprological study. Annals of

Downloaded from http://meridian.allenpress.com/doi/pdf/10.7589/0090-3558-47.1.222 by guest on 01 December 2021

Agricultural and Environmental Medicine 12: 237–245.

León-Vizcaino, L., M. R. Ruíz de Ybañez, M. J. Cubero, J. M. Ortíz, J. Espinosa, L. Pérez, M. A. Simón, and F. Alonso. 1999. Sarcoptic mange in Spanish ibex from Spain. Journal of Wildlife Diseases 35: 647–659.

Mech, L. D., and H. J. Kurtz. 1999. First record of coccidiosis in wolves, *Canis lupus*. Canadian Field-Naturalist 113: 305–306.

Salb, A. L., H. W. Barkema, B. T. Elkin, A. R. C. Thompson, D. P. Whiteside, S. R. Black, J. P. Dubey, and S. J. Kutz. 2008. Dogs as sources and sentinels of parasites in humans and wildlife, northern Canada. Emerging Infectious Diseases 14: 60–63.

Sallows, T. A. 2007. Diet preferences and parasites of grey wolves in Riding Mountain National Park of Canada. Master of Environment Thesis, University of Manitoba, Winnipeg, Manitoba, 99 pp.

Stronen, A. V. 2009. Dispersal in a plain landscape: Wolves in southwestern Manitoba, Canada. Ph.D. Dissertation, University of New Brunswick, Fredericton, New Brunswick, Canada, 243 pp.

———, G. J. Forbes, T. Sallows, G. Goulet, M. Musiani, and P. C. Paquet. 2010. Wolf body mass, skull morphology, and mitochondrial DNA haplotypes in the Riding Mountain National Park region of Manitoba, Canada. Canadian Journal of Zoology 88: 496–507.

Thompson, R. C. A., D. D. Colwell, T. Shury, A. J. Appelbee, C. Read, Z. Njiru, and M. E. Olson. 2009. The molecular epidemiology of *Cryptosporidium* and *Giardia* infections in coyotes from Alberta, Canada and observations on some cohabiting parasites. Veterinary Parasitology 159: 167–170.

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