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Authors: CHOQUETTE, L. P. E., and KUYT, E.

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## SEROLOGICAL INDICATION OF CANINE DISTEMPER AND OF INFECTIOUS CANINE HEPATITIS IN WOLVES (*Canis lupus* L.) IN NORTHERN CANADA

L. P. E. CHOQUETTE<sup>1</sup> and E. KUYT<sup>2</sup>, Canadian Wildlife Service, Environment Canada

**Abstract:** Serum and blood samples from 86 wolves taken in northern Canada were examined for neutralizing antibodies to canine distemper, infectious canine hepatitis and canine herpesvirus. The tests were performed on eluates prepared from paper discs previously saturated with serum or blood from wolf carcasses. Canine distemper and infectious canine hepatitis antibodies were demonstrated in two and eleven samples respectively. No reactors were detected for canine herpesvirus. These findings indicate the existence of the viruses of both CD and ICH in free-living wolves in northern Canada. However, their significance in these animals is not known.

### INTRODUCTION

Adams and Hanson described a procedure for adsorbing virus neutralizing antibodies on paper discs.<sup>1</sup> They found it applicable to serological survey work. Karstad et al. showed that the results of serum neutralization and complement fixation tests conducted with eluates prepared from paper disc adsorbed blood and serum samples compared favourably with parallel tests on fluid samples.<sup>6</sup> This, and the elimination of problems associated with the collection, handling, storing and shipping of material suitable for serological studies, led the Wildlife Disease Association in 1959 to promote the use of adsorbent paper discs in the collection of whole blood and serum from wildlife, even some time after death and even after the carcasses have been frozen and thawed.

The availability of wolf carcasses was an opportunity for the collection of adsorbed blood or serum samples for serological examination to determine whether the animals had been exposed to infectious diseases, particularly canine distemper and infectious canine hepatitis.

### MATERIALS AND METHODS

Carcasses of 86 wolves (*Canis lupus* L.) were available. Most of the wolves had been killed in winter predator control programs in the Mackenzie District, Northwest Territories.

Fifty-six wolves were taken during the winter of 1964 in the vicinity of Bishop Lake, 65°32' N, 116°08' W, approximately 80 km southeast of Great Bear Lake, associated with a large caribou herd that remains in that area for much of the winter. It is probable that most, if not all wolves associated with caribou in winter in this area, are tundra wolves which have followed caribou from their summer range. Twenty-two wolves taken between 1960 and 1964 originated from various localities north and south of Great Slave Lake, in an area between 60°51' N-64°41' N and between 112°28' W - 115°45' W. Three wolves were taken in the winter of 1964, 40 km west of Fort Smith, Northwest Territories, 60°01' N, 112°22' W, and five during the winter of 1965 in the vicinity of the Park's Hay Camp, Peace Point and Sweetgrass stations located north and south of the Peace River.

<sup>1</sup> Ottawa, Ontario K1A 0H3

<sup>2</sup> Fort Smith, Northwest Territories X0E 0P0

The frozen carcasses were shipped to Fort Smith and examined after thawing. Adsorbent paper discs 17 mm in diameter\* were saturated with blood or serum which had separated from coagulated blood. The saturated paper discs were air-dried and stored in glassine envelopes at room temperature.

Serum neutralization tests against three canine viruses, canine distemper (CD), infectious canine hepatitis (ICH) and canine herpesvirus (CHV), using embryonated hen's eggs in the first case and tissue culture systems in the other two, were performed on eluates of the adsorbed samples. The samples were tested against 300 embryo-infective doses (EID<sub>50</sub>) CD virus, 80 tissue culture-infective doses (TCID<sub>50</sub>) ICH virus, and 100 tissue culture-infective doses (TCID<sub>50</sub>) *Herpesvirus canis*. Positive samples (complete neutralization of the test doses of the virus) were retested for repeatability. The tests were conducted by Dr. L. E. Carmichael, Veterinary Virus Research Institute, Cornell University, Ithaca, New York.

## RESULTS

Results of the tests for CD, ICH and CHV antibodies in the 86 wolves are given in Table 1.

The two reactors to the test for CD antibodies were a male wolf over 3 years old taken north of the Great Slave Lake and a 2 year old male wolf taken at Wood Buffalo National Park (Peace Point station), north of the Peace River.

Of the eleven reactors to the test for ICH antibodies, four originated from localities north and south of Great Slave Lake, four from the Bishop Lake area and three from Wood Buffalo National Park, one from the vicinity of each of the Park's stations. Ten of these animals had been taken between 1963 and 1965; the other had been killed in 1960. In four cases the animals were at least 3 years old but none of the others were less than 1 year.

## DISCUSSION

In 1931, Elton<sup>8</sup> reviewed records and observations of disease outbreaks in sled dogs and of cyclical epizootics in Arctic fox (*Alopex lagopus* (L.)) in the eastern Canadian arctic and sub-arctic, as well as in northern Quebec and northern Ontario. It was suggested to Elton<sup>8</sup> that possibly true distemper or infectious canine hepatitis were involved in the disease outbreaks recorded in dogs and Arctic fox. In 1947, Plummer<sup>10,11</sup> and Savage and Isa<sup>12</sup> expressed the opinion

TABLE 1. Serum Neutralization Tests.

Sex	Number animals tested	Reactors*		
		CD	ICH	CHV
Male	52	2	8	0
Female	34	0	3	0
Total	86	2	11	0

\* Complete neutralization of the test doses of virus.

CD = canine distemper

ICH = infectious canine hepatitis

CHV = canine herpesvirus

\* Manufactured by Carl Schleicher and Schuell Co., Keene, N.H., U.S.A.

that pathogens other than the virus of rabies were involved in such disease outbreaks. ICH was involved in the death of dogs in northern Manitoba in 1959 and 1960.<sup>8</sup> Clinical and other evidence indicated the endemicity of both CD and ICH in dogs in northeastern Canada and in Arctic Quebec.<sup>4</sup>

While the present report indicates the existence of the viruses of both CD and ICH in free-living wolves, the results also indicate that few animals have been exposed to both infections and survived. Unfortunately, it is not possible to correlate these findings with clinical or other observations. In Dr. Carmichael's opinion, since animals recovered from CD and ICH have, and maintain, good antibody levels far in excess of the dilution used in the test, the results would not be indicative of the extent of recent infection, at least in the year previous to the killing of the animals.

Commenting on the results of a serological study of fox and racoon in New York State, Parker et al.<sup>9</sup> suggested that a minimum population level may be necessary to allow the spread of the agents of CD and of ICH, as well as of other viruses. Between 1951 and 1961, nearly four thousand wolves were killed in northern Canada, most of them in the Mackenzie District of the Northwest Territories. The samples used in this study were collected between 1960 and 1965 inclusive when the wolf population was sparse compared to what it had been in

the preceding years, and likely with lessened opportunity for exposure. This may account for the low prevalence recorded.

The possibility that antibodies were destroyed by storage cannot be entirely dismissed. However, in one reactor to the test for ICH, the paper dried sample had been stored for nearly 6 years before it was examined; in the other ten cases, the storage period ranged from 1 to 3 years. This bears out the observations by Karstad et al.<sup>9</sup> that samples of serum dried on paper can be stored for long periods of time without deterioration.

Reports cited by Parker et al.<sup>9</sup> and by Trainer and Knowlton<sup>13</sup> show that both CD and ICH may infect captive foxes and coyotes with disastrous severity. CD has been recorded in captive wolves.<sup>2</sup> Wolves have also been experimentally infected with the virus of ICH.<sup>8</sup> However, the significance of both infections in free-living wolves is not known.

Trainer and Knowlton<sup>13</sup> suggested that perhaps CD and ICH are enzootic and not an important mortality factor in free-living coyote populations though they could become important when compounded by other environmental factors such as crowding, malnutrition, parasitism, etc. This could also be the case in free-living wolves under the conditions prevailing in northern Canada and partly explain the seemingly high death rate Kuyt<sup>7</sup> reported in wolves during the first year of life.

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