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SEROLOGIC SURVEY FOR SELECTED VIRAL PATHOGENS IN BROWN BEARS FROM ITALY

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ABSTRACT: Blood samples were collected from six captive bears and nine free-ranging Marsican brown bears (*Ursus arctos marsicanus*) in the Abruzzo National Park, Italy, between 1991 and 1995. Sera were tested for evidence of exposure to canine distemper virus (CDV), canine adenovirus type 2, canine coronavirus, and canine parvovirus type 2 (CPV-2). Serologic evidence of CDV and CPV-2 exposure was found in both captive and free-ranging bears. This may be the first report of CDV exposure in free-ranging bears.

Key words: European brown bear, Marsican brown bear, *Ursus arctos*, serologic survey, viral pathogens.

The Marsican brown bear (*Ursus arctos marsicanus*) is a subspecies of the European brown bear (*Ursus arctos arctos*) according to genetic (Randi et al., 1994), morphologic (Toschi, 1965), ethologic (Fabbri et al., 1983), and ecologic (Zunino, 1976) features. It also is a threatened species. Only about 50 to 70 animals remain in restricted habitat (Boscagli, 1994). Presently, a large percentage of the Marsican brown bear population lives in the Abruzzo National Park. The Abruzzo National Park lies among the Appennine mountains in central Italy (41°48′N, 13°47′E). It covers 44,000 ha within a Protection Area of 60,000 ha. At present, the Park is a safe refuge for several free-ranging species such as Marsican brown bears, Abruzzo chamois (*Rupicata omata*), and Appennine wolves (*Canis lupus italicus*).

Personnel of the Appennine Ecologic Center, Abruzzo National Park, began a scientific study of health conditions of the Marsican brown bear population. Our objective was to determine serologic evidence of viral pathogens which cause mortality in carnivores, including, canine distemper virus (CDV), canine adenovirus type 2 (CAV-2), canine coronavirus (CCV), and canine parvovirus type 2 (CPV-2).

Twenty-three blood samples were taken from six captive bears and nine free-ranging bears between 1991 and 1995. Twelve blood samples were collected from captive bears near the Abruzzo National Park headquarters in Pescasseroli (1,165 m above sea level). Chemical immobilization of bears was obtained by remote injection. Eleven blood samples were collected during capture of free-ranging brown bears within the Abruzzo National Park. Bear capture was performed near the Mainarde mountains (1,400 m above sea level) and near the "Camosciara" wildlife sanctuary (1,135 m above sea level). Bears were captured by Aldrich foot snares and immobilized with drugs. Clotted blood samples were refrigerated at 4 C and were centrifuged to obtain sera which were frozen (−25 C) until analysis. Nasal, ocular, and rectal swabs were also taken from three free-ranging animals.

Bear chemical immobilization was obtained by using three methods. Some were anesthetized with xylazine hydrocloride (Rompun, Bayer, Germany) and ketamine hydrocloride (Inoketam 1000, Virbac, France) (Hatlapa and Wisner, 1988), with doses of 5.6 to 5.9 mg/kg for captive bears and 15.5 to 17.3 mg/kg for wild bears. Other bears were immobilized with tiletamine hydrocloride and zolazepam hydrocloride (Zoletil 100, Virbac, France) (Taylor et al., 1989); median doses were 7.1 mg/kg for captive bears and 9.4 mg/kg for wild bears. Medetomidine hydrocloride (Domitor, Vetem, Italy) (24 μg for captive bears; 149

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μg/kg for wild bears) and ketamine hydrocloride (Inoketam 1000, Virbac, France) (1.4 mg/kg for captive bears; 7.5 mg/kg for wild bears) also were used (Jalanka, 1991).

Age of bears was estimated by counting cement layers around the root of the extracted rudimentary first premolar tooth (Stonenberg and Jonkel, 1966). Teeth were shipped to a commercial laboratory (Matson's Laboratory, Milltown, Montana, USA) for confirmation.

Bears were never vaccinated prior to or during capture.

Serum neutralization tests (Lennette et al., 1988) for CDV antibodies were performed using Vero cell lines (Cell Line Collection from the Istituto Zooprofilattico Sperimentale, Brescia, Italy) and Onderstepoort strain of CDV (isolated from a commercially attenuated CDV vaccine for dogs, Caniffa, Pierzoo-Rhône Mérieux, France). Each serum was heated at 56 C for 30 min. The readings were made after 5 days incubation. The end point titer of each serum was expressed as the reciprocal of the highest dilution that completely inhibited cytopathic effect (CPE). A reciprocal titer of ≥20 was considered positive.

Serum neutralization tests for CAV-2 antibodies were performed using Madin Darby Canine Kidney (MDCK) cell lines (Cell Line Collection from Istituto Zooprofilattico Sperimentale) and CAV-2 vaccinal strain (isolated from a commercially attenuated CDV vaccine for dogs, Caniffa, Pierzoo-Rhône Mérieux). Each serum was heated at 56 C for 30 min. The readings were made after 3 days incubation. The end point titer of each serum was expressed as the reciprocal of the highest dilution that completely inhibited CPE. A reciprocal titer of ≥20 was considered positive.

Serum neutralization tests for CCV antibodies (Mochizuki et al., 1987) were performed using A-72 cell lines (Binn et al., 1980) and CCV strain isolated in Italy (Marsilio et al., 1993). Each serum was heated at 56 C for 30 min. The readings were made after 5 days incubation. The end point titer of each serum was expressed as the reciprocal of the highest dilution that completely inhibited CPE. A reciprocal titer of ≥20 was considered positive.

Hemagglutination-inhibition tests for CPV-2 antibodies (Carmichael et al., 1980) were performed using red blood cells of cats and 17/80/ISS strains of CPV-2 (Buonavoglia et al., 1981). The readings were made following overnight incubation at a temperature of 4 C. The hemagglutination-inhibition titer of each serum was expressed as the reciprocal of the highest dilution that completely inhibited hemagglutination. A reciprocal titer of ≥20 was considered positive.

For viral culture, the supernatant (0.1 ml) from each swab was adsorbed for 60 min at 37 C on confluent and freshly confluent monolayers of Vero, A-72, and MDCK cell lines. Following washing and refeeding of the monolayer with the maintenance medium, the cultures were incubated up to 7 days in a humidified chamber 4.5% CO_2 at 37 C and examined daily for evidence of CPE. All samples were passed three times and samples were harvested by freeze-thawing three times and stored at −70 C. With every assay a negative control was included by adsorbing ordinary growth medium on the cell monolayer.

At the end of the third passage indirect fluorescence antibody tests (IFAT) (Spendlove, 1967) to search CDV or CAV-2 or CCV or CPV-2 antigens were performed on cell monolayers. Absence of a fluorescent cells was considered negative, whereas a fluorescent reaction was considered positive.

No antibodies against CAV-2 and CCV occurred in either free-ranging or captive bears. Antibody titers against CPV-2 were observed in three of nine free-ranging bears (highest titer 320) and in three of six captive bears (highest titer 640). Titers against CDV occurred in three of nine free-ranging bears (highest titer 320) and

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TABLE 1. Reciprocal serum antibody titers of captive brown bears from Abruzzo National Park, Italy, 1991 to 1995.

Bear identification number	Species	Sex	Date of sampling	Age (yr)	CDV[a]	CPV-2
1a[a]	*Ursus arctos marsicanus*	M	8 June 1991	9	<20	640
1b[b]	*Ursus arctos marsicanus*	M	28 Oct. 1993	11	<20	320
2	*Ursus arctos marsicanus*	M	9 Nov. 1993	3	<20	640
3	*Ursus arctos marsicanus*	M	22 Sept. 1994	<1	<20	<20
4a[b]	*Ursus arctos*	F	14 Mar. 1991	19	<20	<20
4b[b]	*Ursus arctos*	F	28 Oct. 1993	21	<20	<20
5a[b]	*Ursus arctos*	M	9 June 1991	2	<20	<20
5b[b]	*Ursus arctos*	M	12 Nov. 1991	2	<20	<20
5c[b]	*Ursus arctos*	M	11 Nov. 1992	3	<20	320
5d[b]	*Ursus arctos*	M	28 Oct. 1993	4	<20	320
6a[b]	*Ursus arctos*	M	11 Nov. 1992	20	40	<20
6b[b]	*Ursus arctos*	M	28 Oct. 1993	21	40	<20

[a] CDV = canine distemper virus; CPV-2 = canine parvovirus type 2.
[b] Different samples from the same bear.

in one of six captive bears (highest titer 20) (Tables 1 and 2).

No viruses were isolated from swabs. In addition, the IFAT was always negative.

The presence of antibodies to CPV-2 and CDV in Marsican brown bears is unusual. In past work, only antibodies against canine adenovirus were observed in bears in the USA (Collins et al., 1984; Zarnke and Evans, 1989).

TABLE 2. Reciprocal serum antibodies titers of free-ranging brown bears (*Ursus arctos marsicanus*) from Abruzzo National Park, Italy, 1991 to 1995.

Bear identification number	Sex	Date of sampling	Age (yr)	CDV[a]	CPV-2
7	M	14 Nov. 1991	6	20	160
8	F	16 Nov. 1991	3	<20	<20
9a[b]	M	28 May 1992	10	320	320
9b[b]	M	16 Nov. 1992	10	160	160
10	M	2 June 1992	12	<20	20
11	M	8 June 1992	12	<20	<20
12	F	2 Nov. 1992	10	20	<20
13a[b]	F	19 Aug. 1994	6	<20	<20
13b[b]	F	15 June 1995	7	<20	<20
14	F	9 June 1995	7	<20	<20
15	M	11 June 1995	12	<20	<20

[a] CDV = canine distemper virus; CPV-2 = canine parvovirus type 2.
[b] Different samples from the same bear.

Serologic evidence of exposure to CPV-2 has been previously found in brown bears (*Ursus arctos*) from Croatia (Madic et al., 1993) and in wolves (*Canis lupus* L. 1758) from Italy (F. Marsilio, unpubl.). Bears, wolves, and other carnivores share habitat. Parvoviruses are remarkably resistant to environmental factors. Apparently, CPV-2 is transmitted among several wildlife species.

Some authors disagree on the susceptibility of bears to CDV infections (Montali et al., 1987; Appel and Summers, 1995). Our data provides evidence for the first time of CDV antibodies in free-ranging bear populations. CDV has low resistence to environmental factors. Therefore, transmission must be relatively direct. We propose that an intra-species transmission cycle among Marsican brown bears has been established.

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