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AVALON VIRUS, SAKHALIN GROUP (*NAIROVIRUS*, BUNYAVIRIDAE) FROM THE SEABIRD TICK *IXODES (CERATIXODES) URIAE* WHITE 1852 IN FRANCE

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Summary. — Nine strains of Avalon virus were isolated from *Ixodes uriae* ticks collected in the Cape Sizun seabird reserve, Brittany, from 1979 to 1985, during a longitudinal study of consequencies of tick-borne infections for kittiwakes (*Rissa tridactyla*). Avalon virus strains isolated in France proved difficult to study owing to the weak infectious titres they exhibited in suckling mice or cultured cells. However, some interesting data concerning the ecology of virus infection and the morphology of the virions were obtained and are discussed.

Key words: *Avalon virus*; *Sakhalin group*; *Ixodes uriae*; *France*

Introduction

Viruses of the Sakhalin group are all tick-borne arboviruses (Berge, 1975) which have been isolated from two species of hard ticks parasiting seabirds, *Ixodes uriae* (= *I. putus*) and *I. signatus*, and from the blood of an apparently healthy herring gull chick (*Larus argentatus*). They occur on the coasts of North Atlantic, North Pacific and South Pacific oceans (Clifford, 1979; Chastel, 1980; Nuttall, 1984).

At the present time, Sakhalin group includes four members: 1. Sakhalin virus (Lvov *et al.*, 1972), prototype of the group; 2. Avalon virus (Main *et al.*, 1976*a*; 1976*b*); 3. Clo Mor virus (Main *et al.*, 1976b; Lvov *et al.*, 1981*b*; Nuttall *et al.*, 1982); and 4. Taggert virus (Doherty *et al.*, 1975). Another agent, Tillamook virus (Yunker, 1975; Thomas *et al.*, 1973) appears antigenically closely related to Sakhalin virus and it has not been registered to the International Catalogue of Arboviruses (Berge, 1975 and further issues). On the opposite, Paramushir virus (Lvov *et al.*, 1976) is registered to this catalogue but it seems to represent only a strain of Avalon virus (Kondrashina, 1980; Lvov *et al.*, 1981*a*; 1981*b*). In addition, Kachemak Bay virus, isolated from Alaska (Ritter and Feltz, 1974) has not been sufficiently characterized.

These viruses are members of the *Nairovirus* genus (Casals and Tignor, 1980. Clerx *et al.*, 1981) of the Bunyaviridae family, on the basis of their serological and molecular properties, and of electron microscopy studies

(Main *et al.*, 1976; Lvov *et al.*, 1981*b*; Poleshuk *et al.*, 1981; Nuttal *et al.*, 1982). The isolation of nine strains of Avalon virus at Cape Sizun, Brittany, France, increases our knowledge of the geographical distribution and adds some informations about the ecology and the ultrastructural aspects of this relatively poorly documented tick-borne virus.

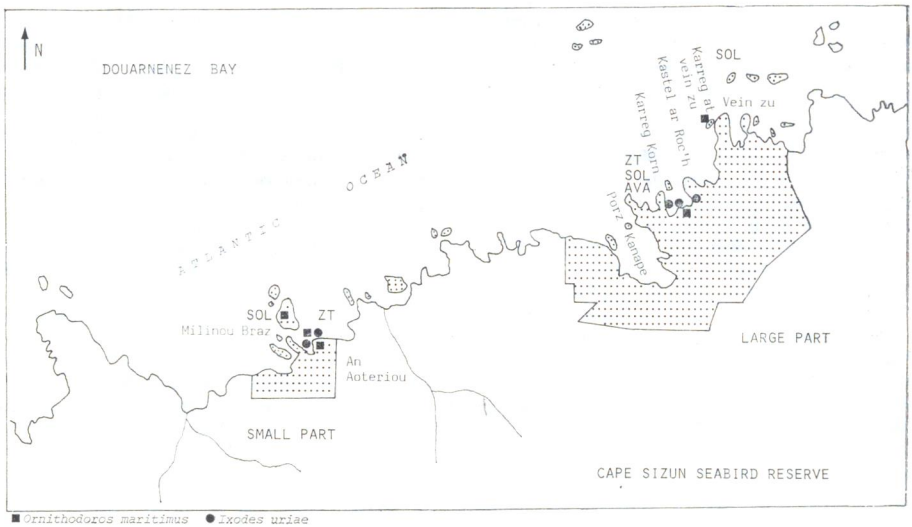

Fig. 5.
The map of the Cape Sizun reserve

Materials and Methods

The study area corresponds to the Michel-Hervé Julien ornithological reserve located near Douarnenez, on the northern coast of the Cape Sizun (48°04′ 75″N — 4° 35′ 25″W), Finistère, France. During the spring, this reserve is a sanctuary for a number of seabird species: kittiwakes (*Rissa tridactyla*), guillemots (*Uria aalge*), razorbills (*Alca torda*), fulmars (*Fulmar glacialis*), shags (*Phalacrocorax aristotelis*) and herring gulls (*Larus argentatus*). Lesser black-backed gulls (*L. fuscus graeslsii*) and great black-backed gulls (*L. marinus*) breed on nearly islets and rocks. Three species of Corvidae, the raven (*Corvus corax*), the chough (*Pyrrhocorax pyrrhocorax*) and the jackdaw (*Corvus monedula*) are also present in the reserve, but in lesser numbers.

Small mammals (rodents, insectivores) are abundant in the falling grasslands located above the cliffs. The reserve is divided in two parts, a small western part (An Aoteriou, Milinou Braz) and a large eastern part (Porz Kanape, Karreg Korn, Kastell ar Roc'h, Vein Zu) (Fig. 5).

Tick collections were performed from 1979 to 1985, in the two parts, during ringing operations of young kittiwakes by one of us (J. Y. Monnat) as a part of a programe of biological studies on *Rissa tridactyla*. For this purpose, it was attributed a letter (P. Q. R, etc...) to each studied cliff and a number to each nest in the cliff, making possible comparisons from time to time. Among thousands of ticks collected in nests, crevices, under stones or on infested chicks, 2,210 *Ornithodoros (Alectorobius) maritimus* and 517 *Ixodes uriae*, or a total of 2,727 ticks, were processed for virus isolation.

Isolation procedures were conducted as previously described, using intracerebral (i.c.) inoculation on to 24—48 hr old suckling mice (s.m.) (Chastel *et al.*, 1981; 1985*a*).

Virological studies of isolates included reisolation from the same material held at −70 °C, titration in s. m. or Vero cells, using the method of Reed and Muench (1938) for the calculation of end points, filtration through Millipores filters with 220 nm pores, lyophilisation, effects on infectivity of diethylether, acidity (pH 3.0) and heating at 60 °C for 1 hr, attemps to adapt the strain in a number of cultured cells including BHK-21 and Vero lines and to obtain plaques in *Xenopus* cells, histological studies and electron microscopy of ultrathin sections of infected s. m. brains or cells (Chastel *et al.*, 1981; 1985*a*). Antigens were prepared by sucrose-aceton extraction of s. m. brains following Clarke and Casals (1958).

Serological studies of isolates were carried out as previously described (Chastel *et al.*, 1981; 1985*a*), using group or type IAF or immune sera supplied by the National Institute of Allergy and Infectious Diseases, Bethesda, by the Yale Arbovirus Research Unit (YARU), New Haven, Connecticut (A. J. Main), and by the Rocky Mountain Laboratory (RML), Hamilton, Montana, USA (C. E. Yunker).

Two of the isolates, Brest/Ar T261 and T439 were identified at the YARU by A. J. Main using Avalon (AVA), Clo Mor (CM), Taggert (TAG), Tillamook (TILL) and Sakhalin (SAK) viruses in cross CF tests. The seven other isolates were identified at the Brest Virus Laboratory by CF tests, using serial dilutions of reference group specific and type specific antibodies to viruses of the SAK group, and 4 units of CF antigen of each isolate. Another strain of virus, Brest/Ar T1267, isolated from *I. uriae* ticks collected on Lundy island, Devon, G. B., in July 1983 (Chastel *et al.*, 1984, unpublished data) was also included in the tests. Paramushir virus was not available in both YARU and Brest Virus Laboratory for comparison with our isolates.

Serological surveys were performed by CF test using T261 strain as antigen on: 474 sera from farmers living in South Brittany in the vicinity of Cape Sizun reserve (Chastel *et al.*, 1983), — — 289 sera from seabirds and landbirds in Brittany (Chastel *et al.*, 1985*b*) and — 129 sera from rodents and insectivores trapped alive into the reserve in November 1983 and May 1984. Each serum found positive by CF test for AVA virus was tentatively studied further in neutralization test (NI) using the T261 strain adapted to VERO cells (Chastel *et al.*, 1983).

Results

General results

As a whole we have isolated 39 strains of virus: 19 strains of Soldado (SOL) virus (*Nairovirus*, Hughes group) from 2,210 *O. maritimus*; 11 strains of Zaliv Terpeniya virus (ZT) virus (*Uukuvirus*, Uukuniemi group) and 9 strains of AVA virus. Only these 9 latter strains (Brest/Ar T261, T439,

Table 1. Results of virus isolations of Avalon virus from Ixodes uriae (Cape Sizun, 1979—1985)

Year of collection:	L	N	♂	♀	Total ticks No.	No. of isolated strains of AVA virus	MIR (*)
1979	—	44	—	7	51	1	1.96
1980	—	—	—	34	34	2	5.88
1981	—	202	50	101	353	6	1.70
1982	—	1	—	34	35	0	—
1984	19	21	—	3	43	0	—
1985	—	1	—	—	1	0	—
Total	19	269	50	179	517	9	1.74

(*) Minimal isolation rate expressed in %.

T465, T695, T699, T722, T723, T725 and T726) will be discussed in this paper. AVA virus was isolated only from specimens collected in 1979, 1980 and 1981, and only from cliffs P, Q, R, on Karreg Korn, in the largest part of the reserve. In contrast, ZT virus was isolated from 1979 to 1984 and SOL virus from 1979 to 1985, in the two parts of the reserve, including cliffs L and K (An Aoteriou) where the infestation of kittiwakes by ticks was observed only recently (map) and where the mortality of young chicks was relatively high.

Another finding of interest is that AVA and ZT viruses were always isolated from *I. uriae* and SOL virus always from *O. maritimus*, even when the two species of ticks infested simultaneously the same nest or the same chick. This scheme was constant for six consecutive years and crossing this species barrier was never observed. AVA virus was isolated from nymphs (N) and females, with a minimal isolation rate (MIR) of 1.86 and 2.23 respectively, in June and July. According to the year of isolation, the highest MIR was found in 1980 (5.88) and the lowest in 1981 (1.7). In 1985 only one specimen of *I. uriae* was virologically studied and was found negative (Table 1). All the strains were reisolated.

Virological properties of isolates

All the 9 isolates exhibited very long incubation period in s. m. inoculated i. c., not only during the original isolation procedure or reisolation (8 to 13 days), but also during the 2nd and the 3rd passages (7 to 12 days), after which antigens were prepared from infected s. m. brains. Paralysis, sometimes regressive, occurred in 100% s. m. after i. c. inoculation and in about 50% after intraperitoneal inoculation. Adult mice were insensitive to these strains. The DL 50 in s. m. was never greater than 10^3 i. c. log/0.02 ml. Strain T261 induced CPE in Vero cells by day 4 p. i., but results were sometimes difficult to reproduce, the DITC 50 being about $10^{3.4}$ log/0.1 ml, making fastidious the use of this cell system for NT tests or other tests. In BHK-21 cells, no obvious CPE was detected after inoculation of T261 strain nor plaques were obtained in *Xenopus* cells.

As briefly mentioned previously, T261 strain was sensitive to di-ethyl-ether, pH 3.0 and heating at 60 °C for 1 hr, the infectious titre falling from 10^3 log/0.02 ml in s. m. controls to less than 10^2 logs and 10^1 logs respectively after exposition (Chastel *et al.*, 1981). In general, preservation of strains for long periods of time was better in form of infected s. m. brains held at −70°C than by lyophilization. Vials of lyophilized material often failed to kill s. m. after sending by post to YARU.

Pathology and electron microscopy

By light microscopy of coronal sections of s. m. infected brains, a mild meningo-encephalitis was found, characterized by a diffuse oedema, an acute vasculitis and occasional necrosis of some neurons. Meningeal infiltration by mononuclear cells was rarely seen.

By electron microscopy, virus particles compatible with those of the

Table 2. Results of CF tests comparing Brest/Ar T439 and T261 with members of the Sakhalin group (from A. J. Main, YARU, 1982) using antibody and antigens from Brest virus Laboratory

Antigens:	Antibody to: T439 (Brest)	T261 (Brest)	AVA	CM	TAG	TILL	SAK
T439 (Brest)	128/≧ 128 (*)	— (**)	1024/16	0 (***)	0	0	0
T261 (Brest)		128/≧ 64	512/16	—	—	4/≧ 64	—
Avalon	128/≧ 128	64/≧ 64	≧ 1024/≧ 128	—	—	—	—
Clo Mor	0	—	—	32/32	—	—	—
Taggert	0	4/4	—	—	≧ 1024/≧ 128	—	—
Tillamook	0	4/4	—	—	—	512/≧ 128	—
Sakhalin	0	4/4	—	—	—	—	64/16

(*) Reciprocal of the highest dilution of antibody/Reciprocal of the highest dilution of antigen.
(**) Not done.
(***) Negative at 1 : 4.

Table 3. Results of CF tests comparing 8 strains isolated from Ixodes uriae with some members of the Sakhalin group

CF antigens (4 units):	SAK group (YARU)	Antibody to: AVA	CM	TILL	SAK type (RML)	T1267
T465	0(*)	**512**(**)	0	0	0	0
T695	0	**8**	0	0	0	0
T699	0	**8**	0	0	0	0
T722	0	**512**	0	0	0	0
T723	0	**16**	0	0	0	0
T725	0	**16**	0	0	0	0
T726	0	**16**	0	0	0	0
T1267 (Lundy island)	0	0	0	0	**16**	**16**

(*) Negative at 1 : 8
(**) Reciprocal of highest dilution of antibody giving positive reaction with 4 units of antigen.

Bunyaviridae family were found, although in a few number, in both infected s. m. brains and VERO cells. The low titres exhibited by these viruses probably explain the difficulties encountered in finding the virus. In infected brains, the virus particles were seen in the cytoplasm of neurons of the cerebral cortex and of the midbrain, one or two particles were located in a cytoplasmic vesicle in the vicinity of polysomes (Figs 1 and 2). Virus particles were spherical with a diameter of 65 to 110 nm and they were delineated by about 12 nm thick envelope. Condensed material (nucleocapsid?) was observed underneath the envelope. Neither obvious surface projection nor any process of budding from Golgi apparatus were observed. Intranuclear filamentous inclusions were exceptionally encountered in the nucleus of infected neurons.

A frequent feature of neuron infection is the accumulation of small pleomorphic particles enclosed by cytoplasmic membrane (Fig. 3). These particles, irregular in shape, are limited by electron dense material and have a diameter of 42 to 66.5 nm (m = 52 nm), smaller than that of virus particles (Fig. 3).

Infected Vero cells exhibited severe signs of cytoskeleton degeneration and virus particles were seen in both cytoplasmic vesicles and extracellular spaces (Fig. 4). Internal condensed material was irregularly distributed as small granules occupying the inner space of virus particles.

Serological identification of isolates

No haemagglutinin was detected using 24 hr-old chick erythrocytes for any of the 9 isolates. However, the antigens exhibited potent CF activity. In CF tests, the same antigens failed to react with IFA corresponding to groups A, B, Bunyamwera, California, Kemerovo, polyvalents 1, 4, 5, 10, Congo, Quaranfil and LCM-rabies, and with immune sera prepared in our Laboratory against a number of tick-borne arboviruses.

At the YARU, A. J. Main established by cross CF tests that strains Brest/Ar T261 and T439 were members of the SAK group, most probably AVA virus (Table 2). In Brest Virus Laboratory, using CF tests and antibody kindly supplied by YARU and RML, it was found that the 7 other isolates were AVA virus or closely related viruses (Table 3). However, reactivity of T695 and T699 for AVA virus antibody was relatively low, indicating some possible antigenic variations among the French strains of AVA virus. Strain T1267 from Lundy Island appears to be not an AVA virus, though exchanges of kittiwakes occur between Cape Sizun and Lundy seabird reserves.

Serological surveys

No CF antibody for the Brest/Ar T261 strain was demonstrated in 289 sera from seabirds (mainly gulls) and landbirds (mainly starlings) from Brittany and in 129 sera from rodents and insectivores from the reserve. In contrast, four sera (or 0.8%) from farmers living in Brittany not very far from the Cape Sizun seabird reserve, exhibited CF antibody with a titer of 1 : 4. Unfortunately, it was not possible to confirm by NT tests the specificity of these reactions, owing to the lack of reliability of the NT test used (Chastel *et al.*, 1983).

Discussion

On the geographical level, multiple isolations of AVA virus, or of a virus very close to it, from *I. uriae* ticks in France increase the known distribution of this agent. Previous isolations have been obtained from the same tick species collected on Great Island, Newfoundland, Canada (Main *et al.*, 1976*a*; 1976*b*) and, as Paramushir virus, from *Ixodes signatus* and *I. uriae* collected on Paramushir, Tyuleniy and Bering Islands, far Eastern U.S.S.R. (Lvov *et al.*, 1976; 1981*a*; 1981*b*; Kondrashina, 1980).

From an ecological point of view, it is of interest to note that some of the northern latitudes from which AVA virus strains were isolated in Newfoundland (47° 11′), Okhostsk sea (48° 30′) and Brittany (48° 04′) are very similar. However, the climatic conditions in Brittany are milder than in the two other countries, owing to the tempering effect of the northern branch of the Gulf Stream.

On the other hand, it is clear that the Cape Sizun seabird reserve is a permanent focus of AVA virus infection as demonstrated by isolations of this virus in 1979, 1980 and 1981. However, only Karreg Korn in the largest part of the reserve seems infected and, apparently, the infection did not spread to other cliffs during the study time, even when SOL and ZT viruses did.

Infection was demonstrated in N and female ticks in Cape Sizun, but in Newfoundland AVA viruses have been isolated from N, males and females (Main *et al.*, 1976*a*). MIR were in similar range in Newfoundland (0.3 to 1.1) and in Brittany (1.7 to 5.9), but MIR observed in these two countries largely encompassed those recorded for Paramushir virus in the Far East, only 1 : 3,300 for adult ticks and 1 : 11,000 for N (Lvov *et al.*, 1979).

AVA virus NT antibody have been demonstrated in 62.1% of adult

Atlantic puffins (*Fratercula artica*), 19% of adult Leach's petrels (*Oceanodroma leucorhoa*) and 8.7% of herring gull chicks (*Larus argentatus*) surveyed in 1972 on Great Island, Newfoundland; AVA was isolated also from the blood of an apparently healthy herring gull chick (Main *et al.*, 1976*a*). We found no CF antibody to AVA among 215 seabirds from different areas of Brittany (Chastel *et al.*, 1985). However, the CF test is relatively insensitive for detecting long-living antibody and most of the surveyed birds were gulls of the *Larus* genus which are not currently parasited by *I. uriae* in this country. In fact, only 6 kittiwakes were included in the survey and not any alcid, the main host of *I. uriae* anywhere.

AVA virus seems to have limited potential of diffusion among vertebrates other than seabirds since no evidence of infection have been obtained in a small number of biologists doing research on Great Island (Main *et al.*, 1976) and among landbirds (Chastel *et al.*, 1985*b*) and small mammals in Brittany. However, our observation of CF antibody in 4 Britton farmers deserve further attention and there is serological evidence that SAK is capable to infect large mammals such as bisons (*Bison bison*) and arctic foxes (*Alopex lagopus*) in Alaska (Zarnke *et al.*, 1983).

Morphology of French isolates of AVA virus replicating in both s. m. brains and VERO cells are compatible with Bunyaviridae family. Especially the size and the shape of virus particles encountered in cytoplasmic vesicles (Figs. 1, 2 and 3) are similar to those of this family. However, the budding of viral particles was never observed in our material when it is a constant feature of the infection by Soldado virus for instance (Chastel *et al.*, 1979), another member of the *Nairovirus* genus. For Paramushir virus, budding was only occasionally seen (Poleshuk *et al.*, 1981).

The electron-dense granular material seen in the virus particles released from infected Vero cells (Fig. 4) might represent cross sections of nucleoprotein strands as suggested by Russian workers for Sakhalin virus (Lvov *et al.*, 1979; Poleshuk *et al.*, 1981), but this remains questionable.

Another question is what actually represents the large cytoplasmic accumulations of irregularly shaped particles enclosed in a single cytoplasmic membrane: precursors of mature virions or by-products of virogenesis during replication of poorly adapted strains of AVA virus?

Answers to these questions would be probably found in molecular biology studies of the different members of the Sakhalin group, only outlined at the present time (Lvov *et al.*, 1979; 1981*a*; Clerx *et al.*, 1981), but difficulties encountered in the study of these fastidious agents (Yunker, 1975) may delay somewhat such studies.

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Explanation of Figures (Plates XLVIII—XLIX):

Fig. 1. Suckling mouse brain infected by strain Brest/Ar T261: two virus particles (V) enclosed in a cytoplasmic vesicle in the vicinity of mitochondria (M) and polysome (P). Magn. × 180,000.
Fig. 2. Suckling mouse brain infected by strain Brest/Ar T439, cytoplasmic vesicle harbouring two virus particles (V) in the vicinity of a polysome (P). Magn. × 96,000.
Fig. 3. Suckling mouse brain infected by strain Brest/Ar T261. Accumulation of small pleomorphic particles enclosed by a cytoplasmic membrane near mitochondria (M). Magn. × 93,000.
Fig. 4. VERO cells infected by strain Brest/Ar T261. Two extracellular virus particles (V). Internal condensed material was seen as small granules occupying the inner space of the particle (sections of nucleoprotein?). Magn. × 89,000.