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Author: STEPHENSON, R.O.

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SEROLOGIC SURVEY FOR CANINE DISTEMPER AND INFECTIOUS CANINE HEPATITIS IN WOLVES IN ALASKA[1]

R.O. STEPHENSON, Alaska Department of Fish and Game, 1300 College Road, Fairbanks, Alaska 99701, USA.
D.G. RITTER, State of Alaska, Division of Public Health, Virology-Rabies Unit, Fairbanks, Alaska 99701, USA.
C.A. NIELSEN, Alaska Department of Fish and Game, 1300 College Road, Fairbanks, Alaska 99701, USA.[2]

Abstract: Sera from 57 wolves *(Canis lupus)* in three areas of Alaska were evaluated for evidence of previous exposure to infectious canine hepatitis virus (ICHV) and canine distemper virus (CDV). Fifty-four sera (94.7%) were positive for ICHV exposure and four (7%) were positive for CDV exposure. All four CDV-reacting wolves also had titres to ICHV. The relatively common occurrence of ICHV exposure may be due to the greater resistance of ICHV to chemical and physical agents and its transmissibility via the urine of infected animals. The ICHV titres observed could indicate enzootic pathogenic ICHV, or exposure to the mildly pathogenic vaccine strain of CAV-1 through contact with the urine of domestic dogs. If CAV-1 is the original source of exposure, the titres could represent an ICHV-protected wolf population.

INTRODUCTION

A number of studies have documented the existence of canine distemper virus (CDV) or infectious canine hepatitis virus (ICHV) in wild canids (Mongeau, 1961; Parker et al., 1961; Trainer and Knowlton, 1968; Choquette and Kuyt, 1974; Gier et al., 1978) and epizootics of these diseases in both captive and wild canids have been documented in arctic and subarctic regions by Elton (1931), Murie (1944), Rausch (1953), Reinhard et al. (1955), and Mongeau (1961). Relatively little is known, however, about the occurrence of these viral agents in wild wolf populations although there are indications that CDV can be a source of mortality (Carbyn, 1982; Peterson, pers. comm.). The objectives of this study were to obtain information on the prevalence of CDV and ICHV in certain wolf populations in Alaska and, to the extent possible, assess the probable effects of these diseases in wild populations.

MATERIALS AND METHODS

Serum samples were obtained from 57 wolves in three areas of Alaska. Thirty-five samples originated from wolves killed in winter 1976-1977 during an Alaska Department of Fish and Game control program on the Tanana Flats south of Fairbanks. Eleven samples were obtained from radio-marked wolves in the Nelchina Basin south of the Alaska Range and six additional samples were obtained in this area from wolves killed in an experimental removal of wolves in a portion of the Basin. Blood was also collected from five wolves that were radio-marked in northwest Alaska in the vicinity of the Utukok River. The Tanana Flats wolves were collected within 165 km of Fairbanks. The Nelchina area lies about 240 km south of Fairbanks and about the same distance northeast of Anchorage. Small cities and villages occur in and around each of these areas. The study area in northwest Alaska is

[1] This study was supported by the State of Alaska and by Federal Aid in Wildlife Restoration.

[2] Present address: Department of Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, Washington, 99164, USA.

located 650 km northwest of Fairbanks, approximately 160 km from the closest human settlements.

Radio-marked wolves were immobilized with Cap-Chur darts containing phencyclidine hydrochloride [2] and promazine hydrochloride [1] administered from a helicopter. Blood was collected from the anesthetized animals via puncture of the saphenous vein, stored in plain glass vacutainers, allowed to clot, and serum was removed by aspiration and stored at -70 C. Blood samples were obtained from wolves collected on the Tanana Flats from 1 to 8 hr post mortem. Blood was drained from the thoracic cavity and after centrifuging the supernatant was collected as serum, although it undoubtedly contained pleural effusions and other contaminants. As described below, special procedures were used to allow these samples to be tested.

A macro-neutralization test (Nt) was initially employed to evaluate wolf sera for canine hepatitis virus (ICHV) antibodies. Chick embryo monolayer cell cultures (CE) were employed as a host system for CDV; and CCL 34, canine kidney (MDCK) cell culture was used as a host system for ICHV.

Because of frank hemolysis, wolf sera were tested for cell toxicity in serial doubling dilutions of 0 to 1:32. Chick embryo cell cultures were inoculated with 0.3 ml of each serum dilution, incubated for 1 hr at 22 C, rinsed with CE maintenance medium, then incubated at 33 C, and read daily for 7 days to evaluate cell condition. Wolf sera diluted 1:8 and greater were found to have minimal toxicity.

Chick embryo fibroblast cell cultures, prepared from 10 to 11-day-old embryos, were inoculated with 0.3 ml per flask (Falcon T-30) with seed virus diluted in log steps; i.e., 10^0 to 10^{-5}, and incubated at 33 C. The flasks were read daily for a cytopathogenic effect (CPE) and the $TCID_{50}$ dose was calculated (Reed and Muench, 1938). CPE was present in CE cell culture and the $TCID_{50}$ was 10^{-4}. Wolf sera were diluted to 1:8 (heat-inactivated at 56 C for 30 min) and combined with 100 $TCID_{50}$ of CDV in equal volumes. The virus-serum mixture was incubated at 22 C for 1 hr and inoculated on confluent CE cell cultures in T-30 flasks. The cell cultures were incubated for 1.5 hr at 33 C for adsorption, rinsed with 1% maintenance medium, incubated at 33 C, and read daily for CPE. Positive CDV, homologous CDV/anti-CDV, and negative reading controls were used. In the ICHV tests the wolf sera were screened initially at 1:8 dilution using MDCK cell culture and ICHV. Heat inactivated sera were tested against 150 $TCID_{50}$ of ICHV. Subsequent NTS were evaluated via microtiter systems employing transfer plates with 0.025 ml of virus and equal volumes of serum at 1:8 dilution. In this modified system, the serum-virus mixtures were incubated at 22 C for 1 hr and then transferred to confluent CE or MDCK cell cultures grown in flat-bottom microtitre plates. The tests were incubated for 1.5 hr under CO_2 and read daily.

RESULTS

Previous exposure to the ICHV antigen with titres of 1:8 or higher was indicated in 54 of the 57 sera (94.7%). Four (7%) of these also had a titre of 1:8 or higher to CDV antigen (Table 1). Antibodies indicative of exposure to ICHV were found in sera from all three areas sampled, while CDV antibodies occurred in two samples from the Nelchina Basin and two from the Tanana Flats. No evidence of exposure to CDV was found in northwest Alaska, but sera from one member of each of two different packs

[2] Bio-ceutic Laboratories, St. Joseph, Missouri 64502, USA.

[1] Wyeth Laboratories, Philadelphia, Pennsylvania 19101, USA.

TABLE 1. Results of virus neutralization tests of serum samples from wolves in Alaska.

Area	No. Packs Sampled	No. Wolves Sampled	No. Wolves CD-Positive[a]	No. Wolves ICH-Positive[b]
Tanana Flats	11	35	2 (5.7%)	35 (100%)
Nelchina Basin	10	17	2 (11.8%)	17 (100%)
Northwest Alaska	3	5	0	2 (40%)
Total	24	57	4 (7.0%)	54 (94.7%)

[a]CDV positive — serum containing antigen to canine distemper virus.
[b]ICHV positive — serum containing antigen to infectious canine hepatitis virus.

were positive for ICHV antibodies at 1:8 and 1:16; respectively. Three members of a third pack were negative for ICHV antibodies. The three ICHV negative wolves were members of a pack inhabiting the Anisak River area which is farther from human habitation than any of the other populations sampled.

Among those wolves that were 1 yr old or less, titres ranged from 1:8 to 1:32 (n = 14). In addition, all three negative reactors to ICHV were 1 yr old. In wolves 1 to 2 yr of age, ICHV titres ranged from 1:8 to 1:128 (n = 12). In wolves 3 yr old and older, titres also ranged from 1:8 to 1:128 (n = 32).

The only observation of what appeared to be clinical signs of either disease involved members of the Delta pack in the Nelchina Basin. Three members of this pack, including an adult female and a yearling male, were very thin and showed abnormal behavior toward humans prior to the time when two of these wolves were killed in June 1975. Although no sera were obtained from the affected wolves, an adult male from the Delta pack showed a titre of 1:32 to ICHV and was negative for CDV antibodies. The Delta pack inhabited an area that appeared to have the lowest abundance of large prey of any of the nine packs studied in the Nelchina Basin and poor nutrition may have rendered some pack members susceptible to disease. Packs in adjacent areas showed no clinical signs of infection, with productivity and survival of pups being normal for the species (Stephenson, 1978).

DISCUSSION

These data suggest that exposure to ICHV is common in Alaskan wolf populations, while exposure to CDV is rare or perhaps fatal. Since the highest titres for ICHV occur in adult wolves, with a wide range of titre levels in each age class, there must be continuing exposure to this antigen in these populations. Eleven of the 54 ICHV-positive wolves were 1 yr of age or less, suggesting that wolves are exposed early in life.

In contrast, the relatively few (4 of 57) wolves with CDV titres ranged widely in age, from 1 to 6 yr, which is consistent with sporadic, short-lived introductions of the virus to populations rather than a continuing enzootic pattern of exposure. When enzootic among domestic dog populations, the infection rate for CDV is very high among pups at 3 to 9 wk of age as passive maternal protection subsides, although up to 75% of all infections may be subclinical (Robson et al., 1959; Gorham, 1966; Gillespie and Carmichael, 1968). By 1 yr of age, protective antibody levels are nearly universal (Gorham, 1966).

While evidence of CDV exposure is based on neutralization titres of only 1:8, corroboration of the occasional presence of this virus in Alaskan wolf populations was obtained recently on the Kenai Peninsula and at Yakutat. In the first area, two radio-marked wolves were found dead with CDV as a possible cause (Peterson, pers. comm.). One of these was a 16-mo-old male that died in September

1977. Fluorescent antibody (FA) testing revealed foci of CDV antigen within cerebral endothelial cells. In January 1979, a 19-mo-old female wolf in the same pack died after remaining separate from other pack members for a least 2 days. In this case, CDV antigen was identified in the urinary bladder mucosa by FA test. The second death occurred during a CDV epizootic among dogs in a populated area adjacent to the pack's territory.

In Yakutat a young adult male wolf trapped near the town in late February 1981 was found by one of us (D.R.) to have CDV antigen in bladder mucosa and cerebral endothelium by FA test. This wolf was emaciated and had lost virtually all its guard hair.

In examining 86 wolves from the MacKenzie District, Northwest Territories (Canada), Choquette and Kuyt (1974) found two (2%) serologically positive for CDV exposure and 11 (13%) positive for ICHV exposure. They noted that relatively few wolves seemed to have been exposed to both CDV and ICHV and survived. In the present study, however, four wolves with evidence of CDV exposure also had ICHV titres.

Of interest in this study was the apparently common occurrence of wolf ICHV exposure compared to the low rate of CDV exposure, particularly when the latter is a serious problem in virtually all unvaccinated dog populations (Gorham, 1966). This difference can probably be related to differences in viral transmission under Alaskan circumstances. CDV is an enveloped paramyxovirus, and is usually transmitted by direct contact and as short-range (less than 15 m) aerosols. This virus survives less than a day in secretions, excretions, or in fomites, and is easily inactivated by sunlight, mild heat (20 C), or chemical agents (Gillespie and Carmichael, 1968). Transmission in urine or feces has never been shown (Budd, 1970). Dogs surviving an infection become immune to reinfection, and there are no persistent carriers (Gorham, 1966; Gillespie and Carmichael, 1968). In contrast, the ICH adenovirus (types 1 or 2) has no envelope and is highly resistant to many chemical and physical agents (Cabasso and Wilner, 1969). In addition, ICHV is commonly excreted in the urine of inapparently infected or clinically recovered dogs due to a persistent infection in the renal interstitium (Osborne et al., 1972). Some dogs vaccinated with the post-1958 standard canine adenovirus-1 (CAV-1) vaccine strain have been shown to shed CAV-1 virus in their urine for variable periods (Bass et al., 1980). Indirect transmission is therefore far more likely for ICHV than for CDV.

Wolves are highly social animals and physical contact among pack members is frequent (Zimen, 1976; Mech, 1977), thus providing considerable opportunity for intra-pack exposure to both CDV and ICHV. Although in most areas in Alaska wolves maintain relatively large territories which are used primarily by members of a single pack, wolves do have occasional contact with alien wolves and wolf packs (Stephenson, 1978) and probably also have a considerable amount of contact with the urine and feces of other packs (Peters and Mech, 1975). In addition, wolves sometimes have direct contact with other canids including foxes and coyotes which are sometimes killed and consumed by wolves (Mech, 1970). Since most far-ranging pack travel occurs in fall and winter, most contact with scent sites, alien packs, or other canids probably occurs at that time. Pups born in the previous May and June would be over 3 mo of age during this fall-winter period, and hence be unprotected by maternal antibodies and fully susceptible to resistant viruses, like ICHV, in their environment.

Comparing our observations with those of Choquette and Kuyt (1974) suggests exposure to ICHV is far more common in Alaska (94.7%) than in northern Canada (13%). Since the latter area has fewer settlements or towns, and

since in our study the pack containing the three ICHV-negative wolves inhabited the area most remote from human habitation, contact with domestic dogs could have an important role in the transmission of ICHV to wolf populations. However, the significance of the adenovirus neutralizing titres attributed by wildlife investigators to "wild-type" ICHV remains to be clarified, since they could actually represent titres to the midly pathogenic respiratory adenovirus (CAV-2), or even to the nonpathogenic vaccine strain of CAV-1. Live-virus vaccination of domestic dogs with attenuated CAV-1 strains has been very common since their introduction in 1958, and since vaccine CAV-1 is known to be shed in the urine of some vaccinates, the titers observed in wolves may in fact represent a widespread "natural vaccination" by CAV-1 strains originally acquired by contact with the urine of vaccinated dogs. In this case, the ubiquity of positive titres observed in the present study would not indicate enzootic pathogenic ICHV but rather an ICHV-protected population. Since 1978, vaccination with CAV-2, which is not shed in urine, has replaced CAV-1 as a protective measure for dogs against ICHV (Bass et al., 1980). The continuing presence of canine adenovirus-neutralizing titres in the wolf populations we surveyed will prove the virus is truly enzootic and independent of domestic sources. Further investigation would be required to determine if virulent ICH virus or a more benign attenuated CAV-1 or CAV-2 form is involved.

Our results as well as those of Choquette and Kuyt (1974) and Trainer and Knowlton (1968) indicate that exposure to ICHV is more comon than exposure to CDV in free-living wolf and coyote populations. This could be explained by the greater ease of ICHV transmission as discussed above, or perhaps by a higher mortality rate from CDV infection. However, the latter possibility seems unlikely since the wolf populations we studied (Stephenson, 1978), the coyote population studied by Trainer and Knowlton (1968), and probably the wolf population studied by Choquette and Kuyt (1974) were for the most part healthy (i.e. showed normal recruitment) despite evidence of exposure to these viruses.

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