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## SEROLOGICAL SURVEY OF HERPESVIRUS INFECTIONS IN WILD RUMINANTS OF FRANCE AND BELGIUM

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**ABSTRACT:** The presence of antibodies against bovine herpesvirus 1 (BHV-1), bovid herpesvirus 6 (BHV-6), herpesvirus of Cervidae type 1 (HVC-1), reindeer herpesvirus, bovine herpesvirus 2 (BHV-2) and bovid herpesvirus 4 (BHV-4) was investigated in wild ruminants of France and Belgium between 1981 and 1986. There were no animals serologically positive for BHV-4. Antibodies against BHV-2 were demonstrated in roe deer (*Cervus capreolus*) (<1%) and chamois (*Rupicapra rupicapra*) (1%) in France. Animals seropositive to the four related viruses (BHV-1, BHV-6, HVC-1, reindeer herpesvirus) were detected in red deer (*Cervus elaphus*) in France and Belgium (1% and 11%, respectively), in roe deer (<1%) from France, in chamois (4%) in France and in ibex (*Capra ibex*) (4%) from France. The presence of antibodies against HVC-1, especially in red deer from Belgium, may suggest that wild ruminants in continental Europe are now infected with this virus, which previously has been isolated only in Scotland.

**Key words:** Serology, bovine herpesvirus 1, bovine herpesvirus 2, herpesvirus of Cervidae type 1, reindeer herpesvirus, bovid herpesvirus 4, bovid herpesvirus 6, wild ruminants, roe deer, *Cervus capreolus*, chamois, *Rupicapra rupicapra*, *Cervus elaphus*, *Capra ibex*.

### INTRODUCTION

Ruminants are infected by a variety of herpesviruses. Infectious bovine rhinotracheitis (bovine herpesvirus 1 [BHV-1]), bovine mammillitis (bovine herpesvirus 2 [BHV-2]) and the African form of malignant catarrhal fever (Alcelaphinae herpesvirus 1 [AHV-1]) are the major diseases caused by herpesviruses in domestic ruminants (Ludwig, 1983), but other herpesviruses have been isolated also from these animals. The prevalence of such infections in wild ruminants is poorly documented, especially in continental Europe. Two herpesviruses which are antigenically related to BHV-1 have been recently isolated from wild ruminants: herpesvirus of Cervidae type 1 (HVC-1) (Reid et al., 1986) was isolated from red deer (*Cervus elaphus*) in Scotland by Inglis et al. (1983) and reindeer (*Rangifer tarandus tarandus*) herpesvirus was isolated in Scandinavia (Ek-kommonen et al., 1982). The isolation of such viruses from different

wild species is important to our understanding of the epizootiology of herpesvirus infections. Serological surveys against BHV-1, before the isolation of these two viruses, may require revision; this has been already conducted in Great Britain by Nettleton et al. (1986). Another herpesvirus isolated from domestic goat, bovid herpesvirus 6 (BHV-6; Ludwig, 1983; also named caprine herpesvirus 2 by Roizman et al., 1981) is antigenically related to BHV-1 (Ludwig, 1983).

Ruminant herpesvirus infections are not always restricted to their natural host species since two cross-infections have been recently demonstrated. BHV-1 may infect domestic goat (Pirak et al., 1983; Ackermann et al., 1986) and red deer (Reid et al., 1986), but the infection is asymptomatic and BHV-1 does not remain in a latent state in goat (Pirak et al., 1983; Ackermann et al., 1986). Reindeer herpesvirus is probably restricted to the reindeer population in Europe (Ek-kommonen et al., 1986) and may be in North America where

caribou (*Rangifer tarandus caribou*) possess neutralizing antibodies against BHV-1 (Elazhari et al., 1981). Therefore, wild species are probably infected by their own herpesvirus, such as HVC-1 in red deer and reindeer herpesvirus in reindeer. Alternatively, they may be infected with a herpesvirus that infects a closely related species.

BHV-2 infection of cattle is frequent in Belgium, but the lesions occur very rarely (Pastoret et al., 1983). Bovid herpesvirus 4 (BHV-4; Ludwig, 1983), also named bovine herpesvirus 3 (Roizman et al., 1981), infection of cattle is widespread in Belgium (Van Opdenbosch et al., 1986), but is often symptomless. Infection of wild ruminants with these two viruses may not be associated with diseases, but could result in the possible transmission of herpesviruses from domestic to wild ruminants.

In the present study, we investigated the prevalence of infections of wild ruminants from Belgium and France with (1) BHV-1, (2) HVC-1, (3) reindeer herpesvirus, (4) BHV-6, (5) BHV-2 and (6) BHV-4. This study is a first step towards the identification of herpesviruses that are capable of infecting wild ruminants in western Europe, in order to determine the role of wild species as maintenance hosts of herpesviruses and the possibility of their spread in wild populations.

## MATERIALS AND METHODS

### Cells and viruses

Georgia Bovine Kidney (GBK) cells, provided by L. A. Babiuk (Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0), were cultivated in Minimum Essential Medium (MEM) as previously described (Thiry et al., 1985). The viruses used in serological tests were: the Los Angeles (LA) strain of BHV-1, the 69/1LO strain of BHV-2, provided by C. Castrucci (Istituto di Malattie infettive, Profilassi e Polizia Veterinaria, Università di Perugia, 06100 Perugia, Italy), the V.Test strain of BHV-4, isolated in Belgium (Thiry et al., 1981), the E/CH strain of BHV-6 provided by H. Ludwig (Institut für Virologie, Fachbereich Veterinärmedizin, Freie

Universität Berlin, 1000 Berlin 65, West Germany), HVC-1 and reindeer herpesvirus provided by P. F. Nettleton (Moredun Research Institute, Edinburgh EH17 7JH, Great Britain).

### Seroneutralization

Seroneutralizations were performed by the micromethod (Jenny and Wessman, 1973). Heat-inactivated (56 C, 30 min) sera were diluted two-fold in microplates (Nunc-Gibco, Paisley, Renfrewshire PA3 4EF, Great Britain) and were mixed with 100 plaque forming units (PFU) of BHV-1, BHV-2, BHV-6, HVC-1 or reindeer herpesvirus. After 2 hr incubation at 37 C, approximately 100,000 GBK cells were added to each microplate well. Each serum was tested in duplicate.

The inoculated cells were examined for cytopathic effect (CPE) after 4 days (BHV-1, reindeer herpesvirus), 5 days (BHV-2) or 7 days (BHV-6, HVC-1). Seroneutralizations were performed simultaneously for BHV-1, BHV-6, HVC-1 and reindeer herpesvirus. Positive controls were added to each test. Titres were expressed as the reciprocal of the highest dilution of serum exhibiting an inhibition of 50% CPE. Titres equal to, or higher than 1:8 were considered as positive. Sera with titres equal to 1:8 were tested three times. Other positive sera were tested two times.

### Indirect fluorescent antibody test

An indirect fluorescent antibody test (IFAT) was performed in GBK cells grown in microplates and infected with the V.Test strain of BHV-4 (50 PFU/well) after fixation in a mixture of 95:5 acetone/distilled water by volume. The fixed plates were incubated 30 min with duplicate 10-fold dilutions of each serum; they were then rinsed with phosphate buffered saline (pH 7.4) and incubated 30 min with rabbit anti-bovine globulin serum conjugated to fluorescein (Dakopatts, 2600 Glostrup, Denmark). The titres were expressed as the end-point dilution of serum exhibiting a positive fluorescent antibody reaction. Positive controls were added to each microplate.

### Serum samples

In Belgium, blood samples were collected either by private veterinarians during hunting seasons or by a member of the Centre de Médecine du Gibier (Faculty of Veterinary Medicine, B-1070 Brussels, Belgium) who accompanied hunters. Samples were collected throughout the southern part of Belgium (south of the river Meuse; 49°0' to 50°30'N; 5°0' to 6°30'E). In France, blood samples were collected throughout the country (43°0' to 50°0'N; 4°0'W to 8°0'E) either by a member of the Centre

TABLE 1. Results of serological tests for six herpesviruses in red deer from France and Belgium, 1981–1986.

Year	n	BHV-1	BHV-6	HVC-1	Reindeer herpesvirus	Total <sup>b</sup>	BHV-2	BHV-4
France								
1982	48	0 <sup>a</sup>	1	1	1	1	0	0
1983	22	0	0	0	0	0	0/21 <sup>c</sup>	0/21 <sup>c</sup>
1984	5	0	0	0	0	0	0	0
1985	5	0	0	0	0	0	0	0
Belgium								
1985	33	3	2	2	3	4	0	0/32 <sup>c</sup>
1986	37	1	3	4	1	4	0	0/36 <sup>c</sup>

<sup>a</sup> Number of seropositive animals.<sup>b</sup> Number of animals positive for one or more of the four viruses, BHV-1, BHV-6, HVC-1 or reindeer herpesvirus.<sup>c</sup> Number of animals examined less than total collected.

National d'Etude sur la Rage et la Pathologie des Animaux Sauvages (F-54220 Malzeville, France) or under the supervision of this centre, essentially on captured ruminants and sometimes on hunted animals. Blood samples were centrifuged and the serum was removed and stored at  $-20^{\circ}\text{C}$  until tested in the laboratory.

Blood samples were collected from 748 wild ruminants belonging to the following species: red deer, 80 from France (1982–1985), 70 from Belgium (1985–1986); roe deer (*Cervus capreolus*), 387 from France (1982–1986), 80 from Belgium (1981, 1985 and 1986); chamois or isard (*Rupicapra rupicapra*), 99 from France (1982–1986); ibex (*Capra ibex*), 28 from France (1984–1986); mouflon (*Ovis ammon musimon*), four from France (1983–1986).

### RESULTS

Results of this serological survey are given in Tables 1–3. It is emphasized that most of the sera positive for BHV-1, BHV-6, HVC-1 or reindeer herpesvirus reacted against several of these related herpesviruses because of the serological cross-reactivity of the four viruses. Table 1 shows the number of red deer sampled in France and Belgium between 1981 and 1986 and records the numbers positive against each of the viruses tested. Only one red deer of the 80 animals tested (1%) was positive in France against three of the serologically related herpesviruses (titres of 1:32 to 1:64). All the other samples collected between 1982 and 1985 were negative against each of the viruses. In contrast, eight of the 70 red deer (11%) sampled in Belgium

in 1985 and 1986 were seropositive against at least one of the four related herpesviruses (titres varying from 1:8 to 1:64). No animal was seropositive for BHV-2 and BHV-4.

The serological results of roe deer sampled in France and Belgium between 1981 and 1986 are listed in Table 2. No antibodies were detected in sera of 80 roe deer sampled in Belgium in 1981, 1985 and 1986. These results were similar to those obtained with 387 roe deer sera sampled in France between 1982 and 1986 where <1% of samples were positive to BHV-1 or related viruses (titres varying from 1:8 to 1:64) and <1% of samples were positive to BHV-2 (titres of 1:16). Antibodies were not detected against BHV-4.

Table 3 presents results obtained for other ruminant species. No sera were positive in mouflon from France, but the number of mouflon investigated was very low. Only one serum of 28 ibex (4%) from France was positive against BHV-6 (titre of 1:8). Four of 99 chamois (4%) were positive to one of the four related herpesviruses (titres of 1:8 to 1:64). They were sampled between 1982 and 1984. Only one (1%) was positive to BHV-2 (titre of 1:8).

### DISCUSSION

The prevalence of herpesvirus infections appears to be very low in wild ruminants from France and Belgium. There

TABLE 2. Serological results of roe deer tested for six herpesviruses in France and Belgium, 1981–1986.

Year	n	BHV-1	BHV-6	HVC-1	Reindeer herpesvirus	Total <sup>c</sup>	BHV-2	BHV-4
<b>France</b>								
1982	7	0 <sup>a</sup>	0	0	0	0	0/4 <sup>b</sup>	0/4 <sup>b</sup>
1983	59	0/58 <sup>b</sup>	0	0	0/58 <sup>b</sup>	0	2/57 <sup>b</sup>	0/58 <sup>b</sup>
1984	52	0	1	0	0	1	1	0
1985	69	2	1	2	1	2	0/68 <sup>b</sup>	0/68 <sup>b</sup>
1986	200	0	0	0	0	0	0	0
<b>Belgium</b>								
1981	21	0	0	0	0	0	0	0
1985	20	0	0	0	0	0	0	0
1986	39	0	0	0	0	0	0	0

<sup>a</sup> Number of seropositive animals.<sup>b</sup> Number of animals examined less than total collected.<sup>c</sup> Number of animals positive for one or more of the four herpesviruses, BHV-1, BHV-6, HVC-1 or reindeer herpesvirus.

were no positive serologies against BHV-4. BHV-2 antibodies were detected only in France in roe deer (<1%) and chamois (1%), but not in wild ruminants from Belgium although the prevalence of seropositive cattle to BHV-2 was 28% (Pastoret et al., 1983). The epizootiology of BHV-2 infection is different in Africa, where this infection is prevalent in >20 species of wild ruminants (Plowright and Jesset, 1971; Hamblin and Hedger, 1982).

BHV-1, BHV-6, HVC-1 and reindeer

herpesvirus are closely related. The prevalence of these collective infections in red deer from France (1%) and Belgium (11%) differs. The experimental inoculation of red deer with BHV-1 produces asymptomatic infection (Reid et al., 1986). Therefore, it may be assumed that red deer seropositive for one of the related herpesviruses has been infected by HVC-1. Our results suggest that HVC-1 could be present in continental Europe, but at a prevalence much lower than that observed in

TABLE 3. Results of serological tests for six herpesviruses in chamois, ibex and mouflons from France, 1982–1986.

Year	n	BHV-1	BHV-6	HVC-1	Reindeer herpesvirus	Total <sup>b</sup>	BHV-2	BHV-4
<b>Chamois</b>								
1982	15	0 <sup>a</sup>	0	1	0	1	1	0
1983	51	0	0	2	0	2	0/39 <sup>c</sup>	0/47 <sup>c</sup>
1984	13	1	1	1	1	1	0	0
1985	3	0	0	0	0	0	0	0
1986	17	0	0	0	0	0	0/16 <sup>c</sup>	0
<b>Ibex</b>								
1984	7	0	0	0	0	0	0	0/6 <sup>c</sup>
1985	3	0	0	0	0	0	0	0
1986	18	0	1	0	0	1	0	0/17 <sup>c</sup>
<b>Mouflon</b>								
1983–1986	4	0	0	0	0	0	0	0

<sup>a</sup> Number of seropositive animals.<sup>b</sup> Number of animals positive for one or more of the four herpesviruses, BHV-1, BHV-6, HVC-1 or reindeer herpesvirus.<sup>c</sup> Number of animals examined less than total collected.

Great Britain (Nettleton et al., 1986). None of the roe deer in Belgium and <1% of those of France were seropositive. During previous surveys in France in 1979, none of the roe deer was seropositive against BHV-1 (Blancou, 1983). The eventual susceptibility of roe deer to HVC-1 needs further investigation. The sample of mouflon and ibex examined in this study was very low, but one ibex had neutralizing antibodies against BHV-6. Because the ibex belongs to the same genus as the domestic goat, this suggests that this latter could be infected with BHV-6.

Neutralizing antibodies against the four related herpesviruses were detected in 4% of the chamois tested, but positive results were obtained only between 1982 and 1984. This corresponds to several outbreaks of keratoconjunctivitis in chamois (Hars and Gauthier, 1984). Viruses were not isolated from clinical cases, but the infectious character of the disease was demonstrated (Blancou et al., 1985). Therefore, there is much similarity between the keratoconjunctivitis of the chamois and the symptoms observed in the red deer infected with HVC-1: conjunctivitis, ocular discharge, oedema of the eyelids, photophobia, hypopyon (Inglis et al., 1983; Blancou et al., 1985; Reid et al., 1986). HVC-1 is the only herpesvirus so far recognized to produce a clinical disease in a European wild ruminant. The chamois may be more susceptible to BHV-6 than HVC-1 because it belongs to the same subfamily as the goat. If HVC-1 infection of chamois is corroborated, it could be considered as an exception.

In contrast to the results reported in the present study, antibodies against BHV-1 and BHV-6 were not detected in wild ruminants in Switzerland, but only a small number of samples (eight chamois, 25 ibex, 61 fallow deer [*Cervus dama*], nine red deer, two roe deer) were tested (Hasler and Engels, 1986).

The epizootiology of herpesvirus infections in wild ruminants must include three essential features: (1) the transmission of

viruses from domestic to wild ruminants; (2) the presence of herpesviruses specific for a wild species; and (3) the persistence of herpesviruses in a latent state. Latency would allow the virus to persist in a restricted population for long periods, after the infective contact between domestic and wild animals or after infection of the population by a specific herpesvirus. A low prevalence of herpesvirus infection in a wild species is therefore significant because of the existence of these latent carriers. Subsequent studies should include the isolation of the respective herpesviruses infecting their respective hosts. This will assess the range of hosts species susceptible to the different ruminant herpesviruses and contribute to a better knowledge of the epizootiology of these viruses under natural conditions.

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