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Authors: Madić, Josip, Huber, Djuro, and Lugović, Branko

Source: Journal of Wildlife Diseases, 29(4) : 572-576

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-29.4.572

Journal of Wildlife Diseases, 29(4), 1993, pp. 572–576

SEROLOGIC SURVEY FOR SELECTED VIRAL AND RICKETTSIAL AGENTS OF BROWN BEARS (*URSUS ARCTOS*) IN CROATIA

Josip Madić,[1] Djuro Huber,[1] and Branko Lugović[2]

[1] Veterinary Faculty, University of Zagreb, Heinzelova 55, 41000 Zagreb, Republic of Croatia
[2] PLIVA, Prilaz baruna Filipovića 89, 41000 Zagreb, Republic of Croatia

ABSTRACT: Sera from 22 (13 wild and nine captive) European brown bears (*Ursus arctos*) from Croatia were tested to 18 viral and rickettsial agents. Serologic evidence of exposure was found to the following agents (number positive/number examined): Bhanja virus (3/15), Tahyna virus (3/15), West Nile virus (4/15), Naples sandfly fever virus (1/15), human adenovirus (1/22), influenza A (1/22) and B (1/22) virus, cytomegalovirus (1/22), parainfluenza virus 1 (2/22), *Chlamydia psittaci* (1/22), *Coxiella burnetii* (2/22), and canine parvovirus 2 (CPV-2) (7/22). Evidence of exposure to arboviruses was found exclusively among free-living bears. Evidence of exposure to agents usually transmitted directly was predominant among captive bears. Canine parvovirus 2 antibodies were the most frequently found antibodies and the only antibody common to both groups of bears. This may be the first report of antibodies to CPV-2 in bears.

Key words: European brown bear, *Ursus arctos*, viruses, rickettsias, Croatia, serology.

INTRODUCTION

European brown bears (*Ursus arctos*) are large, long lived, wide-ranging, omnivorous, opportunistic predators and scavengers. These characteristics give them great opportunity for contact with various infectious agents. Binninger et al. (1980) stated that American black bear (*U. americanus*) may serve as an indicator of infections in other wildlife species, domestic animals and humans. Grizzly bears (*U. arctos*) may be involved in the maintenance cycle of some arboviral infections (Zarnke et al., 1983).

Arboviral infections have been well documented in humans, domestic and wild animals, and hematophagus arthropods in Croatia (Vesenjak-Hirjan, 1980; Punda et al., 1985; Punda and Ropac, 1985). Tick borne encephalitis (TBE) virus (Vesenjak-Hirjan, 1976), Bhanja virus (Vesenjak-Hirjan et al., 1977), Tahyna virus (Vesenjak-Hirjan, 1980), West Nile virus (Vesenjak-Hirjan, 1980), West Nile virus (Vesenjak-Hirjan et al., 1980), and Naples sandfly fever virus (Tesh et al., 1976) all have been reported from Croatia.

Other agents included in this survey are distributed worldwide and are of great importance for public and animal health in Croatia, including canine parvovirus 2 (CPV-2) which is the causative agent of severe disease in canides (Fletcher et al., 1979; Afshar, 1981), as well as canine adenovirus type 1 (CAV-1) which can cause lethal hepatic infection in dogs and wild animals including bears (Pursell et al., 1983; Whetstone et al., 1988). Our objective was to determine the prevalence of antibodies to these microorganisms among brown bear populations in Croatia. Additionally we compared antibody prevalence between free-ranging and captive bears.

The Croatian part of the Dinara mountain range is inhabited by approximately 400 brown bears (Huber and Morić, 1989). Some have been transplanted for repopulating western Europe, and more reintroductions are planned. Knowledge about their health status is thus an important management factor.

MATERIALS AND METHODS

Blood samples were taken from 22 European brown bears in Croatia. Thirteen were collected during capture of free-ranging brown bears for radio-tagging within Plitvice Lakes (44°55′N, 15°39′E: bears P5 to P14 and ET1) and Risnjak (45°27′N, 14°38′E: bears G1 to G3) National Parks (Table 1). Three bears were from the Zagreb Zoo (bears Z2 to Z4) and the remaining six bears (D12, and C1 to C5) were free-born but hand-reared brown bear cubs. For some animals, birth dates were known and ages could be easily calculated. Ages for other bears were determined

TABLE 1. Serum antibody titers against selected agents[a] in 22 European brown bears, (*Ursus arctos*) Croatia, 1984 to 1988.

Bear identification	Sex	Age (yr)	Disease agent: Bhanja virus	Tahyna virus	West Nile virus	Naples Sandfly Fever virus	Human adeno-virus	Influenza A virus	Influenza B virus	Cyto-megalovirus	Parainfluenza virus	*Chlamydia psittaci*	*Coxiella burnetii*	Canine parvovirus
Free-living bears														
P5	m	7	20	0	0	0	0	0	0	0	0	0	0	160
P6	m	5	0	0	20	0	0	0	0	0	0	0	0	0
P7	m	2	0	10	20	0	0	0	0	0	0	0	0	0
P8	m	1	0	0	0	0	0	0	0	0	0	0	0	0
P9	m	5	20	0	0	0	0	0	0	0	0	0	0	0
P10	m	4	0	20	20	0	0	0	0	0	0	0	0	0
P11	m	5	0	0	0	0	0	0	0	0	0	0	0	0
P13	m	8	ND[b]	ND	ND	ND	0	0	0	0	0	0	0	0
P14	m	3	ND	ND	ND	ND	0	0	0	0	0	0	0	0
ET1	m	5	0	0	0	0	0	0	0	0	0	0	0	0
G1	m	3	0	0	0	0	0	0	0	0	0	0	0	1,280
G2	f	5	0	0	0	0	0	0	0	0	0	0	0	160
G3	m	12	20	20	20	20	0	0	0	0	0	0	0	0
Captive bears														
Z2	m	4	0	0	0	0	0	0	0	0	0	0	0	0
Z3	f	3	0	0	0	0	0	0	0	0	8	0	0	0
Z4	f	10	0	0	0	0	0	0	0	0	0	0	0	640
D12	f	0.1	0	0	0	0	0	0	0	0	0	0	0	0
C1	f	0.3	ND	ND	ND	ND	0	8	8	0	8	0	8	0
C2	f	0.3	ND	ND	ND	ND	0	0	0	0	0	0	0	20
C3	f	0.3	ND	ND	ND	ND	0	0	0	8	0	8	8	40
C4	f	0.3	ND	ND	ND	ND	0	0	0	0	0	0	0	20
C5	f	0.3	ND	ND	ND	ND	8	0	0	0	0	0	0	0

[a] Only pathogens with at least one positive bear are included. Sera of all bears were negative to: tick borne encephalitis virus, Sindbis virus, lymphocytic choriomeningitis virus, *Rickettsia prowazekii*, *R. thyphi*, and canine adenovirus.
[b] ND = not determined.

by means of cementum annuli (Stonenberg and Jonkel, 1966). All samples were taken between September 1984 and May 1988. Clotted blood samples were refrigerated at 4 C and were centrifuged within 12 hr to obtain sera which were frozen (−18 C) until analysis.

Serologic tests were performed at the Institute of Public Health Service of Republic of Croatia (Zagreb), except the test for CAV-1 and CPV-2 which were performed at the Department of Microbiology and Infectious Diseases, Veterinary Faculty, University of Zagreb.

Sera were tested for antibodies to the following arboviruses: tick borne encephalitis, Bhanja, Tahyna, West Nile, Naples sandfly fever and Sindbis by the hemagglutination-inhibition (HI) test (Clarke and Casals, 1958). Presence of antibodies to human adenovirus, influenza A and B viruses, human cytomegalovirus, parainfluenza 1 virus, lymphocytic choriomeningitis virus (LCMV), *Chlamydia psittaci*, *Coxiella burnetii*, *Rickettsia prowazekii* and *Rickettsia typhi* were determined by complement fixation tests (Hawkes, 1979). We tested for antibodies to canine adenovirus 1 by immunodiffusion in gel using a modified technique by Coggins and Norcross (1970), and to canine parvovirus 2 by the HI test described by Carmichael et al. (1980). In the modified immunodiffusion test we used 95 mm glass plates, and two layers of Noble's agar (Difco Laboratories, Detroit, Michigan, USA). At the bottom of each plate 6 ml of 2% agar in borate buffer (pH 8.6) was poured. After it became solid, 16 ml of 1% agar was added.

A reciprocal titer of ≥10 was considered positive for antibodies against the arboviruses, ≥4 for human adenovirus, influenza A and B viruses, human cytomegalovirus, parainfluenza 1 virus, lymphocytic choriomeningitis virus, *Chlamydia psittaci*, *Coxiella burnetii*, *Rickettsia prowazekii* and *Rickettsia typhi*, and ≥20 for CPV-2. Chi-squared tests (Burington and

May, 1958) were used for comparisons of results; $P < 0.10$ was considered to be a statistically significant difference.

RESULTS

All sera were negative for antibodies to tick borne encephalitis virus, Sindbis virus, lymphocytic choriomeningitis, *Rickettsia prowazekii*, *Rickettsia typhi*, and canine adenovirus 1. Fifteen (68%) of 22 bears were serologically positive to at least one of the 18 agents (Table 1). Seven bears were serologically negative for all agents. Clinical signs of these diseases were not observed at the time of sampling. Low level tick infestations were observed in several free-living bears.

Antibodies to arboviruses were found in six of 13 free-living bears (Table 1). Only one bear, the oldest animal, was positive for antibodies to all arboviruses included in the survey. No free-living bears were serologically positive to agents which are usually transmitted by direct or indirect contact, except to canine parvovirus 2.

Seven of nine captive bears were serologically positive to one or more agents usually transmitted by direct or indirect contact (Table 1).

DISCUSSION

Arboviruses are maintained in nature by transmission cycles that involve a vertebrate host and a hematophagus or blood-sucking arthropod vector. Some may cause latent and/or clinical infections in humans. An epidemic of hemorrhagic fever with a renal syndrome among humans near the Plitvice Lakes has been reported (Vesenjak-Hirjan et al., 1971) but there are no data on occurrence of other arboviral infections in the area of Plitvice and Risnjak National Parks. No antibodies were found for two of six arboviruses surveyed (TBE and Sindbis). We are unaware of any evidence that Sindbis virus occurs in Croatia (Vesenjak-Hirjan, 1980). Since free-living bears had antibodies to West Nile virus, Tahyna virus, Bhanja virus, and Naples sandfly fever virus, we presume that arboviruses of these serogroups are active in the study areas. Bears may play a role in the circulation of arboviruses in nature as suggested by Zarnke et al. (1983). Antibodies to arboviruses were not detected in captive bears. Because of their large ranges (Huber and Roth, 1987) free-living bears may be more exposed to a variety of arthropods thus increasing the chance for infection with arboviruses when compared to sedentary captive bears.

Antibodies to CPV-2 were detected in both free-living and captive bears. Low antibody titers detected in 3-mo-old sibling cubs (C1 and C2; C3 and C4) are evidence for the transfer of maternal antibodies. Canine parvovirus 2 causes enteritis and myocarditis in dogs. In Croatia, antibodies against CPV-2 were detected in 65% of the dogs tested (Župancić et al., 1987). Evidence of infection has been demonstrated in both captive and free-ranging wild canids (Fletcher et al., 1979; Mann et al., 1980; Gese et al., 1991). Presence of antibodies against CPV-2 in bear sera is evidence for exposure of bears to CPV-2 or other antigenically related parvoviruses. This may be the first report of presence of antibodies against CPV-2 in bears. Possibly, bears were exposed to the virus from wild or domestic canids. However, there have been no reports of CPV-2 exposure in wild canids in Croatia.

In this survey, low antibody titers to Q fever were recorded in two captive bears. Similar results were reported by Binninger et al. (1980) in wild American black bears.

Parainfluenza viruses are associated with upper respiratory tract infections in humans and animals, especially in the young (Fenner et al., 1987). Presence of low antibody titers to parainfluenza virus 1 in two captive bears indicates the possibility of infection of bears with viruses transmitted from humans. Parainfluenza viruses share related antigens and weak crossreactivity may occur.

Low antibody titers and low prevalence were recorded in captive bears for human adenovirus, influenza A and B, *Chlamydia*

psittaci and cytomegalovirus. No sera of free-ranging bears were positive for these agents. Thus, bears may have been infected with potential human pathogens transmitted by direct or indirect contact.

Canine adenovirus 1 may affect bear population dynamics (Zarnke and Evans, 1989). American black bears may die of CAV-1 infection (Pursell et al., 1983; Collins et al., 1984; Whetstone et al., 1988). None of the bears included in this survey was positive to CAV-1. Canine adenovirus 1 has been isolated from dogs in Croatia. Antibody prevalence has reached 20% in the Croatian dog population (Kraft, 1977).

Serum antibody prevalence for arboviruses was higher ($P < 0.10$) in free-ranging than in captive bears. Prevalence of antibodies to agents transmitted by aerosol or direct contact was higher ($P < 0.01$) in captive bears.

In conclusion, the presence of antibodies to a variety of pathogenic agents provides evidence of the circulation of these agents within the European brown bear population in Croatia. Frequent contacts with humans could increase the opportunity for transmission of some agents by aerosol and direct contact.

ACKNOWLEDGMENTS

We thank the officials of the Plitvice Lakes and Risnjak National Parks, and the Zagreb Zoo for permitting us to collect the brown bear blood samples. We are grateful to Dr. Marija Galinović-Weisglass for enabling us to perform the majority of serologic tests, as well as to Dr. Elizabeth Williams for reviewing a draft of the manuscript. Field work was in part sponsored by the National Geographic Society.

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Received for publication 19 November 1992.