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RECENT STATE OF KNOWLEDGE OF ŤAHYŇA VIRUS INFECTIONS

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Abstract. The study summarizes the results of serological tests with the Ťahyňa virus in the territory of Moravia and Slovakia and the results of experimental works with primates. The isolation of Ťahyňa virus from the blood of a sick person proved the pathogenicity of this agent for man. The clinical symptoms as well as the seasonal dynamics of the infection are given.

On the occasion of the 15th anniversary of Ťahyňa virus isolation in Czechoslovakia (Bárdoš and Danielová 1959) we would like to demonstrate one of the numerous examples how the Academician E. N. Pavlovsky's theory of the natural focality of some infections may help discover new arbovirus infections, giving in this way opportunity to the health authorities to prevent these infections in the population. At the same time we would like to repeat also why we have decided to investigate the mosquito population in our lowlands for mosquito borne viruses. We tried without any success for a few years before 1958 to clarify the aetiology of the hospitalized cases of febrile diseases occurring in summer months in the lowlands of eastern Slovakia. In spite of a broad battery of known virus antigens used in our serological tests, we were not able to clarify the aetiology of a substantial proportion of the hospitalized febrile cases.

Despite unsuccessful virus isolation attempts from mosquitoes of mosquito borne viruses undertaken in the past by many virologists (Gutsevich and Vigovsky 1960) and despite the frequently repeated proclamations of our epidemiologists that mosquitoes are not a health hazard but only an annoyance, we have decided to start virus isolation experiments from mosquitoes on a basis of biocenological evaluations of the east-Slovakian lowland. These evaluations were done in close cooperation with Academician B. Rosický.

The results are well known and the isolation of Ťahyňa virus and neutralizing antibodies in many European countries, Africa and Asia brought an evidence that this virus, belonging to the California group, is present on these continents (Kokernot et al. 1962; Likar and Casals 1963; Aspöck and Kunz 1966; Hannoun et al. 1966; Balducci et al. 1968; Gaidamovich et al. 1971; Spieckermann and Ackermann 1972; Molnar et al. 1973; Lvov et al. 1972; Vargin and Motejunes 1972). Fig. 1 demonstrates the distribution of Ťahyňa virus isolations and/or virus neutralizing antibodies in Europe.

It might be said that wherever proper biotopes were selected for virus isolation experiments Ťahyňa virus was isolated. The prevalence of antibodies in man depends on the kind of serological tests used in the survey, on the localities where the tested persons are living and on the age group of the tested persons. If sera are collected from adult persons in localities with a typical biocenosis and with the mass occurrence of *Aedes* mosquitoes, 80—90 % of the tested sera are positive in a virus neutralization

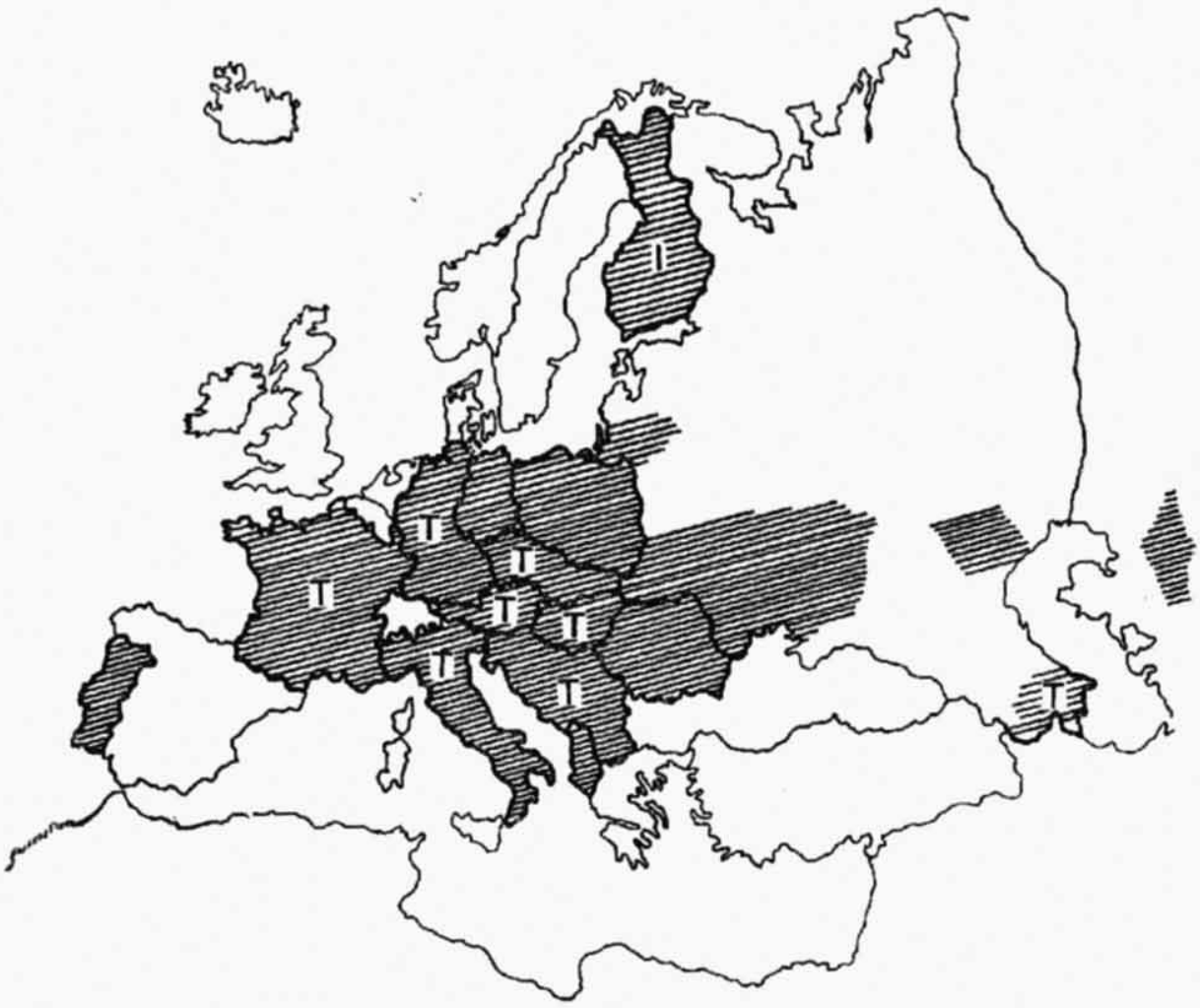

Fig. 1. Geographic distribution of Ťahyňa virus isolations and/or virus neutralizing antibodies in Europe. Cross-hatched areas — antibodies against Ťahyňa virus detected; T — Ťahyňa virus isolated; I — Inkoo virus closely related to Ťahyňa virus isolated (Brummer- -Korvenkontio 1969).

test (Bárdoš et al. 1962). In some countries typical biotopes are only in the vicinity of a few villages, in others whole districts or even regions of several hundred square kilometers may be involved.

Until recently there was an unanimous agreement that the results of serological tests in persons reflect the immunological status after a past mosquito-borne infection, but some doubts were raised whether the clinical picture seen in persons is in direct relation to the Ťahyňa virus infection, although it is the European representative of the California virus group. In the U.S.A., however, there was no doubt at all about the California encephalitis virus pathogenicity.

Since more experimental and diagnostic work has been done and further evidence of Ťahyňa virus pathogenetic action has been accumulated, I have decided to summarize and evaluate so far published evidence in this topic.

From 1959 up to now 4 separate independent teams have undertaken studies on Ťahyňa virus infections in hospitalized patients. Three teams have observed patients in hospitals situated in the lowland with mass occurrence of mosquitoes, in areas where more than 30 % of the serologically tested adult population had virus neutralizing antibodies against Ťahyňa virus (Bárdoš 1960). The fourth team in the Ostrava hospital worked in a region where prevalence of virus neutralizing antibodies in the population has been below 30 %.

In Košice and Valtice a significant increase of antibodies against Ťahyňa virus has been detected in almost 1/4 to 1/3 of susceptible patients in one or two serological tests (Table 1) (Bárdoš et al. 1965 a, b). This proportion of recent Ťahyňa virus infections, detected in the patients in these two hospitals, need not necessarily reflect the situation valid for every year and should be taken only as an evidence of the frequency of Ťahyňa virus infections in the years under study.

Quite different was the position in the study undertaken in the 4 southwestern Slovakia hospitals (Table 1) (Mayerová et al. 1966). Here only virus neutralization tests were done. It is most probable that some recent Ťahyňa virus infections might have escaped detection since it is known that virus neutralizing antibodies appear earlier than other and a significant increase of antibodies is easily missed. The Ostrava cases differ substantially from the cases in other hospitals since here only those patients were tested who manifested syndrom of aseptic meningitis (Heinz et al. 1972: Herzig et al. 1972). Only virus neutralization tests were done here (Table 1). These details are mentioned to stress the fact that the incidence of recent Ťahyňa virus infections in these 4 hospitals cannot be compared.

These results are proving that where the proper screening of the patient's sera has been done, recent Ťahyňa virus infections have been detected. In Košice the team of clinicians and virologists focused their attention also to persons calling at the outpatients clinics (Table 2) (Mittermayer et al. 1965). Therefore in the spring months they screened serologically (in a virus neutralization test) factory workers employed outdoors in summer months. In the winter months of 1961 they tested them again and noticed conversion from negativity to positivity in 17.1 %. In a repeated study in 1962 they confirmed again that almost 1/5 to 1/3 of the exposed susceptible factory workers had been infected in this area.

In the study undertaken in southwestern Slovakia in 1966 we aimed at another goal (Bárdoš et al. 1969). An attempt was made to isolate Ťahyňa virus from the blood of sick children. In this study only children with a history of fever of 1- or 2-day duration were included. Children with a history of a longer fever duration were excluded. The detected percentage of infected children might be considered as lower limit of the incidence rate.

Presented data prove that in all the lowland hospitals and outpatient clinics where the patients sera were tested with Ťahyňa antigen recent infections have been detected.

In the next table (Table 3) data are presented to demonstrate that in the differential diagnostic serological tests a battery of antigens was used to exclude the occurrence of another infection. Since micromethods were not used in the years under study, the supply of serum was in some cases exhausted and serological tests could not be done in all cases. In studies carried out in southwestern Slovakia and Ostrava virus isolation experiments were also included. Since in some patients the syndrom of bronchopneumonia had been observed, antigens of Q-fever, ornithosis and other diseases were also used in the serological tests. The selection of antigens in the study undertaken in the Ostrava hospital was done to exclude infections by other viruses causing aseptic meningitis.

Table 1. Survey of articles on published Ťahyňa virus infections in hospitalized patients in Czechoslovakia

Year of study	Locality	Age group	Number of persons				% †) infected	Literature
			Tested	with antibodies*)	without antibodies**)	infected ***)		
1959	E. Slovakia (Košice)	Adults	53	15	24	14	36.8	Bárdoš et al. 1965 a
1960—64	S. Moravia (Valtice)	Adults	210	69	108	33	23.4	Bárdoš et al. 1965 b
1962—63	Sw. Slovakia (4 hospitals)	Adults and children	476	45	423	8	1.8	Mayerová et al. 1966
1970	N. Moravia (Ostrava)	Adults and children	122	7	95	10	8.6	Heinz et al. 1972

*) Upon arrival and discharge from the hospital the same level or insignificant increase of antibodies detected

**) Upon arrival and discharge from the hospital no antibodies detected

***) Patients with conversion from negativity to positivity or with four fold increase of antibodies recorded in one, two or three different serological tests

†) Calculated from the number of persons tested and infected

Table 2. Survey of articles on published Ťahyňa virus infections in persons calling at out-patient clinics in Czechoslovakia

Year of study	Locality	Age	Tested	Number of infected	%	Literature
1961	E. Slovakia (Košice)	Adults	35	6	17.1	Mittermayer et al. 1965
1962	E. Slovakia (Košice)	Adults	32	10	31.2	Mittermayer et al. 1965
1966	Sw. Slovakia (Bratislava)	Children	80	6	7.5	Bárdoš et al. 1969

Table 3. Diferential diagnostic serological tests and virus isolation experiments done in hospitalized patients

Locality	Number of cases	CFT*)				HIT*)			Agglutinolysin test*)	Agglutination test
E. Slovakia (Košice)	14	TBE (12×)**)	WEE (12×)	EEE (12×)	LCM (6×)	WN (12×)	D1 (12×)		Leptospirosis 10 strains (8×)	BRUC. (8×) LIST. (11×)
		Q. (6×)	LIST. (11×)	TOXOPL. (11×)						
S. Moravia (Valtice)	33	TUL. (6×)	Q. (6×)	ORN. (6×)	INFL. (A. B) (6×)	CAL (32×)	SF (25×)	D1 (32×)***)		
		ADENO (6×)	PARA (1×)	EATON (2×)	RSV (2x)					
SW. Slovakia (4 hospitals)	8	ADENO	INFL. (A, B)		SENDAI				Isolation experiments for entero-viruses, respiratory viruses and bacteriological examinations negative	
N. Moravia (Ostrava)	6**)	PAROT.	TBE	LCM	HERP.					

*) No antibodies or antibodies present but without increase or with insignificant increase in all tests

**) 12 sera tested

***) 1× significant rise

Table 4. Differential diagnostic serological tests done in persons calling at outpatient-clinics

Locality	Number of cases	CFT					HIT		
SW. Slovakia (Bratislava)	6	SF	D1	BUN	INFL (A, B)	ADENO	SF	D1	BUN

No antibodies or antibodies present but without increase or with insignificant increse in all tests

In persons calling at outpatient clinics differential diagnostic tests were done only in the studies carried out in southwestern Slovakia (Table 4).

To summarize: with the exception of one case in the Valtice study (where significant increase in HIT against dengue 1 antigen was detected) the results of all differential serological tests were negative or, either an insignificant increase of antibodies or none at all was detected. Therefore only Ťahyňa virus could have been considered as the only agent responsible for the clinical picture seen in the patients.

Almost all persons with recent Ťahyňa virus infection were admitted to the hospital with fever ranging from 37.2 to 40.9 °C (Table 5). In the adult patients in the hospitals

Table 5. Clinical symptoms seen in hospitalized patients

Locality	Number of cases	Temperature at admission in °C	Number of cases with influenza-like symptoms, headache, dizziness, nausea, pharyngitis, conjunctivitis and myalgia	Broncho-pneumonia	Aseptic meningitis
E. Slovakia (Košice)	14	37.3—40.5	10	2	2
S. Moravia (Valtice)	33	37.2—40.9	24	7	2
SW. Slovakia (4 hospitals)	8	38.0—40.0	8 (without myalgia)	0	0
N. Moravia (Ostrava)	10	38.5—39.0	0	0	10

of Valtice and Košice most frequently observed symptoms were those of influenza-like disease with myalgia and in a few patients syndrom of bronchopneumonia or aseptic meningitis was observed. It should be mentioned, however, that in the Valtice hospital cases of aseptic meningitis were hospitalized exceptionally only, since under the poliomyelitis surveillance programe all CNS infections were concentrated elsewhere. Cases treated in the 4 hospitals in southwestern Slovakia differed from those in other hospitals since here myalgia was not mentioned in the clinical picture of the sick children.

In persons calling at outpatient clinics only influenza-like symptoms were observed with fever of 3 to 4 days duration (Table 6). In the Košice study the adults complained of myalgia.

First cases of Ťahyňa virus infections were observed in the second half of May (Table 7). Increasing incidence of infections appeared in June to August and a decline was noticed in September with the last cases in October. This recorded seasonal distribution does not necessarily reflect the real seasonal distribution of cases for every year and every locality. 9.1 % of all the hospitalized cases occurred in May. This is seemingly contradicting the experience of entomologists and virologists since the first isolation of Ťahyňa virus from mosquitoes in Central Europe has been so far recorded in June only (Aspöck et al. 1971; Danielová 1972). It should be mentioned, however, that the majority of isolation experiments from mosquitoes is done in one territorially restricted biotope mostly, but patients are admitted to the hospitals from a considerably larger area.

In the tables presented there has been so far only a serological evidence that the clinical

Table 6. Clinical symptoms seen in persons calling at outpatient-clinics

Locality	Number of cases	Temperature at admission in °C	Number with symptoms of influenza-like disease
E. Slovakia (Košice)	6	not tested	6
E. Slovakia (Košice)	10	37.3—37.8	10 with myalgia
SW. Slovakia (Bratislava)	6	37.2—39.0	6

Table 7. Seasonal distribution of cases of Ťahyňa virus infections according to the onset of symptoms

Months	May	June	July	August	September	October
Number of cases	6	18	16	14	7	4

picture seen in the patients could be attributed to a recent Ťahyňa virus infection. No virus was isolated from the blood of the hospitalized patients. Experimental infections in primates were undertaken to answer the question whether Ťahyňa virus is pathogenic and if so what are the symptoms in experimentally infected primates. First experiments in 19 primates brought evidence that the species *Macaca rhesus*, *M. radiata* and *Cercopithecus aetiops* were not suitable for this kind of experiments (Šimková and Bárdoš 1969). They reacted to the experimental infection with a viraemia and production of antibodies only and showed no clinical symptoms.

Therefore five chimpanzees, *Pan troglodytes*, (of 3.0 to 14.5 kg of body weight) were subcutaneously infected (with 2.4 to 5.4 log units of LD_{50}) with an extraneurally passaged strain of Ťahyňa virus (Šimková and Bárdoš 1969). This strain has been constantly extraneurally passaged in hamsters since the time of its isolation from mosquitoes (Bárdoš and Danielová 1959). It is believed that the biological qualities of this strain (236-H) are similar to the strains circulating in nature since no intracerebral passage of this strain in mice has been performed (Bárdoš 1965). In all five inoculated chimpanzees fever of 3 to 4 day's duration and in three of them weakness and decreased mobility was observed during the fever period. In two chimpanzees an acceleration of the erythrocyte sedimentation was recorded. Viraemia lasted from 3 to 8 days and reached in some chimpanzees titres above 3.0 log units of LD_{50}. The production of specific antibodies (VN, HI, CF) was detected in all inoculated primates. Thus in chimpanzees a suitable model was found for the future experimental studies. The results of the experimental infection of the above mentioned five chimpanzees could be used for the interpretation of the clinical picture seen in the naturally infected patients with some caution only since Ťahyňa virus was artificially inoculated in these experiments.

To simulate the conditions occurring in nature as close as possible Ťahyňa virus infected mosquitoes of the species *Culiseta annulata* (Schrk.) were used for the experimental infection of another five chimpanzees (Šimková and Danielová 1969). Again

extraneurally passaged strain of Ťahyňa virus was used. In four chimpanzees a febril illness, similar to the one seen in the previously mentioned chimpanzees, was observed. The results of other laboratory tests and immunological response were also similar.

In two separate experiments on chimpanzees the pathogenetic action of Ťahyňa virus was proved and the human beings' reaction was expected to be similar. However, Ťahyňa virus isolated from a naturally infected sick person was still the missing link.

Finally, in autumn of 1972 Ťahyňa virus was isolated from the blood of a sick person (Šimková and Sluka 1973). The sick man was exposed to natural mosquito bites during his work in the open, done on September 8. In the following 4 days he was suffering from an influenza-like disease (with a splitting headache, loss of appetite, general weakness and fatigue). Two days later i.e. 6 days after the exposure (i.e. on Sept. 14) Ťahyňa virus was isolated from his blood in i.c. inoculated suckling white mice. The virus was reisolated from the stored blood again. In this person a conversion from negativity to positivity of VN and CF antibodies was detected (Table 8). The course of the disease

Table 8. Results of serological tests in the human case with Tahyňa virus isolation from the blood (according to Šimková and Sluka 1973)

Serological tests	Titres of antibodies at days after virus isolation		
	0	21	33
VN	negat	64*)	128
CF	< 4	< 4	8

*) 1 : 64 against 100 CPD_{50} in GMK cells

and the immunological response was similar to that seen in the experimentally infected chimpanzees. Thus the last link in the process of proving that Ťahyňa virus is pathogenetic for human beings has been presented. Still unanswered are questions on the relation of Ťahyňa virus to the complications (bronchopneumonia and aseptic meningitis) observed during this infection in some infected persons. But this is another chapter and will be not dealt with in this paper.

The above mentioned results of experimental Ťahyňa virus infections of chimpanzees and the isolation of Ťahyňa virus from the blood of a sick person have dissipated remaining doubts and proved that Ťahyňa virus pathogenicity in Europe does not differ from that of California encephalitis virus in the U.S.A. It has been proved that in Central Europe mosquitoes are no longer an annoyance only but a definite health hazard.

СОВРЕМЕННЫЕ ЗНАНИЯ О ИНФЕКЦИЯХ ВИРУСОМ ТЯГИНЯ

В. Бардош

Резюме. Работа подытоживает результаты серологических тестов с вирусом Тягиня на территории Моравии и Словакии и результаты экспериментальных работ с приматами. Выделение вируса Тягиня из крови больного подтвердило его патогенное значение даже человека. Приводятся клинические симптомы и сезонная динамика заболевания.

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