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Serologic Survey of Selected Viral Pathogens in Free-Ranging Eurasian Brown Bears (*Ursus arctos arctos*) from Slovakia

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ABSTRACT: We tested sera of 24 free-ranging European brown bears (*Ursus arctos*) from six regions of Slovakia for antibodies to 10 viral agents. We tested sera by an indirect fluorescence antibody test for antibodies to canine distemper virus (CDV), canine coronavirus (CCV), canine parvovirus type 2 (CPV-2), canine adenovirus, canine parainfluenza virus type 2 (CPIV-2), and canine herpesvirus type 1 (CHV-1). We used an enzyme-linked immunosorbent assay for detection of antibodies to hepatitis E virus, bluetongue virus, West Nile virus (WNV), and Aujeszky's disease virus (ADV). We detected antibodies to CDV, CHV-1, CPV-2, CPIV-2, CCV, WNV, and ADV in seven (29%), three (12%), two (8%), two (8%), one (4%), one (4%), and one (4%) bear, respectively. Evidence of exposure of free-ranging European brown bears to CCV and ADV has not been reported.

Key words: Aujeszky's disease virus, coronavirus, distemper virus, herpesvirus, parvovirus, parainfluenza, West Nile virus.

The brown bear (*Ursus arctos*), distributed across much of northern Eurasia and North America and with the world population estimated to be around 200,000 individuals, is classified into several subspecies. Slovakia is inhabited with the Eurasian brown bear (*Ursus arctos arctos*) subspecies. Currently, around 17,000 individual European brown bears live in 10 separate populations in Europe (Chapron et al. 2014); the population in Slovakia accounts for 1,256 individuals inhabiting an area of 13,000 km^2 (Paule et al. 2015). In recent years, bears are being more frequently observed near human dwellings where they compensate for a lack of food by consumption of garbage. This behavior can facilitate pathogen transmission between the bears and domestic animals. Serologic studies focusing on infectious agents in European brown bears have been done in Croatia and Italy (Madić et al. 1993; Marsilio et al. 1997; Di Francesco et al. 2015). Similar studies in Slovakia have not been done. Our aim was the testing of sera of free-ranging European brown bears to expand knowledge of exposure to pathogens to assess the overall health of the brown bear population in middle Europe.

We collected 24 blood samples from free-ranging brown bears that were either killed for public safety reasons or hunted in various regions of Slovakia in years 2011–15 (Table 1 and Fig. 1). Ages of bears were determined by means of cementum annuli (Stoneberg and Jonkel 1966) and ranged from 3–15 yr with an average of 5 yr. Immediately after hunting, we collected blood from the femoral or jugular vein and placed it into serum separator tubes, allowed it to clot, and then kept it cool on ice packs until centrifugation. Time from collection to centrifugation and separation of serum varied based on the site of collection from 1–4 h. We stored serum in a conventional freezer at –25 C.

We detected antibodies to canine distemper virus (CDV), canine coronavirus (CCV), canine parvovirus type 2 (CPV-2), canine adenovirus (CAV), canine parainfluenza virus type 2 (CPIV-2), and canine herpes virus type 1 (CHV-1) by an indirect fluorescence antibody test using glass slides coated with cells lines infected with respective viruses (VMRD, Pullman, Washington, USA) and goat anti-cat fluorescein isothiocyanate conjugate (Sigma-Aldrich, St. Louis, Missouri, USA), which have good applicability for Ursidae specimens (Sedlák and Bártová 2006). We diluted serum

TABLE 1. Serologic survey of selected viral pathogens in free-ranging Eurasian brown bears (*Ursus arctos arctos*) in Slovakia. We detected antibodies to canine distemper virus (CDV), canine coronavirus (CCV), canine parvovirus type 2 (CPV-2), canine parainfluenza virus type 2 (CPIV-2), and canine herpesvirus type 1 (CHV-1) by indirect fluorescence antibody test (IFAT). We detected antibodies to West Nile virus (WNV) and Aujeszky's disease virus (ADV) by enzyme-linked immunosorbent assay, also confirmed by a virus neutralization test (VNT).

Sample no.	Age (yr)	Sex[a]	Locality	Titers in samples with antibodies (IFAT) to different viruses[b]	Titers in samples with antibodies (VNT) to ADV or WNV[b]
1	3	F	Poľana	320 CDV	—
2	4	M	Fatra	320 CPV-2	—
3	6	M	Vysoké Tatry	640 CDV	—
4	8	F	Orava	—	—
5	4	F	Orava	80 CDV	—
6	3	M	Nízke Tatry	—	—
7	7	M	Poľana	—	—
8	5	F	Orava	—	—
9	5	M	Muránska Planina	—	—
10	4	M	Fatra	160 CHV-1	—
11	4	M	Muránska Planina	40 CHV-1	—
12	4	M	Nízke Tatry	—	4 ADV
13	3	F	Poľana	—	—
14	4	F	Vysoké Tatry	—	—
15	5	M	Muránska Planina	—	—
16	4	M	Nízke Tatry	—	256 WNV
17	5	F	Fatra	320 CDV	—
18	5	F	Orava	40 CDV	—
19	7	M	Muránska Planina	40 CHV-1	—
20	15	M	Muránska Planina	—	—
21	3	M	Poľana	—	—
22	3	M	Vysoké Tatry	—	—
23	4	M	Nízke Tatry	40 CDV, 80 CCV, 80 CPIV-2	—
24	5	F	Fatra	40 CDV, 160 CPV-2, 320 CPIV-2	—

[a] F = female; M = male.

[b] — = samples with negative results.

in twofold series from 1:40 as the basic dilution and included canine and feline positive and negative control sera in each test. We considered samples with titers ≥40 to be positive. We detected antibodies to hepatitis E virus (HEV) by ID Screen hepatitis E indirect multispecies enzyme-linked immunosorbent assay (ELISA) and to bluetongue virus (BTV) by ID Screen Bluetongue Competition ELISA (cELISA, IDvet, Grabels, France). Antibodies to West Nile virus (WNV) and Aujeszky's disease virus (ADV) were detected by ID Screen West Nile Competition Multi-Species ELISA and ID Screen Aujeszky gB cELISA, respectively (IDvet). Samples positive for antibodies to WNV and ADV were subsequently confirmed by virus neutralization tests (VNT; Sedlák et al. 2014).

We detected antibodies to at least one virus in 54% (13/24) bears; two animals had antibodies simultaneously to three viruses (Table 1). Positive bears were a mean age of 4.4 yr, more males than females were

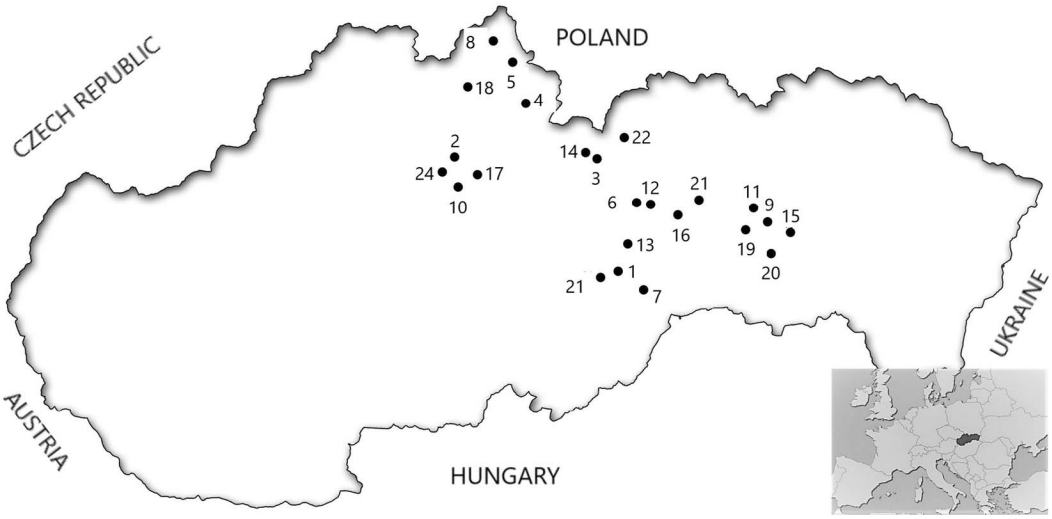

FIGURE 1. Locations in Slovakia of 24 Eurasian brown bears (*Ursus arctos arctos*) where blood samples were taken for testing for antibodies to selected viral diseases. Numbers of bears in map correspond to numbers of bears in Table 1. Four bears came from Polana (48°38′37′′N, 19°29′52′′E), four bears from Fatra (Veľká and Malá Fatra; 49°2′29′′N, 19°4′49′′E), three bears from Vysoké Tatry (National Park Vysoké Tatry; 49°7′40′′N, 20°6′17′′E), four bears from Nízke Tatry (National Park Nízke Tatry; 48°54′42′′N, 19°23′7′′E), four bears from Orava (49°20′41′′N, 19°11′54′′E), and five bears from Muránska Planina (National Park Muránska Planina; 48°38′37′′N, 19°29′52′′E).

antibody-positive, and more bears from Fatra and Nízke Tatry were antibody-positive than were bears from other areas.

Canine distemper is a highly contagious disease of carnivores, regularly detected in red foxes (*Vulpes vulpes*), martens (*Marten* spp.), raccoons (*Procyon lotor*), and other wild mammal species (Frölich et al. 2000), and it may serve as a source of infection for brown bears. Though clinical manifestation of CDV has been rarely described in bears (Marsilio et al. 1997; Bronson et al. 2014; Di Francesco et al. 2015), our finding of high antibody prevalence (29%, 7/24) was not unusual. Canine coronavirus causes intestinal disease in canids. We detected antibodies to CCV in 4% (1/24) of the bears but with a low titer. Antibodies to CCV were also detected in giant pandas (*Ailuropoda melanoleuca*; Mainka et al. 1994) and in raccoons (Aoki et al. 2017). Canine parvovirus type 2 causes enteritis and myocarditis. We detected antibodies to CPV-2 in 8% (2/24) of the bears. Serologic evidence of exposure to CPV-2 has also been found in brown bears from Croatia (Madić et al. 1993) and in Marsican brown bears (*Ursus arctos marsicanus*) from Italy (Marsilio et al. 1997; Di Francesco et al. 2015).

Canine parainfluenza is a typical infection of dogs. We detected antibodies to CPIV-2 in 8% (2/24) of the bears. Antibodies to various parainfluenza viruses have been detected in bears from Croatia and Canada (Madić et al. 1993; Philippa et al. 2004). Parainfluenza viruses share related antigens and weak cross-reactivity may occur (Madić et al. 1993). Further study with a molecular biologic approach is therefore recommended. Canine herpesvirus causes respiratory and reproductive tract illnesses in dogs. We detected antibodies to CHV-1 in 12% (3/24) of the bears. Antibodies to CHV-1 have been detected in red foxes (Robinson et al. 2005) and in North American river otters (*Lontra canadensis*; Kimber et al. 2000) but not in ursids. It is difficult to say whether weak seropositivity is due to CHV-1 seroconversion or a cross-reaction between herpesviruses. West Nile virus is transmitted by mosquito vectors among bird populations and from

birds to susceptible mammalian species, including humans. Antibodies to WNV have been detected in many animal species, including bears (Madić et al. 1993; Farajollahi et al. 2003; Bronson et al. 2014). We detected antibodies to WNV by cELISA in 4% (1/24) of the bears. However, the interpretation of cELISA results is complicated by cross-reactivity, typical for flaviviruses, and results should be interpreted as the prevalence of antibodies to the *Flavivirus* genus. In Slovakia, the most widespread representative of *Flaviviridae* is the tick-borne encephalitis virus (TBEV), so positive serum samples were further examined by VNT for WNV and TBEV. The samples were found to be positive to WNV but negative to TBEV.

Aujeszky's disease affects the central nervous system and other organs in a variety of mammals. We detected antibodies to ADV by cELISA in 4% (1/24) of the bears, confirmed by VNT. Although there are known cases of ADV infections in captive brown bears following consumption of raw pork (Zanin et al. 1997; Banks et al. 1999), the natural infection of free-ranging bears has not been reported. We assume the same source of infection in our case due to the occurrence of Aujeszky's disease in wild boar (*Sus scrofa*) in Europe, including Slovakia (Boadella et al. 2012). Canine adenovirus causes canine infectious hepatitis and infectious laryngotracheitis in dogs, but the virus may infect also foxes, coyotes (*Canis latrans*), and wolves (*Canis lupus*). Infection caused by CAV is confirmed in captive black bears (*Ursus americanus*; Collins et al. 1984), Marsican brown bears (Di Francesca et al. 2015), and American black bears (Bronson et al. 2014). We did not detect antibodies to CAV. Hepatitis E is a food- and water-borne disease of various mammals including humans. Antibodies to HEV have also been detected in wild boars, wild rabbits, and hares (Leporidae; Larska et al. 2015; Hammerschmidt et al. 2017). We did not detect antibodies to HEV; however, wild boars can serve as a reservoir. Bluetongue is a noncontagious disease causing infections mainly in domestic and wild ruminants. We did not detect antibodies to BTV; however, antibodies to BTV have been detected in black bears from Florida (Dunbar 1998).

Detection of antibodies to CCV and ADV in free-ranging brown bears from Europe is a novel finding. Our results corresponded with results of other studies showing the most frequent viral pathogens in brown bears to be CDV and CPV. Evidence that CDV and ADV are circulating in wildlife of Slovakia can have negative implications for the populations of other large carnivores such as wolf (*Canis lupus*) and lynx (*Lynx lynx*). Detection of antibodies to WNV in bears from National Park Nízke Tatry shows that infection is not limited only to the southern part of the state and to lowlands; this can have an impact on both animal and human populations. The use of reverse transcription-PCR from swabs is recommended in case of CCV and CPIV-2–positive samples to exclude cross-reactions.

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LITERATURE CITED

Aoki E, Soma T, Yokoyama M, Matsubayashi M, Sasai K. 2017. Surveillance for antibodies against six canine viruses in wild raccoons (*Procyon lotor*) in Japan. *J Wildl Dis* 53:761–768.

Banks M, Torraca LS, Greenwood AG, Taylor DC. 1999. Aujeszky's disease in captive bears. *Vet Rec* 145:362–365.

Boadella M, Gortázar CH, Vicente J, Ruiz-Fons F. 2012. Wild boar: An increasing concern for Aujeszky's disease control in pigs? *BMC Vet Res* 8:7.

Bronson E, Spiker H, Driscoll C. 2014. Serosurvey for selected pathogens in free-ranging American black bears (*Ursus americanus*) in Maryland, USA. *J Wildl Dis* 50:829–836.

Collins JE, Leslie P, Johnson D, Nelson D, Peden W, Boswell R, Draayer H. 1984. Epizootic of adenovirus infection in American black bears. *J Am Vet Med Assoc* 185:1430–1432.

Chapron G, Kaczensky P, Linnell JDC, Von Arx M, Huber D, Andrén H, López-Bao JV, Adamec M, Álvares F, Anders O, et al. 2014. Recovery of large carnivores in Europe's modern human-dominated landscapes. *Science* 346:1517–1519.

Di Francesco CE, Gentile L, Di Pirro V, Ladiana L, Tagliabue S, Marsilio F. 2015. Serologic evidence for selected infectious diseases in Marsican brown bears. *J Wildl Dis* 51:209–213.

Dunbar MR, Cunningham MW, Roof JC. 1998. Seroprevalence of selected disease agents from free-

ranging black bears in Florida. *J Wildl Dis* 34:612–619.

Farajollahi A, Panella NA, Carr P, Crans W, Burguess K, Komar N. 2003. Serologic evidence of West Nile virus infection in black bears (*Ursus americanus*) from New Jersey. *J Wildl Dis* 39:894–896.

Frölich K, Czupalla O, Haas L, Hentschke J, Dedek J, Fickel J. 2000. Epizootiological investigations of canine distemper virus in free-ranging carnivores from Germany. *Vet Microbiol* 74:283–292.

Hammerschmidt F, Schwaiger K, Dähnert L, Vina-Rodriguez A, Höper D, Gareis M, Groschup MH, Eiden M. 2017. Hepatitis E virus in wild rabbits and European brown hares in Germany. *Zoonoses Public Health* 64:612–622.

Kimber KR, Kollias GV, Dubovi EJ. 2000. Serologic survey of selected viral agents in recently captured wild North American river otters (*Lontra canadensis*). *J Zoo Wildl Med* 31:168–175.

Larska M, Krzysiak MK, Jabłoński A, Kęsik J, Bernarski M, Rola J. 2015. Hepatitis E virus antibody prevalence in wildlife in Poland. *Zoonoses Public Health* 62:105–110.

Madić J, Huber D, Lugović B. 1993. Serologic survey for selected viral and rickettsial agents of brown bears (*Ursus arctos*) in Croatia. *J Wildl Dis* 29:572–576.

Mainka SA, Xianmeng Q, Tingmei H, Appel MJ. 1994. Serologic survey of giant pandas (*Ailuropoda melanoleuca*), and domestic dogs and cats in the Wolong Reserve, China. *J Wildl Dis* 30:86–89.

Marsilio F, Tiscar PG, Gentile L, Roth HU, Boscagli G, Tempesta M, Gatti A. 1997. Serologic survey for selected viral pathogens in brown bears from Italy. *J Wildl Dis* 33:304–307.

Paule L. 2015. Rozšírenie veľkých šeliem v Európe a na Slovensku. In: *Ochrana a manažment veľkých šeliem na Slovensku*, Lešová A, Antal V, editors. Štátna ochrana prírody SR, Banská Bystrica, Slovakia, pp. 52–59.

Philippa JD, Leighton FA, Daoust PY, Nielsen O, Pagliarulo M, Schwantje H, Shury T, Van RH, Martina BE, Kuiken T, et al. 2004. Antibodies to selected pathogens in free-ranging terrestrial carnivores and marine mammals in Canada. *Vet Rec* 155: 135–140.

Robinson AJ, Crerar SK, Sharma NW, Muller WJ, Bradley MP. 2005. Prevalence of serum antibodies to canine adenovirus and canine herpesvirus in the European red fox (*Vulpes vulpes*) in Australia. *Aust Vet J* 83:356–361.

Sedlák K, Bártová E. 2006. Seroprevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in zoo animals. *Vet Parasitol* 136:223–231.

Sedlák K, Zelená H, Křivda V, Šatrán P. 2014. Surveillance of West Nile fever in horses in the Czech Republic from 2011 to 2013. *Epidemiol Mikrobiol Imunol* 63:307–311.

Stoneberg RP, Jonkel CJ. 1966. Age determination of black bears by cementum layers. *J Wildl Manage* 30: 411–414.

Zanin E, Capua I, Casaccia C, Zuin A, Moresco A. 1997. Isolation and characterization of Aujeszky's disease virus in captive brown bears from Italy. *J Wildl Dis* 33:632–634.

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