FOLIA PARASITOLOGICA (PRAHA) 17: 337–340, 1970.

UUKUNIEMI VIRUS IN WESTERN SLOVAKIA AND NORTHERN MORAVIA*)

O. KOŽUCH, M. GREŠÍKOVÁ, J. NOSEK, J. CHMELA

Institute of Virology, Slovak Academy of Sciences, Bratislava and
District Sanitary-Epidemiological Station, Olomouc

Abstract. Eight strains of Uukuniemi virus were isolated from *Ixodes ricinus* nymphs and adults collected in western Slovakia and northern Moravia. The strains were isolated in suckling mice and in CEC using interference method. All strains kill suckling mice after i. c. inoculation, but no symptoms of disease were observed in mice infected i. p.

In Czechoslovakia Poteplí virus was isolated by KOLMAN et al. (1966). This virus is identical with the Uukuniemi virus (CASALS, personal communication) isolated by OKER—BLOM et al. in Finland (1964).

In the years 1967—1968, ecological investigations were carried out in several foci of tick-borne encephalitis (TE), namely at three localities from the Tribeč Mountains—Topoľčianky, Žirany and Horné Lefantovce, further at the locality of Lamač situated on the slopes of the Little Carpathians, and at the locality of Lašťany in the Jeseníky foreland. During these studies, several strains of Uukuniemi virus were isolated.

The Tribeč focus localities are situated on the south-eastern and south-western slopes of the Tribeč Mountains covered with characteristic oak and oak-hornbeam woods (*Quercetum* and *Querceto-Carpinetum*), here and there mixed with black locust (at Topoľčianky, Horné Lefantovce). The locality of Lamač is situated in oak-hornbeam woods. On the other hand the locality of Lašťany is covered with *Picetum excelsae* community. Whereas the Jeseníky natural foci are situated mainly in cultivated clearings the focality of the Carpathian region refers mainly to edges of the forests.

*) Presented at the International Symposium on Tick-borne Arboviruses, Smolenice, Czechoslovakia, 9—12 September 1969.

MATERIAL AND METHODS

The ticks used for isolation experiments were being collected throughout whole period of ticks incidence. Isolation experiments were performed in pools prepared from individual developmental stages of ticks (10 nymphs, 5 females, 5 males). Ticks were rinsed with physiological saline with added penicillin and streptomycin. Suspensions were prepared in a mixture of 1 ml of Earle solution with 5 % inactivated calf serum. The suspension was centrifuged and the supernatant inoculated into suckling white mice, 1—4 days old, and into chick embryo cell (CEC) tube cultures, respectively. The presence of virus in CEC was detected after the 2nd passage, using interference method. Positive were regarded those pools which caused death of suckling mice in the 1st passage and/or in subpassages, and CEC showed interference of 100 CPD_5- of Sindbis virus (VILČEK 1960). All the isolates obtained in CEC were intracerebrally (i.c.) inoculated into suckling white mice (0.01 ml per mouse). All the suckling mice were followed up to 14 days p.i.

RESULTS

In 1967 seven strains of this virus were isolated from *Ixodes ricinus* nymphs (one strain), females (5 strains) and males (1 strain) collected from the above 5 localities (Topoľčianky, Žirany, Horné Lefantovce, Lamač, Lašťany). One virus

Table 1. Isolation of Uukuniemi virus from *Ixodes ricinus* ticks

Locality	Date of collection	Nymphs	Females	Males
Topoľčianky	April—November 1967	1/52/515*)	0/10/47	0/11/56
Žírany	April—October 1967	0/27/272	2/33/163	0/31/156
Horné Lefantovce	May 1967	0/1/12	1/6/27	0/5/24
Lamač	April 1967	0/13/131	1/4/19	1/4/21
Lašťany	April—September 1967	0/2/20	1/3/12	0/4/20
Topoľčianky	April—July 1968	0/173/1732	0/10/49	0/9/45
Žírany	April—October 1968	0/41/412	0/4/19	0/8/38
Lašťany	March—August 1968	1/26/257	0/4/21	0/21/103
Lamač	April 1968	0/3/28	0/1/1	0/1/1
Horné Lefantovce	May 1968	0/29/294	0/5/23	0/4/20

*) Number of positive / number of pools / number of examined ticks.

Table 2. Mode of isolation of Uukuniemi virus strains and their pathogenicity for white mice

Strains	Locality	Isolation in		Pathogenicity in 2nd passage			
		CEC	suckling mice	suckling mice		adult mice	
				i.c.	i.p.	i.c.	i.p.
265	Lamač	○	+	+	○	○	○
268	Lamač	+	+	+	○	○	○
293	Žírany	+	+	+	○	○	○
301	Topoľčianky	+	○	+	○	○	○
364	H. Lefantovce	+	+	+	○	○	○
428	Žírany	○	+	+	○	○	○
767	Lašťany	○	+	+	○	+	○
880	Lašťany	+	○	+	○	+	○

+ isolation, pathogenicity positive.
○ isolation, pathogenicity negative.
CEC chick embryo cell culture.

strain was isolated from *I. ricinus* nymphs collected from Lašťany surroundings in 1968 (Table 1). Five virus strains were isolated in CEC using interference method, and 6 strains in suckling mice (Table 2). All these isolations exerted partial cytophatic effect (CPE) on CEC on the 5th day p.i. reaching titres of $10^{2,5}$—$10^{3,5}$ CPD_{50} per ml; If $_{50}$ was 10^5—10^7/ml. Incubation period in the 1st passage was 8—13 days, in further passages it was only 5—7 days. The titre of virus in suckling white mice amounted to $10^{6,5}$—$10^{8,5}$ mouse LD_{50} per ml. All the strains isolated were found to kill suckling mice after i.c. inoculation; however, no symptoms of disease were observed in mice infected via i.p. route. The strains isolated at the locality of Lašťany (No. 767 and 880) were found to be lethal for 6—8 g white mice after i.c. inoculation. The titre of 767 strain in 6—8 g juvenile mice attained the value 10^6 mouse i.c. LD_{50} per ml. The other strains did not cause any symptoms of disease in adult mice neither after i.c. nor after i.p. inoculation (Table 2). Reisolation attempts were positive.

Identification of isolated agents was performed in hemagglutination-inhibition and complement-fixation tests. Using the method of acetone extraction by CLARKE and CASALS (1958), antigen was prepared from mouse brain suspension. The strains isolated in the Tribeč region in 1967 were found not to hemagglutinate goose erythrocytes in low passages. The antigens prepared from 767 and 880 virus strains (isolated at the locality of Lašťany) hemagglutinated goose erythrocytes after the 2nd and 3rd passage, respectively, in the titre of 1 : 160, pH 5.7—6.0. Hy-

Table 3. Identification of isolated strains by the CF test

Antigen	Antiserum	CF titre**
Poteplí*	Poteplí	128/64
Poteplí	268	32/32
	301	128/64
	293	128/64
	767	32/32
	429	128/64
	364	128/64
	265	128/64
	880	256/64
Tick-borne encephalitis	Poteplí	0

* Poteplí strain is identical with Uukuniemi virus.
** Reciprocal of serum dilution/reciprocal of antigen titre.

perimmune sera were prepared in white mice weighing 18—20 g. The mice were immunized with 5 doses of 10 % mouse brain suspension, administered at one week intervals. The first dose (0.03 ml) was administered i.c., the following doses (0.5 ml) were applied intraperitoneally. On the 7th day after they received the last dose, the mice were bled.

Results of complement-fixation reaction confirmed a close relationship between these isolations and Uukuniemi virus (Table 3). The immune sera prepared from these isolations did not react with the following virus antigens: Semliki, TE, West Nile, Tribeč and LCM, in complement-fixation reaction.

The presented results show that *Ixodes ricinus* ticks are vectors of Uukuniemi, TE and Tribeč viruses.

REFERENCES

Clarke D. H., Casals J., Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. Hyg. 7: 561—573, 1958.

Kolman J. M., Málková D., Smetana A., Isolation of a presumably new virus from unengorged *Ixodes ricinus* ticks. Acta virol. 10: 171—172, 1966.

Oker-Blom N., Salminen A., Brummer-Korvenkontio M., Kääriäinen L., Weckström P., Isolation of some viruses other than typical tick-borne encephalitis viruses from *Ixodes ricinus* ticks in Finland. Ann. Med. exp. Fenn. 42: 109—112, 1964.

Vilček J., Interference between tick-borne encephalitis and western equine encephalomyelitis viruses in chick embryo tissue cultures. Acta virol. 4: 308—310, 1960.

Received 24 September 1969.

O. K. Institute of Virology,
Slovak Academy of Sciences,
Bratislava 9, ČSSR