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ISOLATION AND CHARACTERIZATION OF SEDLEC VIRUS, A NEW BUNYAVIRUS FROM BIRDS

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Summary. — A pathogenic agent designated AV 172 was isolated from the blood of a Reed Warbler (*Acrocephalus scirpaceus*) out of 767 samples from birds belonging to 35 species and 14 families. The birds (largely wetland passerines) were captured in the reed-belt littoral of Nesyt fishpond in southern Moravia, Czechoslovakia, during the years 1984 to 1987. Virus AV 172 has been found to represent probably a new species (designated virus "Sedlec") of family *Bunyaviridae*. Sedlec virus is pathogenic to suckling and adult mice when inoculated intracerebrally (i.c.) but not intraperitoneally (i.p.) and its ether-sensitive spherical particles measure 90—100 nm.

Key words: *Sedlec virus*; *Bunyaviridae*; *birds*; *free-living*; *Acrocephalus scirpaceus*

Introduction

The study site, Nesyt fishpond (315 ha), is situated at Sedlec village (48°46′ N, 16°43′ E) in southern Moravia at 175 m a.s.l. A detailed description of the locality and habitat has been given elsewhere (Květ, 1973; Hubálek *et al.*, 1989). Briefly, the mean annual temperature of the area is 9 °C, with the mean January and July temperatures of —2 °C and 19 °C, respectively. Annual precipitation is 570 mm, with a maximum in June and a minimum in January. The leading plant alliance of the littoral reed-belt vegetation is *Phragmition communis* Koch covering ca. 50 ha, with dominating species *Phragmites communis* and *Typha angustifolia*. Homeotherm vertebrates recorded in the littoral zone comprise 33 species of mammals (Pelikán, 1975) and 54 species of birds (Hudec, 1975; Hájek, 1981). Many species of potential arbovirus vectors have been recorded within, or close to, the habitat (Minář, 1973; Knoz and Vaňhara, 1982; Málková *et al.*, 1986): four species of ixodid ticks (*Ixodes ricinus*, *I. hexagonus*, *I. apronophorus*, *Haemaphysalis concinna*); 22 species of mosquitoes (*Culex modestus* — dominating, *C. pipiens*, less *C. territans*, *Aedes vexans*, *Ae. cinereus*, *Ae. sticticus*, *Ae. cantans*, *Ae. excru-*

Table 1. Numbers of birds examined

Bird family and species	No. of birds
ANATIDAE	
Anser anser (L.)	27
RALLIDAE	
Rallus aquaticus L.	5
ALCEDINIDAE	
Alcedo atthis (L.)	1
HIRUNDINIDAE	
Riparia riparia (L.)	7
Hirundo rustica L.	40
MOTACILLIDAE	
Motacilla flava L.	2
M. alba L.	5
TURDIDAE	
Luscinia luscinia (L.)	1
L. svecica (L.)	1
Saxicola torquata (L.)	1
Turdus merula L.	2
T. philomelos Bream	1
SYLVIIDAE	
Locustella naevia (Bodd.)	1
L. fluvialitis (Wolf)	1
L. luscinioides (Savi)	7
Acrocephalus paludicola (Vieill.)	1
A. schoenobaenus (L.)	218
A. scirpaceus (Herm.)	266
A. arundinaceus (L.)	34
A. palustris (Bechst.)	21
Sylvia borin (Bodd.)	1
S. atricapilla (L.)	6
Phylloscopus trochilus (L.)	1
P. collybita (Vieill.)	4

cians, *Ae. flavescens*, *Ae. dorsalis*, *Ae. communis*, *Ae. caspius*, *Ae. geniculatus*, *Ae. annulipes*, *Conquillettidia richiardii*, *Anopheles maculipennis*, *An. messeae*, *An. atroparvus*, *An. labranchiae*, *An. claviger*, *An. plumbeus and Culiseta annulata*); ceratopogonid flies (*Culicoides obsoletus*, *C. pictipennis*); simuliid flies (*Eusimulium angustitarse*, *E. latigonium*, *E. securiforme*, *Boophthora erythrocephala*, *Odagmia ornata*, *Simulium argyreatum*), and 13 species of tabanid flies (genera *Haematopota*, *Chrysops*, *Tabanus*, *Hybomitra*).

Table 1.-continued

Bird family and species	No. of birds
TIMALIIDAE	
Panurus biarmicus (L.)	42
PARIDAE	
Parus caeruleus L.	8
REMIZIDAE	
Remiz pendulinus (L.)	23
LANIIDAE	
Lanius collurio L.	3
STURNIDAE	
Sturnus vulgaris L.	1
FRINGILLIDAE	
Fringilla coelebs L.	1
Varduelis chloris (L.)	8
C. carduelis (L.)	5
C. cannabina (L.)	3
EMBERIZIDAE	
Emberiza schoeniclus (L.)	18
Miliaria calandra (L.)	1
Total	767

Materials and Methods

A total of 767 birds, largely wetland passerines, were captured in mist nets erected across the reed belt between May and September of the years 1984 to 1987. The examined birds involved 14 families and 35 species: *Sylviidae* as much as 73.1 %, *Hirundinidae* 6.1%, *Timaliidae* 5.5%, *Anatidae* 3.5%, *Remizidae* 3.0%, *Emberizidae* 2.5%, the other avian families in sum 6.3% (Table 1).

The captured birds were identified, aged, ringed, and released after collecting a sample of blood by puncture of the wing vein (*vena ulnaris cutanea*). The blood (50—200 μl) was collected into 75 μl hematocrite capillary tubes (Juřicová *et al.*, 1986), mixed with 200 μl of cooled phosphate buffered saline pH 7.2 containing 0.75% bovine serum albumin, antibiotics, and heparin (10 u./ml), kept on wet ice, transported to the laboratory within 1—3 hr and then stored at —60°C until tested. Aliquots (0.1 ml) of blood samples taken generally from three (one to five) birds of the same species, were pooled and inoculated by i.c. route (0.02 ml) into suckling ICR mice (SPF grade). Standard characterization and identification procedures (Lennette and Schmidt 1969; Gaidamovich, 1986) were used: filtrability through Millipore membranes; sensitivity to, diethyl ether and 0.1% sodium deoxycholate (SDC); pathogenicity to suckling and adult mice at different routes of inoculation; ability to cause cytopathic effect (CPE) in various cell cultures; electron microscopy of ultrathin sections of infected cell cultures; formation of haemagglutinating antigen after saccharose-acetone extraction of infected suckling mouse brains (SMB). The identification included the use of complement-fixation tests (CFT), virus neutralization tests (VNT) or counter immuno-electrophoresis (CIEP) (Bárdoš *et al.*, 1980) against sets of immune sera and ascitic fluids. Mouse serum to AV 172 isolate was prepared by three weekly i.p. doses of 10% infectious SMB to adult ICR mice and their bleeding 8 days after the last injection.

Results

One isolation only of a mouse pathogenic agent (AV 172) was made, namely in a pool of the blood samples taken from four young (yearlings) reed warblers (*Acrocephalus scirpaceus*), collected on July 30, 1984. This pool killed one out of nine suckling mice (SM) inoculated, on day 20 post-inoculation (p.i.). The first passage (SM_1) of SMB resulted in the death of all 7 i.c. inoculated SM on day 8 p.i., while the next passages SM_2 and SM_3 caused a shortened average survival time of 4 days. The SM_6 passage killed all SM on day 3 p.i.

The infectious titre of the agent was $10^{8.2}$ LD_{50}/ml in SM: a subcutaneous (s.c.) or i.p. injection of 10% SMB did not kill SM or adult mice, whereas adult mice (even 70-day old) were killed at i.c. infection within 4—6 days p.i. (the infectious titre was $10^{7.3}$ LD_{50}/ml). Adult rabbit (3.5 kg) was refractory to the agent when inoculated intravenously. AV 172 did not produce a readily visible CPE in Vero cells (at 37 °C) or XTC-2 cells (at 28 °C). However, a partial CPE was observed in SPEV cells (pig kidney embryo cell line) either at 37 °C or (more marked) at 41 °C (i.e., at the avian body temperature); nevertheless, this CPE revealed a tendency to disappear after 4—5 days p.i. Apparent CPE was produced within 3 days in CV-1 cells at 37 °C (titre $10^{6.2}CD_{50}$/ml).

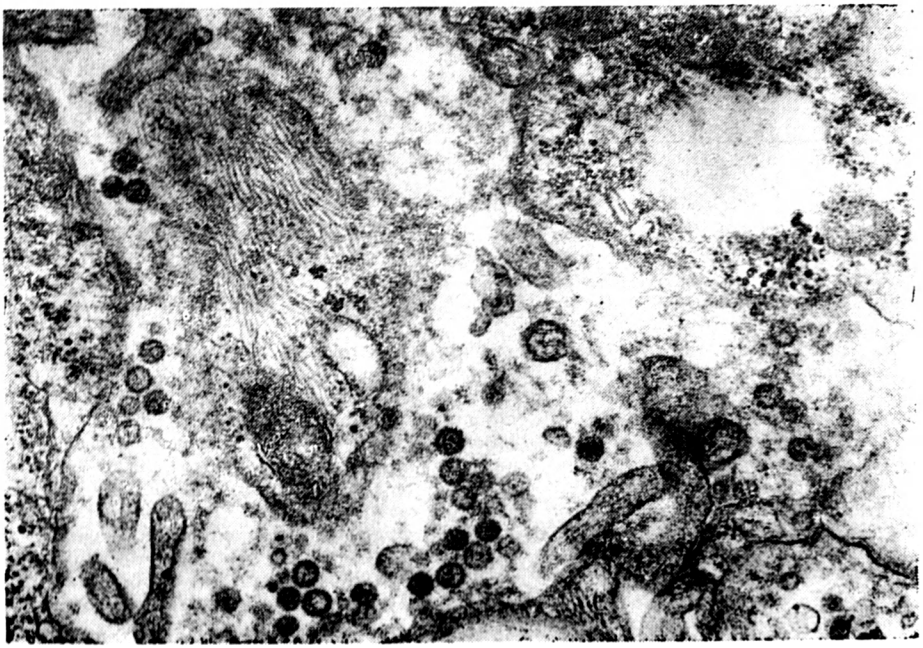

Fig. 1

Sedlec virus particles (90—100 nm in diameter) in an ultrathin section of SPEV cells 72 hr p.i.

The infectious titre of the agent decreased from $10^{8.0}$ to $10^{5.1}$ after treatment with diethyl ether, from $10^{7.4}$ to $10^{2.2}$ after exposure to SDC, and from $10^{7.3}$ to $10^{6.9}$ and below $10^{3.4}$ after filtration through Millipore membranes with pore sizes 220 nm and 100 nm, respectively. It means that virions contain a lipid envelope and their size is between 90 and 200 nm. Electron microscopy of ultrathin sections of infected SPEV cells then showed spherical to subspherical particles 9—100 nm in diameter, morphologically related to viruses of the *Bunyaviridae* family (Fig. 1).

Saccharose-acetone (SA) extraction of infectious SMB did not produce an antigen able to agglutinate goose erythrocytes in the range of pH 5.7—7.4. CF antigen was prepared by both SA procedure or as a clarified suspension of SMB in borate buffer pH 9. By box CF titration against immune mouse serum to AV 172, the titres of both antigen and antiserum were 1 : 128 (for both types of antigens — SA or borate). SA antigen was then used in CF tests against sets of immune sera or ascitic fluids (starting dilutions were 1 : 8) to a wide spectrum of arboviruses, but with negative results: alphaviruses Sindbis, Chikungunya, Western equine encephalomyelitis, Eastern equine encephalomyelitis, Venezuelan equine encephalomyelitis, Whataroa; flaviviruses Dengue 1, Dengue 2, Japanese encephalitis, West Nile, Yellow fever, Powassan, tick-borne encephalitis, Tyuleniy, Apoi; bunyaviruses Batai, Čalovo, Ťahyňa, Mahogany, Hammock, Mirim, Simbu, Bahig, Matruh, Tete, Tsuruse, Lednice; phleboviruses Karimabad, Sandfly fever-Naples, Sandfly fever-Sicilian, Tehran; nairoviruses Hazara, Dera Ghazi Khan, Hughes, Soldado, Dugbe, Paramushir, Sakhalin, Taggert, Tillamuk; uukuviruses Grand Arbaud, Uukuniemi; bunyavirus-like viruses Bhanja, Upolu, Kaisodi, Bakau, Lone Star, Razdan, Tamdy, Wanowrie, Bimbo, Burg el Arab, Zevashen, Artashat, Caspiy; orbiviruses Eyach, Kemerovo, Tribeč, Aniva, Matucare; rhabdoviruses Piry, Kwatta, Mossuril, Kolongo, Chandipura, Barur; orthomyxovirus Dhori; unclassified viruses Burana, Tyulok, Yaba-7.

Using VNT, mixtures of inactivated undiluted sera and serially tenfold diluted virus AV 172 were incubated for 90 min at 37 °C and then inoculated i.c. into adult mice. Log_{10} neutralization indices (NI) as compared with normal serum of particular antisera were: a) AV 172 (mouse immune serum), 4.1; b) Lednice 6118 (freeze-dried immune mouse serum with a given NI of 3.0, supplied by courtesy of Dr. D. Málková, DrSc), 0.4; c) tick-borne encephalitis (hyperimmune serum purchased by Imuna), 0.1.

In CIEP, SA antigen AV 172 formed a clear precipitation line with homologous mouse antiserum but did not react with either normal mouse serum or several samples of immune mouse sera against mouse hepatitis coronavirus MHV-3.

Discussion

The characterization tests revealed that the agent AV 172 is an ether-sensitive virus with spherical (subspherical) particles 90—100 nm in diameter, morphologically resembling members of the family *Bunyaviridae* (Murphy *et al.*, 1973). While the pattern of its mouse pathogenicity, i.e. the

killing of both suckling and adult mice after an i.c. application only, is rather unusual among arboviruses and similar to that of Lednice bunyavirus (Málková *et al.*, 1973, 1986; Karabatsos, 1985) of the Turlock antigenic group, CFT and VNT did not demonstrate antigenic similarity either to this virus or to any other arbovirus tested. The agent AV 172, therefore, can be regarded for a presumably new virus; the name Sedlec has been assigned to it according to the village closest to the site of its isolation. The virus has recently (October 1989) been registered in the International Catalogue of Arboviruses (Karabatsos, 1985), and the prototype strain AV 172 deposited in the arbovirus collections at Institute of Virology in Moscow, at CDC in Fort Collins and at the Yale University.

A re-isolation attempt was impossible to carry out because of insufficient quantity of the original blood sample, but no agent with similar properties was handled in the laboratory where the isolation procedures were made. Sedlec virus does not appear to be an endogenous murine virus (a pick-up contamination) because of 1. the bunyavirus morphology; 2. a better replication (CPE) at 41 °C than at 37 °C in SPEV cells; 3) serological exclusion of MHV. Validity of isolation is further supported by the occurrence of antibody to Sedlec virus in local population of wetland passerines. Among 109 birds of 6 species, caught and examined in 1988 (not included in Table 1) at the study site, 25 (i.e. 22.9%) demonstrated VN antibody (reciprocal titre 10 or higher) when tested against ca. 100 CD_{50} in CV-1 cells (unpublished observations): *Acrocephalus scirpaceus* 19/89 (no. positive/no. tested), *A. schoenobaenus* 3/12, *A. arundinaceus* 0/1, *Locustella luscinioides* 2/2, *Emberiza schoeniclus* 1/3, and *Remiz pendulinus* 0/2.

No other viruses were recovered in the blood samples of as much as 767 birds examined, but this seemingly low isolation rate is in accord with that reported by many other authors (cf. Hubálek *et al.*, 1989) who attempted to isolate arboviruses from the avian blood. On the other hand, the isolation of a single strain of Sedlec virus could have been affected by the method used, i.e. by pooling (for the sake of economy) of the blood samples taken from several birds prior to inoculation into mice. Under these conditions, the antibody present in one bird might neutralize the virus originating from another bird in the pooled sample.

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