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SEROLOGIC SURVEY FOR CANINE CORONAVIRUS IN WOLVES FROM ALASKA

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ABSTRACT: Wolves (*Canis lupus*) were captured in three areas of Interior Alaska (USA). Four hundred twenty-five sera were tested for evidence of exposure to canine coronavirus by means of an indirect fluorescent antibody procedure. Serum antibody prevalence averaged 70% (167/240) during the spring collection period and 25% (46/185) during the autumn collection period. Prevalence was 0% (0/42) in the autumn pup cohort (age 4–5 mo), and 60% (58/97) in the spring pup cohort (age 9–10 mo). Prevalence was lowest in the Eastern Interior study area. A statistical model indicates that prevalence increased slightly each year in all three study areas. These results indicate that transmission occurs primarily during the winter months, antibody decay is quite rapid, and reexposure during the summer is rare.

Key words: Canine coronavirus, *Canis lupus,* serology, survey, wolf.

INTRODUCTION

Population dynamics of wolves (*Canis lupus*) in North America are influenced by a number of factors (Mech, 1970). The two most important factors regulating wolf abundance in Alaska (USA) are availability of food and human harvest (Ballard et al., 1981; Stephenson and James, 1982; Peterson et al., 1984; Mech et al., 1998). However, infectious diseases may play a role, as well (Neiland, 1970; Carbyn, 1982; Peterson et al., 1998).

Canine coronavirus (CCV) is an enteric pathogen of canids (Tennant et al., 1993). The primary means of transmission is via exposure of susceptible hosts to virus shed in feces (Carmichael and Binn, 1981). Clinical signs of CCV infection in dogs include diarrhea and dehydration (Evermann and Benfield, 2000). Mortality rate from CCV infection in otherwise healthy dogs is low. Past serologic surveys of free-ranging canids in North America indicate low levels of exposure to CCV (Davidson et al., 1992; Garcelon et al., 1992; Holzman et al., 1992). Domestic dogs and coyotes (*Canis latrans*) with enteritis are often infected with both canine parvovirus (CPV) and CCV (Green et al., 1984; Evermann et al., 1989). Clinical signs of dual infection are similar to signs for CCV, but may be more severe (Evermann et al., 1980; Appel, 1988).

Wolves in Alaska are exposed to a wide spectrum of infectious agents (Stephenson et al., 1982; Zarnke and Ballard, 1987). There are no previously published reports regarding serologic surveys of wolves for evidence of exposure to CCV.

The primary objective of the current serologic survey was to determine if there was any relationship between antibody prevalence of CCV in wolves from Interior Alaska and the following host parameters: (1) sex, (2) age, (3) location, (4) year of collection, and (5) season of collection.

MATERIALS AND METHODS

During the mid- to late 1990s, three independent studies of wolf population ecology were conducted in Interior Alaska (64°30′N, 143°30′W; 64°00′N, 147°30′W; and 63°30′N, 150°30′W). These respective areas were designated as Western Interior, Central Interior, and Eastern Interior, respectively (Fig. 1). Wolves were captured by darting from helicopters.

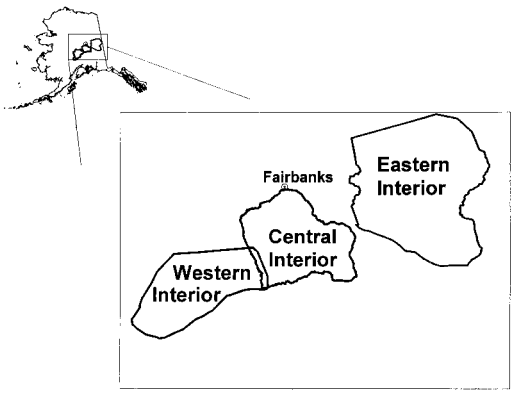

FIGURE 1. Capture areas for wolves tested for serologic evidence of exposure to canine coronavirus.

Blood was collected by venipuncture. Blood samples were kept at ambient or refrigerator temperature for 6–36 hours before centrifugation. Sera were separated and frozen. Sera were tested for evidence of previous exposure to CCV by means of the indirect fluorescent antibody method (Foreyt and Evermann, 1985). Specimens with titers ≥25 were considered indicative of previous exposure. These samples are referred to as "positive." All other samples are referred to as "negative." Titers ≥100 were considered indicative of recent exposure.

A generalized linear model with a logit link (McCullagh and Nelder, 1989) and a binomial distribution was used to determine if there was a significant dependence of a positive CCV serologic test result on the following host variables: (1) sex, (2) age, (3) location, (4) year of collection, and (5) season of collection. Serologic test result is a binary response variable. Year was treated as a continuous variable. Age was treated as a categorical variable with the following classes: (a) <8 mo, (b) 8–16 mo, (c) 17–24 mo, (d) 25–36 mo, (e) 37–60 mo, and (f) >60 mo. Sex, season (spring versus autumn) and geographic location were treated as categorical variables. All main and interaction effects of these variables were examined. During the modeling process, all higher order terms were removed from the model if they did not substantially ($P > 0.05$) increase the fit of the model based on the deviance function compared to a chi-square distribution (McCullagh and Nelder, 1989). The GENMOD procedure of version 6.12 SAS statistical software package was used to fit the model with maximum likelihood parameter estimates (SAS Institute, Cary, North Carolina, USA).

RESULTS

One hundred sixty-one samples were collected from wolves in both the eastern and central study areas. One hundred three were collected from the western area. Two hundred thirteen of the 425 samples had antibody titers ≥25. Forty-four samples had titers ≥100. All of the samples with high titers were collected during the spring period.

Antibody prevalence for CCV followed a distinct seasonal pattern (Fig. 2). Prevalence within packs followed the same seasonal pattern, i.e., low in autumn and high in spring. There were no packs that maintained a consistently high or low prevalence over the course of several collection periods.

Prevalence averaged 32% (46/143) for the adult cohort during the autumn period. Prevalence for the adult cohort in spring was 76% (109/143). Prevalence for the pup cohort in autumn (age 4–5 mo) from all three areas was 0% (0/42). Prevalence increased to 60% (58/97) for the pup cohort in spring (age 9–10 mo).

Fourteen animals were sampled during successive periods (either spring-to-autumn, $n = 5$; or autumn-to-spring, $n = 9$). Seven animals maintained their CCV exposure status (either positive or negative) during these subsequent periods. Four changed from negative in autumn to positive in spring. Two changed from positive in spring to negative in autumn. Only one changed from negative in spring to positive in autumn.

The fitted probability model for wolves included four of the covariates: age, location, year of collection, and season of collection. Sex of the wolf was not a significant factor ($P = 0.2993$). The fitted model for the autumn pup cohort was zero, regardless of year and location. For all other age cohorts, age was not a factor. For all wolves older than pups, the fitted model was: $\mu = \tau_i + 0.2105 \times \text{yr}$, where τ_i is −21.1008 if the animal was from Central Interior in autumn, −18.8959 if the animal

TABLE 1. Age-specific, season-specific, and year-specific serum antibody prevalence for canine coronavirus in wolves from three areas of Interior Alaska.

Area	Year						Total (%)
	1994	1995	1996	1997	1998	1999	
Eastern Interior							
Autumn Pup			ND[a]	0/7[b]	0/4	ND	0/11 (0)
Autumn Adult			1/9	4/25	8/32	2/8	15/74 (20)
Spring Pup			1/2	4/12	14/20	3/13	22/37 (59)
Spring Adult			ND	7/13	9/11	3/5	19/29 (66)
Central Interior							
Autumn Pup		0/6	0/6	0/8	0/10	ND	0/30 (0)
Autumn Adult		2/4	ND	3/17	5/13	3/3	13/37 (35)
Spring Pup		8/11	8/12	7/10	2/2	3/3	28/38 (74)
Spring Adult		1/1	9/11	15/18	7/7	17/19	49/56 (88)
Western Interior							
Autumn Pup	0/1	ND	ND	ND	ND	ND	0/1 (0)
Autumn Adult	6/7	3/6	2/6	6/11	1/2	ND	18/32 (56)
Spring Pup	1/1	3/3	2/4	2/4	ND	ND	8/12 (67)
Spring Adult	2/7	8/14	3/3	12/13	9/12	7/9	41/58 (71)
Combined							
Autumn Pup	0/1	0/6	0/6	0/15	0/14	ND	0/42 (0)
Autumn Adult	6/7	5/10	3/15	13/53	14/47	5/11	46/143 (32)
Spring Pup	1/1	11/14	11/18	13/26	16/22	6/16	58/97 (60)
Spring Adult	2/7	9/15	12/14	34/44	25/30	27/33	109/143 (76)

[a] ND = No data.
[b] Number positive/number tested.

was from Central Interior in spring, −19.9191 if the animal was from Western Interior in autumn, −19.4431 if the animal was from Western Interior in spring, −21.9077 if the animal was from Eastern Interior in autumn, and −20.4387 if the animal was from Eastern Interior in spring. Because the model is on the logit scale, the predicted value is,

$$p(\mu) = \frac{\exp(\mu)}{1 + \exp(\mu)}.$$

For example, if an animal were from Western Interior in spring 1999, then $\mu = 1.3946$, so the probability of a positive test result is predicted to be $p(\mu) = 0.80$. The significance of location, season, and their interaction in the model was $P < 0.0001$, the significance of age in the model was $P < 0.0001$, and the significance of year in the model was $P = 0.0309$. None of the other higher level interactions were significant.

DISCUSSION

The seasonal pattern of low antibody prevalence in the autumn and high prevalence in the spring indicates that winter is the primary period for CCV transmission. Additional support for this conclusion is provided by the fact that all high titers (indicative of recent exposure) were from the spring collection period. The individual animals whose serologic status changed from negative in the autumn to positive the following spring further support this conclusion. The dramatic increase in antibody prevalence from 0% for the pup cohort in the autumn to 60% for the pup cohort in the spring provides additional support. Only a few samples were available for wolves captured during January and February in any of the three study areas.

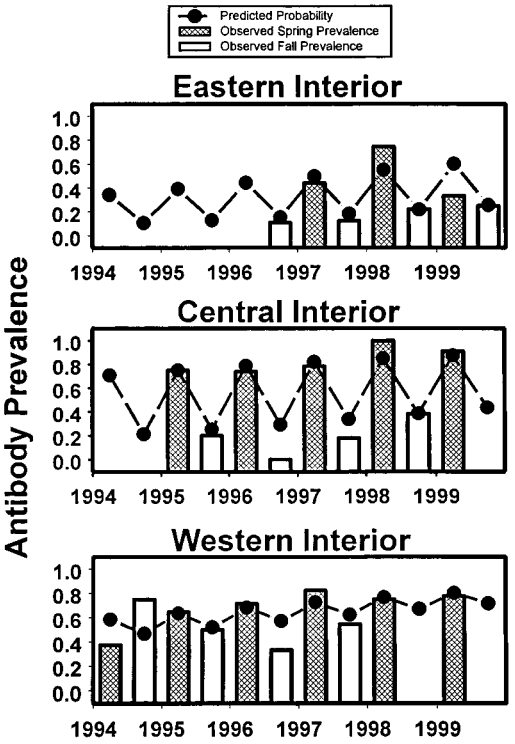

FIGURE 2. Observed and predicted time-specific antibody prevalence for canine coronavirus in wolves from three areas of Interior Alaska.

All were negative. Therefore, we conclude that the peak of transmission occurs during late February and/or early March in these areas.

The dramatic decline in antibody prevalence from spring to autumn indicates that decay of serum antibody is quite rapid. In addition, it seems that re-exposure during the summer months is rare. The individual animals whose serologic status changed from positive in the spring to negative the following autumn support these conclusions.

Passively-acquired maternal antibody may protect young wolf pups (<4-mo-old) from clinical CCV disease. As a parallel example, adult female coyotes transfer protective antibody to their offspring (Green et al., 1984). Maternal antibody may also preclude wolf pups from actively producing their own antibody prior to the autumn capture period. In coyotes, maternal antibody wanes by 11-wk of age (Green et al., 1984). Unfortunately, no sera were available from wolf pups <16 wk of age for the current study. Therefore, it was not possible to evaluate the role of maternal antibody in these wolf pups.

Incidence of clinical CCV disease in domestic dogs increases in winter months (Stott, 1999). Virus shed in dog feces during winter months survives longer than virus shed during summer months, presumably due to lower temperatures and lower ultraviolet exposure in winter (Carmichael and Binn, 1981; Tennant et al., 1991; Holmes and Lai, 1996). In addition, some breeds exhibit increased coprophagic tendencies in winter when dog feces is frozen and more readily available (V. Stuve, pers. comm.). This preference for frozen feces is unexplained. Perhaps wolves also practice coprophagy during the winter. Either one or both of these factors may be responsible for the increase in antibody prevalence for wolves during the winter months.

The magnitude of the seasonal changes in antibody prevalence was not uniform for the three areas. Sled dogs are commonly used in all three areas and we have no reason to suspect that sled dog activity differs among these areas. Wolf and coyote (*Canis latrans*) population densities are higher in the central Interior, as compared to the eastern and western study areas (Mech et al., 1998; P. Valkenburg, pers. comm.). Perhaps the higher wolf and coyote densities in the central study area contributes to higher rates of CCV transmission.

The model indicated a slight but consistent increase in antibody prevalence during the years of this survey. Previous surveys of wolves from Alaska found location-specific CCV antibody prevalences ranging from 0%–19% (R. Zarnke, unpubl. data). Those values are substantially lower than found during the current survey. All of the sera from both surveys were tested by the same laboratory, using the same methods. However, sampling periods for the previ-

ous survey were not aligned with the spring and autumn periods incorporated in the current survey. In addition, sample sizes were often small in the previous study. Therefore, results of the two surveys are not directly comparable. Perhaps CCV has been introduced into wolves in Alaska only recently. The virus could have been spreading rapidly in a predominantly susceptible population during the prior survey. Under this scenario, a large increase in antibody prevalence during a decade would not be unexpected. Conversely, the slow rate of increase observed during the current survey indicates that prevalence may be reaching equilibrium.

The positive test results in the current survey are believed to be the result of exposure to prototype CCV. Coronaviruses are found in a variety of mammalian families (Evermann and Benfield, 2000). Many of these coronaviruses are antigenically related, and serologic cross-reaction is a recognized phenomenon (Tsunetitsu et al., 1995; Herreweigh et al., 1998). However, the serologic test procedure used in the current survey is quite specific for CCV. Based on experience in this laboratory, the high titers found in many wolf samples would not occur with heterologous antigen/antibody reaction. Dual exposure to CCV and other coronaviruses is also a possibility.

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