

EFFECTS OF SEX AND DOSE OF INOCULUM ON THE ANTIBODY RESPONSE OF MICE TO ŤAHYŇA VIRUS (*BUNYAVIRIDAE*)

Z. HUBÁLEK

Institute of Parasitology, Czechoslovak Academy of Sciences, 166 32 Prague, Czechoslovakia

Received September 25, 1979; revised February 20, 1980

Summary. — Six weeks old random bred ICR mice were inoculated intraperitoneally with two different doses of Ťahyňa virus (125 and 12 500 smicLD₅₀). The higher dose caused marked involvement of the central nervous system in a few animals and induced high virus neutralization (VN) antibody titres in more animals than the lower dose. The dose of inoculum had no effect on the height of VN antibody titre in mice with antibody titres > 64. The proportion of mice with VN antibody titres > 64 was 72.7% in males and 56.8% in females. Male mice thus appeared to be somewhat more susceptible to Ťahyňa virus infection than female mice.

Key words: Ťahyňa virus; Bunyaviridae; mouse; sex; virus dose; neutralization antibody

Human infections caused by California group arboviruses occur mainly in children and the morbidity in males is frequently higher than in females (Bárdoš *et al.*, 1965; McGowan *et al.*, 1973). On the other hand, serological surveys on human populations in North America and Europe revealed a mostly uniform prevalence of antibodies against these viruses among both sexes. A higher frequency of antibody in men, especially in certain population groups, has been reported less frequently (Henderson and Coleman, 1971; Brummer-Korvenkontio, 1974).

The present model experiments in mice were initiated in view of the reported discrepancies concerning the effect of sex on morbidity and incidence of California group viruses in humans.

Virus. The T 16 strain of Ťahyňa virus isolated from the blood of a sick boy in south Moravia (Bárdoš *et al.*, 1975) was used in its 3rd suckling mouse (sm) brain passage. From the brains of dead mice a 10% suspension was prepared in phosphate buffered saline (PBS) containing 0.75% bovine albumin and antibiotics. This suspension had a titre of 10^{7.1} smicLD₅₀/0.02 ml and after appropriate dilution was used both for infection of mice and in VN tests.

Animals. Six weeks old random bred SPF white mice of the ICR strain (Velaz, Prague) were kept in groups of 4 animals of the same sex in separate cages and fed Larsen diet ad libitum. Their mean weight (\pm standard deviation) before inoculation was 29.6 \pm 1.48 g (males — M) and 24.1 \pm 1.54 g (females — F). The room temperature in the course of the experiment varied from 18 to 24 °C.

Table 1. Distribution of VN antibody titres as revealed by the PRNT

Titre	Males		Females	
	<i>d</i>	<i>D</i>	<i>d</i>	<i>D</i>
8	0	0	3	0
8-16	1	0	6	0
16	4	0	4	3
16-32	5	0	1	1
32	2	0	1	0
32-64	0	0	0	0
64	0	0	0	0
64-128	0	0	0	0
128	0	2	0	0
128-256	0	1	0	1
256	3	8	1	5
256-512	4	4	1	3
512	3	6	2	6
512-1024	0	1	2	2
1024	0	0	1	1
Total	22	22	22	22

d and *D*: inocula of 125 and 12 500 smicLD₅₀, respectively.

The mice were inoculated intraperitoneally with 0.2 ml of virus diluted in PBS with 0.75% bovine albumin and antibiotics: 22 M and 22 F were given 125 smicLD₅₀ (dose *d*) and another 22 M and 22 F received 12 500 smicLD₀₅ (dose *D*). In addition, 4 M and 4 F (controls) were given 0.2 ml of diluent without virus. Two weeks after inoculation (p. i.) the mice were weighed and bled from the left axillary vein under ether anaesthesia. The sera were kept at -20 °C; before examined, they were heated for 30 min at 56 °C. In 3 mice dead in the course of the experiment, blood plasma from heart eluate was examined.

The plaque-reduction neutralization test (PRNT) was carried out in Linbro IS-FB-96 microplates on XTC-2 cells incubated for 3 days at 28 °C (Hubálek *et al.*, 1979b). The sera were diluted in twofold steps and incubated with Tahyňa virus (10-25 plaque forming units - PFU - per well) for 70 min at 37 °C before adding the cells. Antibody titre was expressed as reciprocal of that serum dilution which caused 50% reduction in plaque number as compared with the test dose of virus. Serum from each mouse was assayed in the PRNT twice (each dilution in 1 well) on different days.

The mean weight increase in mice of the *Md* group was 3.87 g (13.0%) while in *MD* mice it was 4.55 g (15.4%). The difference between both mean increases approached the limit of significance ($t = 1.98$; $0.05 < P < 0.06$) and suggested that the weight increase of males given the higher dose of virus could have been higher. The corresponding difference in females was insignificant: 10.7% in *Fd* and 11.4% in *FD* mice.

The experimental infection remained symptomless in all animals with the exception of four (3M, 1F) inoculated with dose *D*. These mice showed marked symptoms of infection of the central nervous system (CNS): 2M and 1F died 10 days p. i., while 1M survived, although on day 14 p. i. it still showed evident symptoms of neuroinfection. The brains from all these 4 mice contained Tahyňa virus in a comparatively high titre ($> 10^3$ smicLD₅₀/0.02 ml). Specificity was checked by VN tests with immune mouse anti-Tahyňa serum.

Table 2. GMT of VN antibody titres (\log_2 values), their standard deviations (SD) and significance of differences

Variant*	n	GMT	SD	Significance**
M	44	7.273	1.903	
F	44	6.625	2.575	1.34
M <i>S</i>	32	8.375	0.596	
F <i>S</i>	25	8.780	0.678	2.40
M <i>W</i>	12	4.333	0.444	
F <i>W</i>	19	3.789	0.535	2.94
M <i>d</i>	22	6.227	2.164	
F <i>d</i>	22	5.409	2.626	1.13
M <i>D</i>	22	8.318	0.664	
F <i>D</i>	22	7.841	1.892	1.12
M <i>d S</i>	10	8.500	0.408	
F <i>d S</i>	7	9.071	0.673	2.19
M <i>D S</i>	22	8.318	0.664	
F <i>D S</i>	18	8.667	0.664	1.65
M <i>d W</i>	12	4.333	0.444	
F <i>d W</i>	15	3.700	0.561	3.19
M <i>D W</i>	0	—	—	
F <i>D W</i>	4	4.125	0.250	
<i>d</i>	44	5.818	2.414	
<i>D</i>	44	8.080	1.422	5.36
<i>d S</i>	17	8.735	0.589	
<i>D S</i>	40	8.475	0.679	1.37
<i>d W</i>	27	3.981	0.596	
<i>D W</i>	4	4.125	0.250	0.47

* M = males; F = females; *S* = strong reactor (titre > 64); *W* = weak reactor (titre < 64); *d* = inoculum of 125 smicLD₅₀; *D* = inoculum of 12 500 smicLD₅₀

** t-values in bold face type: difference significant at $P < 0.05$.

PRNT revealed in blood plasma from the 3 dead mice and in serum from the mice that survived antibody titres from 128 to 512.

As concerns morbidity, males thus appeared somewhat more susceptible to Tahyňa virus infection than females. Being conscious of a certain simplifica-

tion, the result of this experiment can be correlated with findings concerning human infections with California group viruses. For example in the U.S.A. the CDC Encephalitis Surveillance Report mentioned a total of 278 CNS infections caused by California group viruses in the period 1963-68, 60.1% in men and 39.9% in women (Henderson and Coleman, 1971) and 504 cases in the period 1955-71, 60.5% in men and 38.5% in women (McGowan *et al.*, 1973). Also in Czechoslovakia Ťahyňa virus infections are relatively more frequent in male persons, as observed e. g. in south Moravia by Bárdoš *et al.* (1965) - 27M and 6F - and Sluka (1969) - 38M and 20F.

The distribution of VN antibody titres in all 4 groups of mice is presented in Table 1. In each group with the exception of MD there occurred in addition to mice with high titres (> 64 ; strong reactors, S) also mice with low antibody titres (8-32; weak reactors, W). In W mice, evidently specific VN antibody was involved. Sera from control mice given diluent without virus "neutralized" the virus in lower dilutions: 1:4 and less (4 cases), 1:4 - 1:8 (3 cases) and 1:8 (1 case). As distinct from weak reactors, none of these control sera caused an 80-100% plaque reduction even in the lowest dilution (1:2).

The proportion of S was in males higher than in females, both after inoculation with doses *d* (45.5% vs. 31.8%) and *D* (100.0% vs. 81.8%), as well as irrespective of the dose (72.7% vs. 56.8%). No weak reactor was recorded among males given dose *D*, while 18% of females reacted to this dose weakly (W).

The virus dose had a marked effect on immune response (irrespective of sex). High antibody titres (> 64) were found in 38.6% of animals given the lower dose as compared to 90.6% of animals given the 100-fold higher dose. The difference was significant ($\chi^2 = 9.28$; $P < 0.01$). In both M and F, the increase in dose from *d* to *D* was followed by a more than twofold increase in the number of strong reactors.

Table 2 presents the geometric mean titres (GMT) of VN antibody in the individual mouse groups. An analysis of the differences in GMT by the *t*-test showed that among S reactors females had a significantly higher GMT than males, while the reverse was true in weak reactors. There was no difference in GMT after doses *d* and *D* either among strong or weak reactors. The dose of Ťahyňa virus thus had an effect on the frequency of high antibody titres but no effect on the height of titre in strong reactors. Omitted from Table 2 were: a significant difference ($P < 0.05$) between GMT (FdS vs. MDS) and insignificant ($P > 0.05$) differences MdS vs. MDS, MdS vs. FDS, FdS vs. FDS, MdW vs. FDW and FdW vs. FDW.

Among serologically positive persons, Hubálek *et al.* (1979a) found VN antibody to Ťahyňa virus in the following GMT: boys ($n = 28$) 672, girls ($n = 20$) 750, men ($n = 6$) 203 and women ($n = 15$) 467. Although the differences in GMT between the sexes were not significant, there was a tendency to somewhat higher titres in female persons, which could be in agreement with the results of the present experiments.

Acknowledgement. Thanks are due to Dr. V. Bárdoš for helpful comments to the manuscript and to Mrs. B. Křivánková for skilful technical assistance.

References

- Bárdoš, V., Čupková, E., Šefčovičová, L., and Sluka, F. (1965): A serological study on the medical importance of the Czechoslovak mosquito-borne viruses Ťahyňa and Čalovo, pp. 84—99. In: *Atti XIII Congr. Nazion. Soc. Ital. Microbiol., Parma-Salsomaggiore*, vol. 4.
- Bárdoš, V., Medek, M., Kania, V., and Hubálek, Z. (1975): Isolation of Ťahyňa virus from the blood of sick children. *Acta virol.* **19**, 447.
- Brummer-Korvenkontio, M. (1974): Bunyamwera arbovirus supergroup in Finland. A study on Inkoo and Batai viruses. *Comment. biol. Soc. Sci. fenn. (Helsinki)* **76**, 1—52.
- Henderson, B. E., and Coleman, P. H. (1971): The growing importance of California arboviruses in the etiology of human disease. *Progr. med. Virol.* **13**, 404—461.
- Hubálek, Z., Bárdoš, V., Medek, M., Kania, V., Kychler, L., and Jelínek, E. (1979a): Ťahyňa virus-neutralizační protilátky pacientů na jižní Moravě. *Čs. Epidem.* **23**, 87—96.
- Hubálek, Z., Chanas, A. C., Johnson, B. K., and Simpson, D. I. H. (1979b): Cross-neutralization study of seven California group (*Bunyarividae*) strains in homoiothermous (PS) and poikilothermous (XTC-2) vertebrate cells. *J. gen. Virol.* **42**, 357—362.
- McGowan, J. E., Bryan, J. A., and Gregg, M. B. (1973): Surveillance of arboviral encephalitis in the United States, 1955—1971. *Amer. J. Epid.* **97**, 199—207.
- Sluka, F. (1969): The clinical picture of the Ťahyňa virus infection, pp. 311—314. In V. Bárdoš *et al.* (Eds): *Arboviruses of the California complex and the Bunyamwera group*, Publ. House Slovak Acad. Sci., Bratislava.