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Source: Journal of Wildlife Diseases, 33(3) : 628-631

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-33.3.628

Journal of Wildlife Diseases, 33(3), 1997, pp. 628–631

Detection of Canine Parvovirus in Wolves from Italy

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ABSTRACT: One hundred fifteen samples of wolf (*Canis lupus*) feces were collected during 1994 to 1995 from four free-living populations of the north central Apennines Mountains, Italy. The samples were tested for canine parvovirus by antigen-capture enzyme-linked immunosorbent assay (ELISA), hemagglutination, and virus isolation. Four of these samples were positive by virus isolation as confirmed by electron microscopy. All positive samples were from Casentino Park in Tuscany. This is the first definitive observation of canine parvovirus in wolves from Europe.

Key words: Parvovirus, wolf, *Canis lupus*, virus isolation, electron microscopy.

The wolf population in Italy consists of about 400 individuals ranging throughout the Apennines Mountains (Francisci and Guberti, 1992). The presence of infectious diseases could interfere with the natural demography of this species which has been close to the threshold of extinction.

Canine parvovirus (CPV) infects domestic dogs all over the world (Appel et al., 1978) as well as captive and free-ranging wild canids (Eugster et al., 1978; Fletcher et al., 1979; Mann et al., 1980) including the wolf (*Canis lupus*) in which it may cause gastroenteritis and death of puppies (Mech and Goyal, 1993). The disease is transmitted via the fecal-oral route, and maintenance of infection is secured by the high environmental viral resistance in the feces and by the possible presence of carrier animals (Swango, 1983; Thomas et al., 1984). The presence of CPV in wolves in the United States has been assessed both indirectly through finding CPV antibodies in wolf sera (Goyal et al., 1986) and by detection of the virus in wolf feces (Muneer et al., 1988).

Fico et al. (1996) found CPV antibodies in four wolves captured during 1993 to 1994 in central Italy; however, the virus has never been directly ascertained either in Italy or in other European countries. Herein, we describe the direct detection of CPV in feces of wolves from the north central Apennines, Italy.

One hundred and fifteen samples of wolf feces from four populations in north central Apennines, Italy (Fig. 1), were submitted for testing for CPV. There were four collection sites: Gigante Regional Park (44°25′N, 10°16′E) and Orecchiella Natural Park (44°11′N, 10°23′E) in which three wolves were radio-collared; Alta Val Parma Preserve (44°30′N, 09°56′E) and Casentino Park (43°56′N, 11°48′E) in which feces were collected in order to assess food habits of the species (Mattioli et al., 1995). In the Gigante and Orecchiella parks, the risk of contact with dogs was negligible; whereas in the Casentino and Alta Val Parma parks this risk was possible. Moreover, the Casentino hosts one of the largest wolf population of Italy. Samples were collected in the same areas during the spring of 1994 (n = 50) and 1995 (n = 65), respectively. Samples belonged to the same packs but it was impossible to identifie them at the individual level. All were stored at −20 C until testing.

Each sample was examined by three different methods: double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), hemagglutination (HA), and virus isolation (VI) on permissive feline embryonic fibroblast cells (FEA). These procedures were used at the same time to increase the sensitivity of the investigation.

The technique DAS-ELISA (Ingenasa, Madrid, Spain) is an antigen-capture ELISA for CPV antigen; both the coating antibodies as well as those conjugated with the enzyme are a mixture of highly specific monoclonal antibodies that allow detection

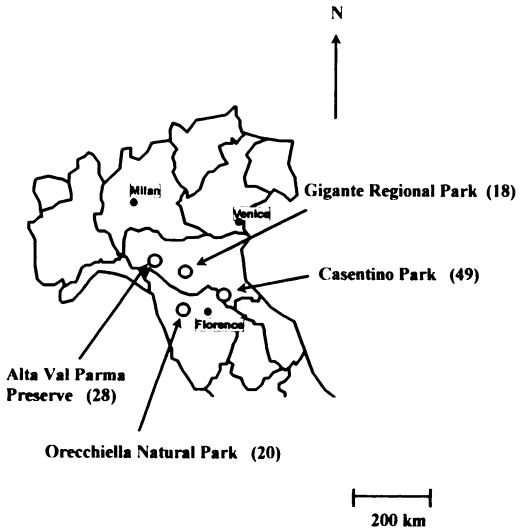

FIGURE 1. Sites in northern Italy where wolf feces were collected. Number of samples is in parentheses.

of any of the CPV strains isolated from dogs in Europe (Rimmelzwaan et al., 1990). All samples were run in duplicate wells and the results were read by a spectrophotometer (Titertek Multiskan Plus, Eflab, Helsinki, Finland) at a 405-nm wavelength; samples with an absorption greater than or equal to 20% of the positive control were considered positive.

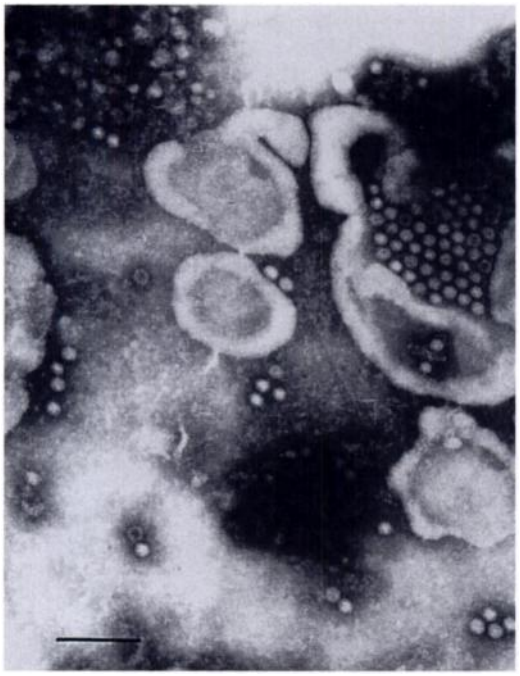

FIGURE 2. Parvoviral particles isolated in cell culture from wolf feces. Negatively stained electron microscopy. Bar = 100 nm.

The HA technique applied to fecal material was conducted in 96 V-bottom wells plastic plates (Greiner Labor-Technik, Germany) by using 1% swine erythrocytes (Carmichael et al., 1980). Agglutinating samples were tested for specific hemagglutination inhibition (HI) using an anti-CPV serum. With this test, all agglutinating samples specifically inhibited were considered positive for CPV.

For VI, fecal samples were diluted 1:10 w/v in phosphate buffer (pH 7.4) containing 1% antibiotic antimycotic solution (Sigma Chemical Co., St. Louis, Missouri, USA). After shaking and centrifuging at 1,000 × *G* for 15 min at 4 C, the supernatant was filtered through 450 nm pore filters. We added 100 µl of each treated sample to a suspension of FEA cells (Mochizuki and Hashimoto, 1986) freshly seeded in 24-wells plates (Corning Laboratories Sciences Co., New York, USA). Cell cultures were incubated at 37 C, 5% CO_2, and observed daily for cytopathic effect (CPE) until the negative control cell monolayer was confluent; this generally occurred within 72 to 96 hr. Each sample underwent two further blind passages following a similar procedure and using as inoculum 100 µl of the respective culture at the preceding passage which had been rapidly frozen and thawed. Some samples had CPE between 2 and 4 days after inoculation.

The third-passage supernatant of each sample was tested for CPV by DAS-ELISA and HA-HI. Positive samples were stained for 2 min by adding 50 µl of 2% potassium phosphotungstate to 50 µl of supernatant. Stained samples were transferred to colloid carbon-coated copper grids, blotted, dried in air and examined at 126,000 × in a Zeiss-109 (Zeiss, Oberkochen, Germany) electron microscope (Fig. 2).

TABLE 1. Detection of canine parvovirus from fecal samples of wolves in Italy, 1994 and 1995.

Area	Year	ELISA	Hemagglutination	Viral isolation	Confirmed by electron microscopy
Gigante Regional Park	1994	0/18[a]	0/18	0/18	ND[b]
Orecchiella Natural Park	1994	0/20	0/20	0/20	ND
Alta Val Parma Preserve	1994	0/12	0/12	0/12	ND
	1995	0/16	0/16	0/16	ND
Casentino Park	1995	2/49	3/49	4/49	4/49
Total		2/115	3/115	4/115	4/49

[a] Number positive/number sampled.
[b] ND = not done.

Canine parvovirus was isolated in FEA cells from four samples (Table 1); all were collected in the spring of 1995 in different areas of the Casentino Park. The CPV positive fecal samples were soft in nature. Of these, the virus was detected from one sample by all three techniques; two samples were positive by HA but not by ELISA, one was positive by ELISA but not by HA. The discordance between DAS-ELISA and HA tests in three fecal samples could not be explained by false positive reactions, because the virus was detectable by VI and electron microscopy (EM). Differences could be due to low sensitivity of the two methods: poor presence of free viral antigen in the sample affecting the DAS-ELISA, and presence of antibodies in feces which would affect the HA. The presence of two DAS-ELISA positive samples is evidence for a high virus titer associated with clinical disease (Mathys et al., 1983). Based on these results, the presence of CPV in the wolves of Casentino Park is established.

Further research is needed to determine the distribution of CPV in wolves in Italy and to evaluate the possible impact of the virus on the dynamics of wolf populations as reported elsewhere (Mech and Goyal, 1993; Johnson et al., 1994).

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Received for publication 15 May 1996.