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# DEMOGRAPHIC EFFECTS OF CANINE PARVOVIRUS ON A FREE-RANGING WOLF POPULATION OVER 30 YEARS

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ABSTRACT: We followed the course of canine parvovirus (CPV) antibody prevalence in a subpopulation of wolves (*Canis lupus*) in northeastern Minnesota from 1973, when antibodies were first detected, through 2004. Annual early pup survival was reduced by 70%, and wolf population change was related to CPV antibody prevalence. In the greater Minnesota population of 3,000 wolves, pup survival was reduced by 40–60%. This reduction limited the Minnesota wolf population rate of increase to about 4% per year compared with increases of 16–58% in other populations. Because it is young wolves that disperse, reduced pup survival may have caused reduced dispersal and reduced recolonization of new range in Minnesota.

*Key words:* Canine parvovirus (CPV), demography, dispersal, population, wolf.

## INTRODUCTION

Canine parvovirus (CPV) may have originated from a feline panleukopenia-like virus in a wild carnivore (Steinel et al., 2001). The earliest evidence of CPV infection in a canine species comes from the detection of CPV antibodies in wild wolves (*Canis lupus*) that were sampled in northeastern Minnesota during 1973 (Mech and Goyal, 1995). Antibodies to CPV subsequently were detected in domestic dogs (*Canis lupus familiaris*) in Greece in 1974 (Koptopoulos et al., 1986) and from dogs in the Netherlands in 1976 (Schwers et al., 1979). The virus has been detected in wild and domestic canids worldwide (Steinel et al., 2001).

Mortality related to CPV in domestic canids primarily is associated with younger animals (1–12 wk old; Eugster and Nairn, 1977; Meunier et al., 1981), but almost nothing is known about the epidemiology of CPV in wild canid populations or its potential to impact populations negatively. The disease can be fatal to wolves (Mech et al., 1986; 1997) and is suspected to have caused declines or attenuation of wolf populations in Wisconsin (Wydeven et al., 1995) and on Isle Royale, Michigan (Peterson et al., 1998). In a small area (2%) of Minnesota's wolf range, wolf population changes were highly related ($r^2$=0.83) to CPV antibody prevalence from 1984 through 1993 (Mech and Goyal, 1995). Now, after following the course of CPV infection in wolves in that study area (intensive study area [ISA]; 48°N latitude and 91°15′W longitude) since the first appearance of CPV some 30 yr ago, we document its long-term effect in the ISA and demonstrate its effect on the entire Minnesota wolf population of some 3,000 animals over an 88,325-km$^2$ range.

Our data consist of the following: 1) an annual antibody prevalence of CPV in wolves in our ISA from 1973 through 2004, 2) an index of annual wolf pup survival to 4 mo of age in the ISA, 3) an annual winter census of wolves in the ISA from 1972 through 2004, 4) intermittent CPV antibody prevalence estimates in wolves in the total Minnesota wolf range from 1979 to 2004, 5) an index of annual pup survival to 4 mo in the entire Minnesota wolf range from 1979 to 2004, and 6) histories of CPV antibody status in wolves sampled multiple times in the ISA.

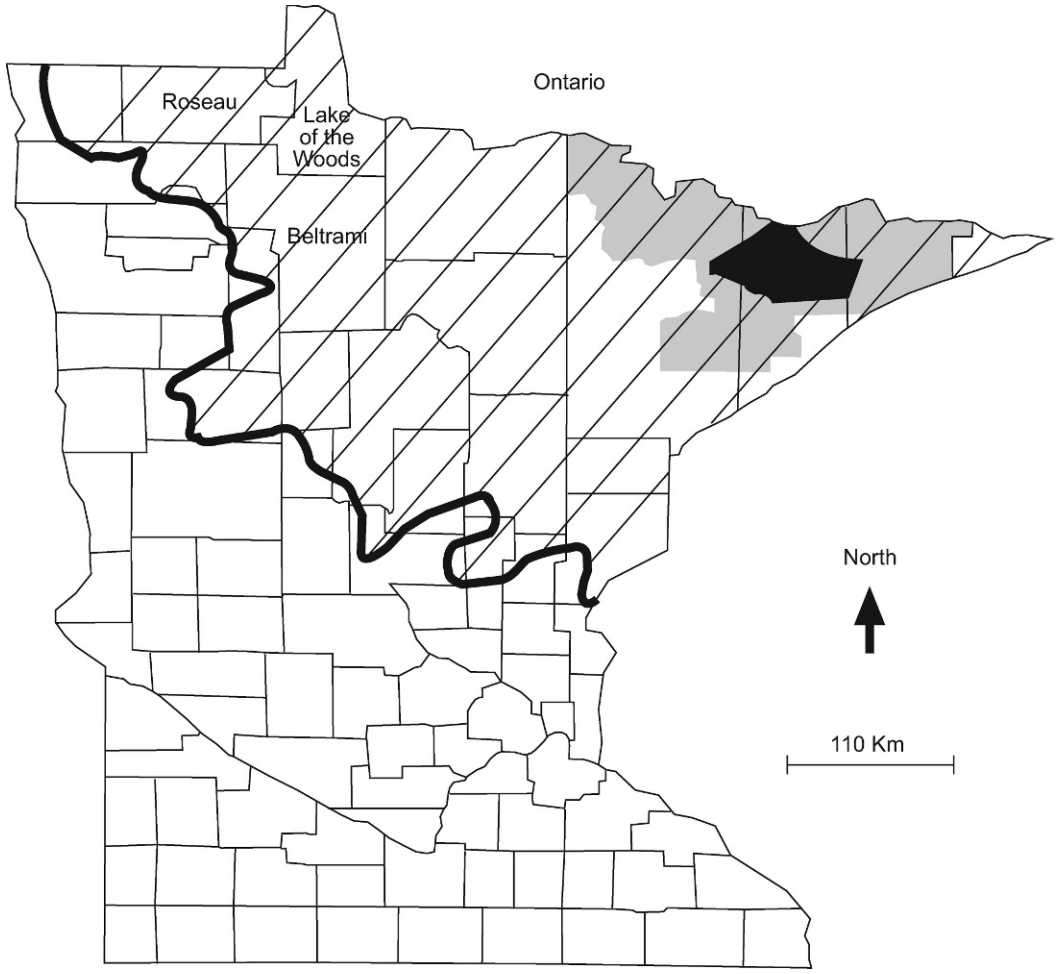


FIGURE 1. The study areas. Minnesota wolf range is lined. The intensive study area (ISA) is black, and the Superior National Forest is gray.

## MATERIALS AND METHODS

### Study area

The ISA is a 2,060-km$^2$ part of the Superior National Forest northeast of Ely, Minnesota (Fig. 1). The wolf population on the ISA is part of the much larger Minnesota wolf population that represents the southernmost extension of the Canadian wolf population, and has never been exterminated. Humans and dogs inhabit the western and southern edges of the ISA, and the entire area is used for recreation. The wolves feed primarily on white-tailed deer (*Odocoileus virginianus*), moose (*Alces alces*), and beavers (*Castor canadensis*). The Minnesota wolf range (Fig. 1) occupies the northeastern 40% of Minnesota, including wilderness and semiwilderness forest interspersed with farms, towns, and cities. Dispersing and nomadic wolves travel throughout this range and into neighboring Ontario, Manitoba, Wisconsin, and Michigan.

### Wolf demography

We live-trapped wolves (Mech, 1974) from May to October or November in the ISA and the immediately adjacent area. In the greater Minnesota wolf range, we trapped and euthanized wolves as part of a government depredation-control program. We distinguished wolf pups from adults by the presence of milk canine teeth (Van Ballenberghe and Mech, 1975). We also attached radio collars to most of the wolves captured in the ISA (Mech, 1974) and later located them from a fixed-wing

aircraft. We aerially observed radio-tagged wolves and their packmates throughout each winter and counted all members of each pack in the census area. Numbers presented here represent the maximum observed members per radioed pack during December–January each year supplemented by tracks or observations of nonradioed packs whose territories fell wholly or partly in the census area (Mech, 1986). Annual changes in estimated populations were related to annual changes in mean size of radioed packs ($r^2$=0.35; $P$<0.01), which were error-free knowns. We were able to obtain some information on pup litter size in our ISA by aerially observing radioed wolves with pups near their dens in late spring and summer. Because of poor visibility, these counts only represented minimum pup numbers.

### Serology

We weighed, examined, and sexed each wolf caught and collected blood, which was processed for CPV antibodies as described below. We removed serum from blood samples and stored it at −15 C to −20 C until assaying for CPV antibody by the hemagglutination inhibition (HI) test (Carmichael et al., 1980). Sera were heat activated, treated with 25% kaolin, and absorbed with packed porcine erythrocytes to remove nonspecific hemagglutinins. We mixed serial twofold dilutions of sera in 96-well microtiter plates with eight hemagglutination units of CPV and incubated them at 4 C overnight. A 1% suspension of porcine erythrocytes was added, and after 2 hr of incubation at 4 C the test was read. Antibody titer represented the reciprocal of highest serum dilution that completely inhibited hemagglutination; titers of ≥256 were considered positive. The HI test has not been validated in wolves and the CPV strain used in the test was derived from dogs; it is not known how close that strain is to CPV strains infecting wolves. Our positive threshold titer of ≥256 was conservative; titers of 128 are considered positive for CPV antibodies in dogs (Carmichael and Binn, 1981). We considered the percentage of wolves that were positive on their first capture as the antibody prevalence for that year (Goyal et al., 1986; Mech et al., 1986). Data from recaptured and retested wolves were counted in our antibody prevalence analyses only during the wolves' first year of capture and testing.

Simple linear and polynomial regression were used to relate trends in antibody prevalence with year, percent of pups caught, and percent annual changes in the wolf population, and chi-square tests were used to compare antibody prevalence among ages, sex, study areas, and years. Changes in CPV antibody prevalence were monitored over time for periodicity by comparing nine models under an information-theoretic model selection approach (Burnham and Anderson, 2002). We posed a set of nine plausible models to describe the observed trajectory of CPV antibody prevalence in the ISA (Table 1). Model 1 (Table 1) is a simple linear regression, model 2 is a quadratic regression, model 3 is a logistic growth model, and models 4–9 are combined logistic growth models with various trigonometric transformations with parameter $P$ an estimate of period (Graybill, 1976). We used the PROC NLIN of SAS (SAS Institute Inc., 2004) to fit all models with the default Gauss–Newton iterative method to compute parameter estimates. Akaike's information criteria for small samples ($AIC_c$) was used to determine which models best described the trajectory. This modeling effort is descriptive only, with no implications beyond the years of this study or to other wolf populations. Our goal was to determine how much evidence there is for periodicity in the nonlinear, increasing seroprevalence trajectory.

TABLE 1. Summary of plausible models examined for modeling canine parvovirus prevalence in wolves from 1972 to 2004. The response variable ($y$) is the percentage of sampled wolves with canine parvovirus, year ($t$) is the explanatory variable (rescaled to year—1972). The number of parameters ($k$) includes one for the residual variance parameter $\sigma^2$ ($\pi$=3.14).

| Model no. | Model structure | $k$ |
|---|---|---|
| 1 | $y=\beta t$ | 2 |
| 2 | $y=\beta_1 t+\beta_2 t^2$ | 3 |
| 3 | $y=\alpha(1-\exp(\beta t))$ | 3 |
| 4 | $y=\alpha(1-\exp(\beta t))+\delta\cos(2\pi t/P)$ | 5 |
| 5 | $y=\alpha(1-\exp(\beta t))+\gamma\sin(2\pi t/P)$ | 5 |
| 6 | $y=\alpha(1-\exp(\beta t))+\delta\cos(2\pi t/P)+\gamma\sin(2\pi t/P)$ | 6 |
| 7 | $y=\alpha(1-\exp(\beta t))+\gamma/(t+1)\sin(2\pi t/P)$ | 5 |
| 8 | $y=\alpha(1-\exp(\beta t))+\delta/(t+1)\cos(2\pi t/P)$ | 5 |
| 9 | $y=\alpha(1-\exp(\beta t))+\delta/(t+1)\cos(2\pi t/P)+\gamma/(t+1)\sin(2\pi t/P)$ | 6 |

## RESULTS

### Intensive study area

Wolves were live-trapped (Mech, 1974) and bled ($n$=542 wolves) in the ISA area

TABLE 2. Data on canine parvovirus (CPV) effect on measures of wolf population change in an intensive 2,060-km$^2$ study area of northeastern Minnesota.

| Year | $n$[a] | % CPV | % pups | Population[b] | % change[b] | No. packs radioed this year and next | $\bar{X}$ pack size | | Radioed pack size change[b] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | This year | Next year | |
| 1973[c] | 9 | 56 | 67 | 65 | −7 | 5 | 6.2 | 3.0 | −52 |
| 1974 | 11 | 45 | 44 | 44 | −32 | 7 | 3.7 | 5.0 | 35 |
| 1975 | 16 | 35 | 41 | 56 | 27 | 7 | 6.6 | 5.1 | −22 |
| 1976 | 15 | 40 | 50 | 45 | −20 | 5 | 5.8 | 5.6 | −3 |
| 1977 | 18 | 11[d] | 67 | 50 | 11 | 4 | 4.5 | 2.5 | −44 |
| 1978 | 12 | 8 | 10 | 46 | −8 | 3 | 3.3 | 3.7 | 10 |
| 1979 | 15 | 20 | 44 | 54 | 17 | 4 | 3.3 | 3.0 | −8 |
| 1980 | 23 | 73 | 48 | 48 | −11 | 5 | 4.0 | 3.4 | −15 |
| 1981 | 19 | 53 | 43 | 47 | −2 | 6 | 5.7 | 5.2 | −9 |
| 1982 | 18 | 44 | 53 | 50 | 6 | 4 | 6.5 | 5.0 | −23 |
| 1983 | 14 | 36 | 50 | 35 | −30 | 5 | 2.6 | 4.8 | 85 |
| 1984 | 14 | 21 | 70 | 54 | 54 | 6 | 5.2 | 5.5 | 6 |
| 1985 | 18 | 44 | 45 | 47 | −13 | 7 | 5.7 | 4.4 | −23 |
| 1986 | 11 | 64 | 25 | 48 | 2 | 5 | 3.8 | 4.6 | 21 |
| 1987 | 25 | 36 | 28 | 59 | 23 | 5 | 4.6 | 8.2 | 78 |
| 1988 | 21 | 14 | 42 | 79 | 34 | 3 | 8.7 | 7.0 | −19 |
| 1989 | 17 | 100 | 11 | 51 | −35 | 3 | 7.0 | 4.0 | −43 |
| 1990 | 30 | 60 | 20 | 56 | 10 | 6 | 4.2 | 6.0 | 44 |
| 1991 | 26 | 46 | 46 | 53 | −5 | 6 | 6.0 | 6.5 | 8 |
| 1992 | 17 | 47 | 47 | 55 | 3 | 5 | 7.0 | 7.6 | 9 |
| 1993 | 20 | 65 | 25 | 55 | 0 | 5 | 8.6 | 7.6 | −12 |
| 1994 | 14 | 57 | 7 | 55 | 0 | 3 | 7.7 | 8.7 | 13 |
| 1995 | 16 | 56 | 44 | 69 | 25 | 4 | 7.5 | 7.3 | −3 |
| 1996 | 11 | 73 | 6 | 56 | −19 | 7 | 7.6 | 6.1 | −19 |
| 1997 | 28 | 64 | 40 | 55 | −2 | 8 | 5.0 | 4.4 | −13 |
| 1998 | 11 | 55 | 40 | 50 | −9 | 7 | 4.9 | 4.3 | −13 |
| 1999 | 9 | 67 | 25 | 44 | −12 | 6 | 4.7 | 4.8 | 0 |
| 2000 | 18 | 72 | 28 | 52 | 18 | 6 | 5.2 | 5.0 | −3 |
| 2001 | 9 | 78 | 0 | 53 | 2 | 6 | 4.7 | 5.2 | 11 |
| 2002 | 8 | 80 | 10 | 58 | 9 | 7 | 4.0 | 4.9 | 21 |
| 2003 | 14 | 64 | 7 | 62 | 7 | 7 | 5.3 | 7.9 | 49 |
| 2004 | 11 | 82 | 21 | 74 | 19 | | | | |

[a] $n$ = wolves tested for CPV on their first capture; recaptured are only counted once.

[b] The following winter.

[c] The only wolves tested in 1972 ($n$=2) had CPV titers of 64 and 128.

[d] $X^2$=3.72; $P$=0.05; df=1 for 1976 versus 1977 and $X^2$=3.48; $P$=0.06; df=1 for 1976 versus 1978.

from 1972 through 2004. Some of the wolves were captured multiple times, and hence 720 samples were tested for CPV antibody (Mech and Goyal, 1995). From 1973 through 2004, 9–30 (mean=16) wolves (total=518) were serologically tested per year during their first capture (Table 2). We recaptured and CPV-tested 54 females and 44 males two–nine times each over intervals of 2 days to 5.9 yr, for a total of 175 capture/recapture pairs. Of those, 49 involved 36 individual pups.

We captured 233 different pups during 290 pup captures, but not all were CPV-antibody tested. Pups comprised 8–70% of the wolves captured each year. Radio-tagged wolves inhabited 4–10 (mean=6.8) packs of 2–15 wolves per year (Mech, 1986, and unpublished data).

Five of nine wolves captured in 1973 tested positive for CPV (titer=256) and five of 11 in 1974 (titers=256–2,048). The earliest CPV-positive wolf, and the first animal of any species anywhere docu-

TABLE 3. Canine parvovirus (CPV) titers for 518 wolves tested and retested (720 wolf tests) in the Superior National Forest (SNF) from 1972 through 2004 and 221 wolves tested throughout Minnesota from 1979 to 2004.

| | SNF | | Minnesota | |
|---|---|---|---|---|
| CPV | *N* | % | N | % |
| Negative | 54 | 7.5 | – | – |
| | 7 | <1.0 | 4 | 1.8 |
| 8 | 23 | 3.1 | 4 | 1.8 |
| 16 | 35 | 4.9 | 15 | 6.8 |
| 32 | 80 | 11.1 | 20 | 9.0 |
| 64 | 68 | 9.4 | 17 | 7.7 |
| 128 | 68 | 9.4 | 17 | 7.7 |
| 256 | 94 | 13.1 | 17 | 7.7 |
| 512 | 112 | 15.6 | 36 | 16.3 |
| 1,024 | 111 | 15.4 | 47 | 21.3 |
| 2,048 | 54 | 7.5 | 37 | 16.7 |
| 4,096 | 13 | 1.8 | 6 | 2.7 |
| 8,192 | 1 | <1.0 | 1 | <1.0 |

mented with CPV antibodies, was male wolf 5053 sampled on 18 May 1973 in eastern Lake County. Four other wolves from two–three other packs captured within 18 km of where wolf 5053 was trapped and within the next 5 mo also tested antibody positive (titer=256). Five other wolves sampled in the same area during the same period tested negative (titer=128).

Of the 720 CPV tests (including recaptures) in the ISA, 335 (46.5%) were negative (titers 0–128) and 385 (53.5%) were positive (titers 256–8,192; Table 3). Of 350 males tested, 183 (52%) were positive and 167 were negative. Of the 370 females, 201 (49%) were positive and 169 were negative; prevalence differences between genders were not statistically significant. Fifty-four (25%) pups tested positive and 165 negative versus 330 adults positive and 171 adults negative, a significant difference between adults and pups ($P<0.01$; $X^2=103.98$, df=1). For both adults and pups, almost equal proportions of males and females were positive.

CPV-antibody prevalence (adults and pups) increased in this population through 2004 (Table 2), with evidence of periodicity of 7.33 (SE=0.3) yr and dwindling amplitude (Fig. 2). Table 4 presents the

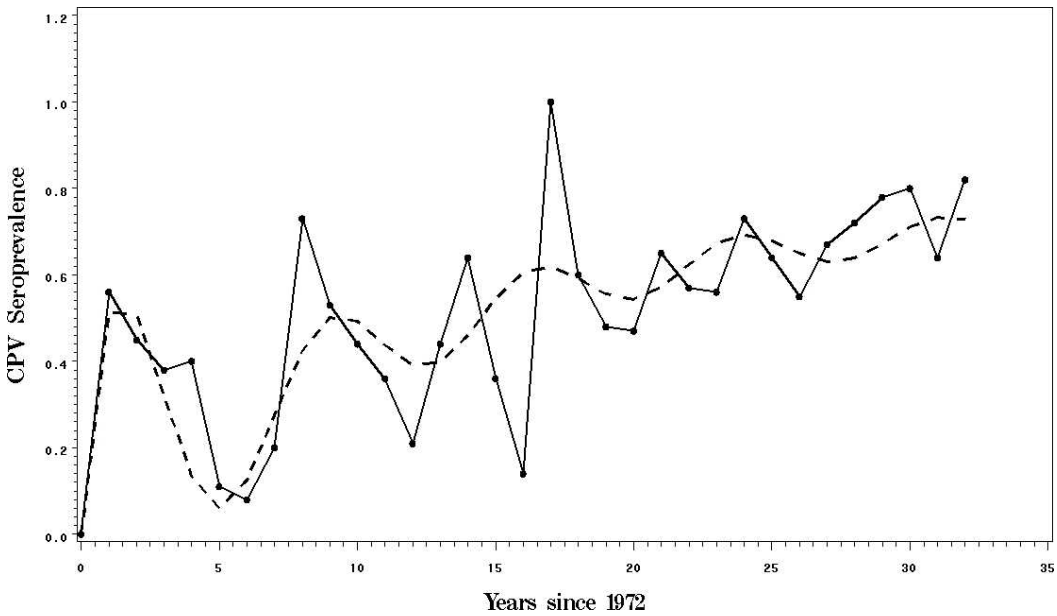


FIGURE 2. Canine parvovirus (CPV) seroprevalence in adult and pups (>2 mo old) in the Superior National Forest of northeastern Minnesota compared with a logistic growth model (dashed line) with sine transformation (adjusted $R^2=0.85$).

TABLE 4. Results of an information-theoretic approach for assessment of models from Table 1 (model numbers are from Table 1 and are sorted by Akaike's information criteria [$\Delta AIC_c$]). RSS=residual sum of squares, total sum of squares=10.2275 for all models, $n$=33 yr.

| Model no. | $k$ | RSS | $AIC_c$ | $\Delta AIC_c$ | Weight | Adjusted $R^2$ |
|---|---|---|---|---|---|---|
| 1 | 2 | 1.5760 | −22.23 | 0.00 | 0.4071 | 0.79 |
| 3 | 3 | 1.2677 | −21.59 | 0.64 | 0.2961 | 0.81 |
| 2 | 3 | 1.3271 | −21.00 | 1.23 | 0.2199 | 0.81 |
| 7 | 5 | 0.7468 | −18.57 | 3.66 | 0.0652 | 0.85 |
| 4 | 5 | 1.1327 | −13.15 | 9.10 | 0.0044 | 0.78 |
| 5 | 5 | 1.1894 | −12.52 | 9.71 | 0.0032 | 0.77 |
| 8 | 5 | 1.2361 | −12.02 | 10.21 | 0.0025 | 0.76 |
| 9 | 6 | 0.7462 | −11.15 | 11.08 | 0.0016 | 0.82 |
| 6 | 6 | 1.1325 | −3 | 16.50 | 0.0001 | 0.73 |

number of parameters in each of the nine models, the residual sum of squares, computed $AIC_c$, $\Delta AIC_c$ (models sorted by), Akaike model weights, and adjusted $R^2$ values. Four models (models 1, 2, 3, and 7) had $\Delta AIC_C$ values <4.0, with the remaining models offering less evidence of plausibility. Models 1, 2, and 3 indicated an increase in CPV with the latter two models accounting for the nonlinearity in the trajectory. The inclusion of model 7 in this set of plausible models provides evidence of periodicity.

Some recaptured wolves seroconverted over short periods. Adult male 6041 had a titer of 128 on 6 September 1983 and 512 on 9 September; female pup 17 had a titer of 16 on August 7, 1987 and 1,024 on 19 August. The proportion of wolf recaptures converting from negative to positive or retaining their positive status increased with time (Fig. 3). Of the 44 male and 54 female wolves tested 2–9 times over periods of up to 5.9 yr, 62% of the recaptures either seroconverted or remained positive, 29% remained negative, and one (9%) converted from positive to negative. Some wolves failed to seroconvert over long periods. Of the 83 male recaptures, six (7%) failed to seroconvert over periods of ≥1 year, including one after 1,753 days. Of the 92 female recaptures, eight (9%) failed to seroconvert over periods of ≥1 yr, including one

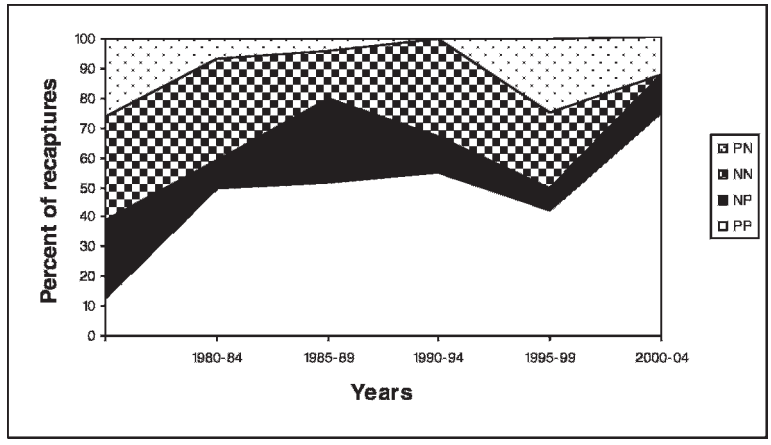


FIGURE 3. Progression of changes in canine parvovirus (CPV) seroprevalence in wolves tested multiple times in the Superior National Forest of northeastern Minnesota. PP ($n$=52) = positive (≥256) to positive; NP ($n$=21) = negative to positive; PN ($n$=12) = positive to negative; NN ($n$=35) = negative to negative.

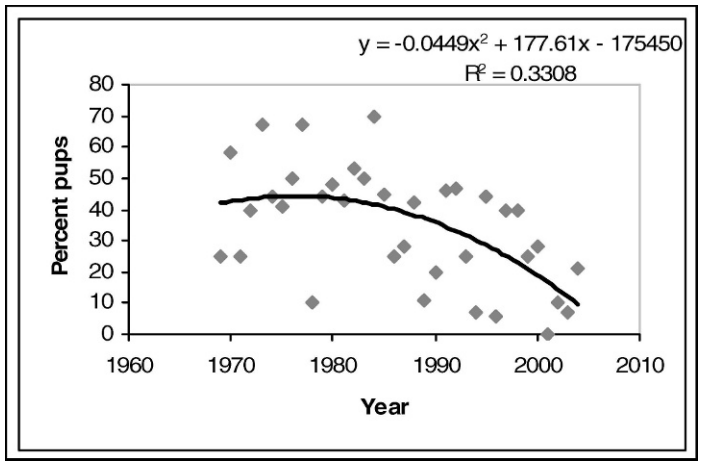


FIGURE 4. Trend in wolf-pup survival index in Superior National Forest of northeastern Minnesota ($P=0.01$).

after 2,139 days. However, all except one of these recaptured animals were tested before 1986. Six (14%) of the male and nine (17%) of the female recaptures converted from positive to negative and these cases were spread over the entire study period. One female and one male seroconverted from negative to positive and back to negative, and one female and one male converted from positive to negative and back to positive.

Nine (25%) of 36 wolves first caught and CPV tested as pups from 1975 to 1998 were positive on retest. Six (17%) of them retained positive status upon recapture 2 days to 3.1 yr later. Within 1 yr, nine (25%) had seroconverted, and by 4 yr, 50% had seroconverted.

The primary effect of CPV in the ISA wolf population could have been mortality of pups <3 mo old (Eugster and Nairn, 1977; Meunier et al., 1981; Johnson et al., 1994), as this would have removed them from our sample. Pups in our area are usually born about 25 April, and first appear outside the den 3 wk later. We were able to observe three CPV-positive (256–1,024) females (one during 2 yr) with litter sizes of four–six pups (mean 5.4 pups/litter). The earliest we caught a seropositive pup was on 13 July 1997. However, this male pup weighed 15 kg, which is heavy for an 11-wk-old pup; the pup may have been born early. We radio-collared most pups >5-mo-old, so we were able to document their survival after that and often their cause of death. We documented the death of a 9-mo-old female from CPV infection (Mech et al., 1997).

Pups 3–7 mo old comprised 0–70% of wolves live-trapped each year (Table 2); the proportion of pups in the total sample significantly declined curvilinearly throughout the study ($r^2=0.33$; $P=0.01$) (Fig. 4). The decline began in about 1984 after CPV became enzootic in the population (Mech and Goyal, 1995) and was inversely related to CPV antibody prevalence from 1984 to 2004 ($r^2=0.51$; $P<0.01$). The annual percent change in the wolf population from winter 1984–1985 to winter 2004–2005 was in turn directly related to the proportion of pups caught during the previous summer ($r^2=0.22$; $P=0.03$) and inversely related to CPV antibody prevalence ($r^2=0.38$; $P<0.01$). A related but more conservative (error-free) component of annual wolf population change, annual change in size of radio-tagged wolf packs, was also related to CPV antibody prevalence ($r^2=0.35$; $P<0.01$).

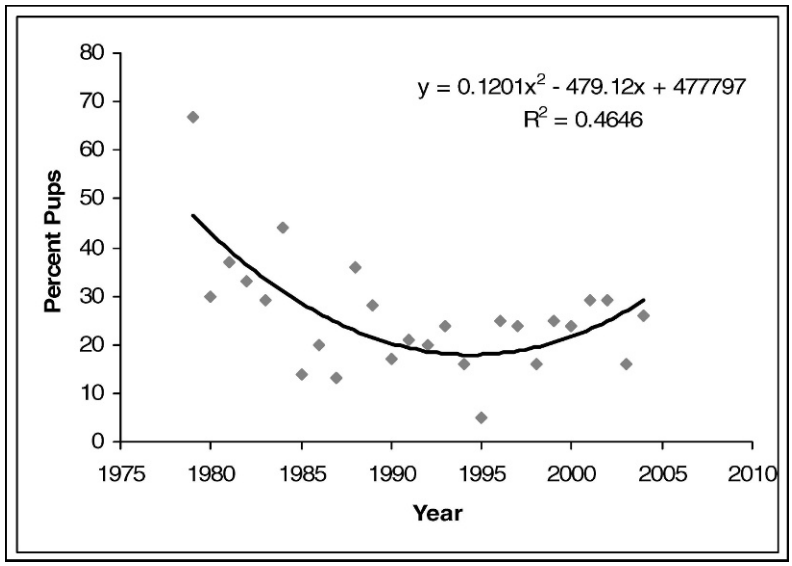


FIGURE 5. Trend in wolf-pup survival index in statewide Minnesota population ($P<0.01$).

#### The greater Minnesota wolf population

In the Minnesota wolf population at large, from 1979 through 2004, we captured 2,562 wolves in 16 counties and tested 221 for CPV antibodies from 1979 to 1989, and in 1991, 1992, and 2004. Sixty-five percent were positive, significantly higher than the percent in the ISA ($X^2=9.38$; $P<0.01$; df=1). Annual CPV antibody prevalence increased with time from 17% to 100% in 2004 ($r^2=0.24$; $P=0.13$). CPV-antibody prevalence in eight counties varied from 33% to 85% with samples of six to 39 wolves per county. Three contiguous counties in extreme northwestern Minnesota had the highest antibody prevalence (69%, 75%, and 85%). Beltrami County had a significantly higher antibody prevalence than all other counties ($X^2=4.06$–$11.49$; $P=0.04$–$0.01$; df=1) with the exception of two neighboring counties; Lake of the Woods and Roseau Counties (Fig. 1).

Twenty-four percent ($n=668$) of the wolves captured in greater Minnesota were pups (range 5–67%/year). The percent of pups captured each year declined ($r^2=0.46$; $P<0.01$) curvilinearly (Fig. 5). The mean proportion of pups captured from 1979 through 1984 was 40, whereas from 1985 through 2004 it was 21 ($P=0.01$). The Minnesota wolf population increased at an average annual rate of 3.0–4.5% from 1979 to 2004 (Fuller et al., 1992; Berg and Benson, 1999; Erb and Benson, 2004).

#### History of CPV

One of the earliest locations where CPV appeared (the earliest on record) was in our northeastern Minnesota ISA. The area where we captured the first antibody-positive wolves is wilderness, but it contains gravel roads and canoe routes and is frequented by humans and dogs. The CPV status of wolves before 1973 is not known. We sampled only two wolves in 1972, neither of which was positive, although one had an antibody titer of 128, which some consider positive (Carmichael and Binn, 1981). In May 1973 we captured the first of five CPV-positive (titer=256) wolves.

CPV-antibody prevalence remained at 38–45% through 1976 in wolves from the same immediate area that we sampled in 1973, and then it dropped significantly ($X^2=3.48$–$3.72$; $P=0.05$–$0.06$; df=1) in

TABLE 5. Parameter estimates, approximate standard errors (SE), and approximate 95% confidence intervals for models 1, 3, 2, and 7 from Table 1.

| Model | Parameter | Estimate | Approximate SE | Approximate 95% confidence intervals |
|---|---|---|---|---|
| 1 | $\beta$ | 0.0275 | 0.0021 | (0.0233, 0.0317) |
| 3 | $\alpha$ | 0.7379 | 0.1275 | (0.4779, 0.9980) |
| | $\beta$ | −0.0860 | 0.0369 | (−0.1612, −0.0107) |
| 2 | $\beta_1$ | 0.0456 | 0.0077 | (0.0298, 0.0614) |
| | $\beta_2$ | −0.0007 | 0.0003 | (−0.0014, −0.0001) |
| 7 | $\alpha$ | 0.7662 | 0.1203 | (0.5202, 1.0122) |
| | $\beta$ | −0.0766 | 0.0276 | (−0.1332, −0.0201) |
| | $\gamma$ | 1.2072 | 0.2684 | (0.6584, 1.7561) |
| | P | 7.330 | 0.3171 | (6.6814, 7.9786) |

1977 and 1978. In addition, wolf 353 (positive at a titer of 256) in November 1975 tested negative in June and August 1977 (titers=8 and 64, respectively) but wolf 5472, which was positive (titer=512) in October 1976 remained positive (titer=1,024) in September 1978. Three other wolves that were negative (titers=64–128) in 1977 remained negative (titers=16–128) in 1978. If foci of CPV infection were still localized in 1977 and 1978, the decreased antibody prevalence might have resulted from bias in area sampled. However, by 1976, we had found CPV-positive wolves 16 km north of the 1973 locations and 26 km west, and our 1977 and 1978 samples included those areas and areas in between, so biased sampling probably was not the cause of the temporary decline in seroprevalence.

In any case, in adult ISA wolves, CPV-antibody prevalence increased ($r^2$=0.47; $P$<0.01) and from 1985 through 2004, it averaged 81%. In 3–7-mo-old ISA pups, however, CPV seroprevalence averaged 43% from 1973 through 1982 ($n$=72), but as increasingly fewer pups survived and entered our sample (Fig. 4), CPV seroprevalence in pups dropped to 12% from 1983 through 2004 ($n$=119; $X^2$=24.39; $P$<0.01, df=1).

## DISCUSSION

The apparent cyclicity and dwindling amplitude of CPV-antibody prevalence in our ISA appears to be a unique observation for a long study of a wildlife disease. In a 14-yr study of canine distemper in raccoons (*Procyon lotor*), epizootics occurred at 4-yr intervals but incidence did not vary during these events (Roscoe, 1993). The Akaike weight model 7 is not strong compared to models 1, 2, and 3, but the adjusted $R^2$ value indicates a good fit to the trajectory (Table 4). Although the evidence is not overwhelming, model 7 indicates a periodicity of 7.33 (SE=0.32) yr (Table 5). We note that model 7 includes structure that accounts for not only the period parameter $P$ but also for the dampening of the fluctuation in the trajectory.

As CPV was infecting wolves in our ISA (Mech et al., 1986), it also began to be detected elsewhere. CPV-antibody-positive dogs were documented in Greece in 1974 (Koptopoulos et al., 1986), in the Netherlands in 1976 (Schwers et al., 1979), and in Texas in 1977 (Eugster and Nairn, 1977). In 1979, one of the three wolves sampled in Koochiching County in the main Minnesota population was positive (titer=1,024), some 160 km west of our ISA, and in 1980, antibody prevalence in 19 wolves was 53%, with positive animals in Roseau County in northwestern Minnesota 275 km from our ISA.

### CPV effect on wolves

We believe that the primary effect of CPV in our study is mortality in young pups. The only other way of explaining a

link between increased CPV-antibody prevalence and decreased number of pups would be through a CPV effect on litter size of CPV-positive adult females. However, the mean litter size of our CPV-positive females (5.4 pups) compares favorably with the normal litter size of 6.0 (Mech, 1970). Although about 10% of the wolves we captured and followed in our ISA had CPV titers ≥2,048, only one (9 mo old) wolf died from CPV. In beagles, CPV titers reached ≥1,280 only in individuals within 2 wk of active infection (Hirasawa et al., 1987). Apparently most of our wolves ≥3 mo old were able to survive infections.

Mortality related to CPV primarily occurs in 1–12-wk-old animals (Eugster and Nairn, 1977; Meunier et al., 1981; Johnson et al., 1994), although CPV mortality has been reported in 15-mo-old captive wolves (Goyal et al., 1986). Maternal antibodies in coyotes (*Canis latrans*) have been reported in 83% of pups of antibody-positive mothers and can persist for up to 8 wk (Green et al., 1984). Titers of maternally derived antibodies in dog pups in large litters were lower than in small litters (Pollock and Carmichael, 1982), but after 8–12 wk such pups were no longer protected (Meunier et al., 1981). In coyote pups, the half life of maternally derived CPV antibody was 6.7 days (Green et al., 1984) and in dogs, 9.7 days (Pollock and Carmichael, 1982). Thus wolf pups, without maternal antibodies, would become vulnerable to CPV when they emerge from the den at about 3 wk of age; pups with maternal antibodies would become susceptible after 6–8 wk.

Canine parvovirus may be maintained in the ISA wolf population by carrier animals. A dog with an HI titer of 4 at 6 mo postinfection, shed virus in feces for ≥6 mo (Komolafe, 1985); CPV can remain infective in feces ≥7 mo (Gordon and Angrick, 1986). It is possible that the virus can remain infective in wolf feces for ≤5 yr (Muneer et al., 1988). Antibodies in dogs persisted ≥2 yr (Carmichael and Binn, 1981). One of our wolves (female 5176) had positive titers all five times tested from 1975 through 1983. Our recapture data indicated that seroconversion can take place within 3–12 days and 25% of recaptured pups had seroconverted within a year.

Because wolf pups remain close to dens until ≥12 wk old (about July 25), our sampling technique, which depended on animals traveling, included few pups that were vulnerable to CPV. Therefore, the proportion of pups in our capture sample became an inverse index to pup survival. This index (i.e., pup survival) declined in our ISA (Mech et al., 1986), especially after CPV became enzootic in the ISA about 1984 (Mech and Goyal, 1995). An additional 11 yr of data from the ISA and an examination of this pup index in the wolf population at large now indicate that CPV has affected not only the local wolf population in the ISA, but also the entire Minnesota wolf population. The number of pups now surviving through summer each year has been reduced from the pre-CPV period by about 70% in the ISA and by 40–60% in the entire Minnesota wolf population. Although this drastic decline in pup survival does influence annual population change, as documented above in our ISA, enough pups still survive to maintain the population. This is because competition for food is keen in this saturated population, and pup starvation (Van Ballenberghe and Mech, 1975) and other types of mortality are high (Mech, 1977). Thus, much of CPV-caused mortality merely compensates for other causes of death (Mech and Goyal, 1995).

#### Population effects of CPV

In a population with the potential to colonize additional areas, the 40–60% pup reduction can seriously retard further colonization. A high proportion of surviving pups disperse from their natal packs when 1–3 yr old (Mech and Boitani, 2003), seek new areas with adequate prey, mate, reproduce, and form new packs,

thus expanding the population. The main prey of wolves in most of Minnesota is white-tailed deer, and although in low density in the ISA, they abound in most of Minnesota. There, the pre-CPV level of pup production would have led to increased dispersal and colonization of new areas similar to adjacent Wisconsin and Michigan, where wolves are increasing at many times Minnesota's rate (Fuller et al., 1992; Wydeven et al., 1995). Although the growth of the Wisconsin wolf population apparently was retarded by CPV early in its recolonization (Wydeven et al., 1995), it has since flourished. From 1981 through 1986, the nascent Wisconsin wolf population dropped from 21 to 16 individuals when its CPV antibody prevalence was 77% (24/31) whereas from 1988 to 1996 it increased an average of 18% per year from 26 to 99 wolves when CPV seroprevalence was down to 35% (22/63) ($X^2=15.02$; $P=0.01$; df=1; Wisconsin Department of Natural Resources, 1999).

The known effects of CPV on young pups, our data on litter sizes, as well as our correlations and the lack of other plausible explanations, suggest that CPV caused the demographic changes we observed. In 2004, CPV antibody prevalence in greater Minnesota was 100% ($n=17$), and from 1997 through 2004 antibody prevalence in ISA adults averaged 87% (72 of 83 animals).

A recent report that CPV antibody prevalence in Yellowstone National Park (YNP) wolves was 100% from 1995 to 2005 (Smith and Almberg, 2007) raises the question of why the CPV antibody prevalence in our area has fluctuated. One explanation is that the YNP wolves are highly isolated from any possible source of non-exposed immigrants. The ISA, conversely, adjoins the entire Canadian wolf population, so it receives immigration from pristine wilderness areas where CPV may not yet have become established. Wolves can disperse straight-line distances of up to 1,092 km (Wabakken et al., 2007).

We still do not understand, however, what determines the spatial variation in CPV antibody prevalence in Minnesota wolves. While CPV was spreading through Minnesota, the prevalence was the highest in the extreme northwest. That area receives less precipitation, mostly <60 cm/yr, whereas most of the rest of Minnesota wolf range receives 66–81 cm/yr (Spatial Climate Analysis Service, 2000). Transmission can occur through contact with infected feces, and dry feces can remain positive for CPV for more than 5 yr (Muneer et al., 1988). Higher precipitation would serve to remove fecal material; the lower precipitation in northwestern Minnesota may explain the higher CPV antibody prevalence there. A higher precipitation of 76–86 cm/yr in Wisconsin's wolf range (Spatial Climate Analysis Service, 2000) also may explain why CPV is more sporadic there and has not become endemic in the wolf population.

One final question our study raises involves the origin of CPV. Although much is known about the molecular changes that allowed the feline panleukopenia-like virus to spread to canids (Shackleton et al., 2005), it is not known where and when that mutation occurred. The initial evidence of this virus in canids, specifically from wolves in northeastern Minnesota in 1973, supports the idea that wild canids may have been involved in this adaptation.

This study appears to be the first to document compelling, circumstantial evidence for long-term effects of CPV on a wildlife population and the only investigation that has followed the course of any wildlife disease from near its inception through its first 30 yr. We conclude, from our ISA data, from the greater Minnesota data, and from the Wisconsin data, that there is compelling circumstantial evidence that CPV may be a major determinant of rate of wolf population increase and recolonization over its current range in the midwestern USA and that it is restricting further recolonization of Minnesota.

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## LITERATURE CITED

BERG, W., AND S. BENSON. 1999. Updated wolf population estimate for Minnesota, 1997–1998. Minnesota Department of Natural Resources, Grand Rapids, Minnesota.

BURNHAM, K. P., AND D. R. ANDERSON. 2002. Model selection and multimodel inference: A practical information-theoretic approach, 2nd Edition. Springer-Verlag, New York, New York.

CARMICHAEL, L. E., AND L. E. BINN. 1981. New enteric viruses in the dog. Advances in Veterinary Science and Comparative Medicine 25: 1–37.

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

ERB, J., AND S. BENSON. 2004. Distribution and abundance of wolves in Minnesota, 2003–04. Minnesota Department of Natural Resources, Grand Rapids, Minnesota.

EUGSTER, A. K., AND C. NAIRN. 1977. Diarrhea in puppies: Parvovirus-like particles demonstrated in their feces. Southwestern Veterinarian 30: 59–60.

FULLER, T. K., W. E. BERG, G. L. RADDE, M. S. LENARZ, AND G. B. JOSELYN. 1992. A history and current estimate of wolf distribution and numbers in Minnesota. Wildlife Society Bulletin 20: 42–55.

GORDON, J. C., AND E. J. ANGRICK. 1986. Canine parvovirus environmental effects on infectivity. American Journal of Veterinary Research 47: 1464–1467.

GOYAL, S. M., L. D. MECH, R. A. RADEMACHER, M. A. KHAN, AND U. S. SEAL. 1986. Antibodies against canine parvovirus in wolves of Minnesota: A serologic study from 1975 through 1985. Journal of the American Veterinary Medical Association 189: 1092–1094.

GRAYBILL, F. A. 1976. Theory and application of the linear model. Wadsworth, Belmont, California, 704 pp.

GREEN, J. S., M. L. BRUSS, J. F. EVERMANN, AND P. K. BERGSTROM. 1984. Serologic response of captive coyotes (*Canis latrans* Say) to canine parvovirus and accompanying profiles of canine coronavirus titers. Journal of Wildlife Diseases 20: 6–11.

HIRASAWA, T., S. IWAKI, K. WATANABE, K. MIKAZUKI, S. MAKINO, AND Y. HAYASHI. 1987. Outbreak of canine parvovirus infection and its elimination in a closed beagle dog colony. Journal of Veterinary Medicine B 34: 598–606.

JOHNSON, M. R., D. K. BOYD, AND D. H. PLETSCHER. 1994. Serology of canine parvovirus and canine distemper in relation to wolf (*Canis lupus*) pup mortalities. Journal of Wildlife Diseases 30: 270–273.

KOMOLAFE, O. O. 1985. The possible existence of an immune-carrier state in canine parvoviral infections. Microbiology Letters 30: 115–118.

KOPTOPOULOS, G., O. PAPADOPOULOS, M. PAPANASTASOPOULOU, AND H. J. C. CORNWELL. 1986. Presence of antibody cross-reacting with canine parvovirus in the sera of dogs from Greece. The Veterinary Record 118: 332–333.

MECH, L. D. 1970. The wolf: The ecology and behavior of an endangered species. Natural History Press, Garden City, New York, pp. 389.

———. 1974. Current techniques in the study of elusive wilderness carnivores. *In* Proceedings of the 11th International Congress of Game Biologists, Stockholm, Sweden, 3–7 September 1973, pp. 315–322.

———. 1977. Productivity, mortality and population trends of wolves in northeastern Minnesota. Journal of Mammalogy 58: 559–574.

———. 1986. Wolf numbers and population trend in the Superior National Forest, 1967–1985. Research Paper NC-270. Forest Service, North Central Forest Experiment Station, US Department of Agriculture, St. Paul, Minnesota.

———, AND L. BOITANI. 2003. Wolf social ecology. *In* Wolves: Behavior, ecology, and conservation, L. D. Mech and L. Boitani (eds.). University of Chicago Press, Chicago, Illinois, pp. 1–34.

———, AND S. M. GOYAL. 1995. Effects of canine parvovirus on gray wolves in Minnesota. Journal of Wildlife Management 59: 565–570.

———, S. M. GOYAL, C. N. BOTA, AND U. S. SEAL. 1986. Canine parvovirus infection in wolves (*Canis lupus*) from Minnesota. Journal of Wildlife Diseases 22: 104–106.

———, H. J. KURTZ, AND S. M. GOYAL. 1997. Death of a wild wolf from canine parvoviral enteritis. Journal of Wildlife Diseases 33: 321–322.

MEUNIER, P. C., L. T. GLICKMAN, M. J. G. APPEL, AND S. J. SHIN. 1981. Canine parvovirus in a commercial kennel: Epidemiologic and pathologic findings. Cornell Veterinarian 71: 96–110.

MUNEER, M. E., I. O. FARAH, K. A. POMEROY, S. M. GOYAL, AND L. D. MECH. 1988. Detection of parvoviruses in wolf feces by electron microscopy. Journal of Wildlife Diseases 24: 170–172.

PETERSON, R. O., N. J. THOMAS, J. M. THURBER, J. A. VUCETICH, AND T. A. WAITE. 1998. Population limitation and the wolves of Isle Royale. Journal of Mammalogy 79: 828–841.

POLLOCK, R. V. H., AND L. E. CARMICHAEL. 1982. Maternally derived antibody to canine parvovirus: Transfer, decline and interference with immunization. Journal of the American Veterinary Medical Association 180: 37–43.

ROSCOE, D. E. 1993. Epizootiology of canine distemper in New Jersey raccoons. Journal of Wildlife Diseases 29: 390–395.

SAS INSTITUTE, INC. 2004. SAS OnlineDoc® 9.1.2. SAS Institute Inc., Cary, North Carolina.

SCHWERS, A., P. P. PASTORET, G. BURTONBOY, AND E. THIRY. 1979. Frequence en Belgique de l'infection a parvovirus chez le chien, avant et après l'observation des premiers cas cliniques. Annales de Medecine Veterinaire 123: 561–566.

SHACKLETON, L. A., C. R. PARRISH, U. TRUYEN, AND E. C. HOLMES. 2005. High rate of viral evolution associated with the emergence of carnivore parvovirus. Proceedings of the National Academy of Sciences of the United States of America 102: 379–384.

SMITH, D. W., AND E. ALMBERG. 2007. Wolf diseases in Yellowstone National Park. Yellowstone Science 15: 17–19.

SPATIAL CLIMATE ANALYSIS SERVICE. 2000. *Oregon State University*, http://www.ocs.orst.edu/pub/maps/Precipitation/Total/States.

STEINEL, A., C. R. PARRISH, M. E. BLOOM, AND U. TRUYEN. 2001. Parvovirus infections in wild carnivores. Journal of Wildlife Diseases 37: 594–607.

VAN BALLENBERGHE, V., AND L. D. MECH. 1975. Weights, growth, and survival of timber wolf pups in Minnesota. Journal of Mammalogy 56: 44–63.

WABAKKEN, P., H. SAND, I. KOJOLA, B. ZIMMERMANN, J. M. ARNEMO, H. C. PEDERSEN, AND O. LIBERG. 2007. Multi-stage, long-range natal dispersal by a global positioning system–collared Scandinavian wolf. Journal of Wildlife Management 71: 1631–1634.

WISCONSIN DEPARTMENT OF NATURAL RESOURCES. 1999. Wisconsin wolf management plan. Wisconsin Department of Natural Resources, Madison, Wisconsin.

WYDEVEN, A. P., R. N. SCHULTZ, AND R. P. THIEL. 1995. Gray wolf monitoring in Wisconsin 1979–1991. *In* Ecology and behavior of wolves in a changing world, L. D. Carbyn, S. H. Fritts, and D. R. Seip (eds.). Canadian Circumpolar Institute, Edmonton, Alberta, pp. 147–156.

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