

EXPERIMENTAL INFECTION OF HEDGEHOG (*ERINACEUS EUROPAEUS*) WITH BHANJA VIRUS

Bhanja virus was demonstrated in free-living insectivores (Insectivora) only once: Kemp G. E. et al. (J. Wild. Dis. 10: 279–293, 1974) isolated a strain from the blood serum of the African hedgehog *Atelerix albiventris* (Wagner) captured in Nigeria (locality Bassa on the Jos

Plateau, about 1280 m above sea level) in December 1970, out of the total number of 450 specimens examined in the region where this virus was relatively often isolated from ticks as well as blood of domestic animals (Causey O. R. et al., Bull. Soc. Path. exot. 62: 249–253

Table 1. Survey of hedgehogs inoculated with Bhanja virus

Animal No:	1	2	3	4	5	6	7	8	9
Sex:	M	M	M	F	F	F	M	F	M
Age (months)	3	3	> 12	> 12	> 12	> 12	> 12	> 12	> 12
Weight prior to infection day 10 p.i.	515 530	420 435	1030 1005	920 900	670 600	835 710	610 570	480 460	490 490
Dose of virus (\log_{10} SMiCLD ₅₀)	3.5	3.5	3.5	3.5	3.5	6.0	6.0	6.0	6.0
Viremia day 2.p.i. 4.p.i. 7.p.i. 10.p.i.	0/18 NT 0/9 0/13 0/7	NT NT 0/7 0/14 ^b	0/14 NT 0/14 0/14	NT 0/24 0/15 0/12	NT 0/15 0/8 0/21	NT NT 0/16 0/17	0/17 NT NT 0/17	0/15 NT NT 0/22	
Neutralization antibodies day 2.p.i. 4.p.i. 7.p.i. 24.p.i.	— ^c NT — —	NT — — —	— NT 5 —	NT — — —	NT — 5 —	NT 10 5 10 ^d	— 5 — 5	NT 10–20 5 5	
HI antibodies day 2.p.i. 4.p.i. 7.p.i. 24.p.i.	— NT — —	NT — — —	— NT 5 —	NT — NT —	NT — — —	— NT — 5	— NT — —	— NT — 5–10	

^a Viremia: number of inoculated 2–3-day-old mice in denominator, number of specifically dead mice in numerator

^b one suckling mouse devoured by its mother on day 9 p.i.

^c negative (< 5); ^d 18 days p.i. NT — not tested

1969, Nigerian J. Sci. 5: 37–40, 1971, Kemp G. E. et al., Bull. epizoot. Dis. Afr. 19: 131 to 135, 1971). Camicas et al. (Rev. Elev. Méd. vét. Pays trop. 34: 257–261, 1981) infected a hedgehog *Atelerix albiventris* intracardially with a dose of $10^{3.7}$ SMiCLD₅₀ Bhanja virus strain ArD 9540 (isolated from *Amblyomma variegatum* in Dakar). They did not find any viremia between day 2 to 20 p.i., but on day 21 they detected seroconversion by complement fixation test. Chunikhin and Karaseva (Vopr. med. virusol. 11: 128–131, 1971) demonstrated haemagglutination-inhibition and neutralization antibodies to Bhanja virus in 36 % of 22 hedgehogs *Erinaceus europaeus concolor* Martin, tested in the Turkmen, Uzbek and Tajik SSR.

Following up these reports we tried to find out what role could be played by the hedgehog in natural foci of Bhanja virus in Europe. We carried out the experiment in August 1982 with nine hedgehogs *Erinaceus europaeus* L., captured in Central Bohemia and kept in an open-air enclosure for three weeks prior to experiment. During the experiment the animals were placed, singly or in pairs, in cages with room temperature ranging from 20 to 26 °C. Table 1 gives the details on the animals tested. Before and during the experiment they were fed dead laboratory mice, and their drinking water was changed daily.

The animals were inoculated subcutaneously (s.c., 0.2 ml) in the region of the right shoulder joint away from the spiky cover, with the strain Bg 326 (Pavlov et al., Folia parasit. (Praha) 25: 67–73, 1978) of Bhanja virus at the sixth brain passage in buffered phosphate solution (PBS), pH 7.4 with 0.75 % of bovine serum albumin and antibiotics, under halothane anaesthesia (Narcotan Spofa). The inoculum was bacterially sterile and was distributed in two different doses, whose titre had been checked before and after administration by intracerebral inoculation of 2–3-day-old SPF mice ICR, and amounted to $10^{3.5}$ and $10^{6.0}$ SMiCLD₅₀. The blood for serological and virological tests was taken by cardiac puncture under halothane anaesthesia in two test tubes (one with heparine 5 u/ml). Heparinized blood was inoculated into 0–2-day-old mice of ICR strain mainly on the day of blood taking, after dilution ana partes with PBS plus 0.75 % of bovine serum-albumin and antibiotics intracerebrally (0.02 ml) and s. c. (0.02 ml). Only at an interval of 7 days p.i. the blood was inoculated after one-week storage at –60 °C. The sera were stored until examination at –20 °C; they were inactivated for 30 min/56 °C for neutralization test (VNT), while acetone-treated and absorbed with goose

erythrocytes for haemagglutination-inhibition test (HIT). The details on test-tube VNT on Vero cells were described in the previous paper (Hubálek Z., Rödl P., Folia parasit. (Praha) 28: 93–96, 1981); homologous strain Bg 326 was used as antigen in the dose of 50–100 TCD₅₀. HIT was carried out by standard micromethod (Clarke D. H., Casals J., Am. J. trop. Med. Hyg. 7: 561–573, 1958) with four units of saccharose-acetone antigen of Bhanja virus strain Bg 335/336 (Pavlov P. et al., 1978). 20 % suspensions of internal organs of an animal which had died during the experiment, were prepared in PBS with albumin and antibiotics and after light centrifugation inoculated i.e. (0.02 ml) into 2–3-day-old mice ICR.

Results of the experiment are given in Table 1. No viremia was demonstrated in the hedgehogs tested. Seroconversion to very low titres (max. 1:10–1:20) of antibodies was detected only in one of the five animals infected with a low dose of virus, and in all four animals infected with a larger dose. VNT showed seroconversion in five animals, HIT—in three animals: antibodies appeared as early as 7 days p.i. The infected animals appeared to be clinically normal and their rectal temperature did not rise, except for hedgehog No 8 which suffered from paroxysms of hind legs since day 10 p.i., of left foreleg and hypothermia since day 13 p.i. until death (18 days p.i.). Isolation tests were carried out from 20 % suspensions of brain, lungs, liver and blood coagulum from heart with negative results.

Our experiment showed that the hedgehog *E. europaeus* was evidently no "amplifying host" sensu Bárdos and Rosický (Folia parasit. (Praha) 26: 89–91, 1979) of Bhanja virus due to the absence of viremia, and that even antibody formation against this virus was very low in this animal. The mechanisms of unspecific resistance to infection in the hedgehog are probably developed to such a degree that specific immune humoral response is no more necessary. On the other hand, the low formation of antibodies might attribute to the hedgehog a role of suitable passive distributor of ticks infected with Bhanja virus, because the virus need not have been neutralized even during their long-term feeding.

Acknowledgement. The authors wish to thank Dr. J. Halouzka for his collaboration in the experiment.

Z. HUBÁLEK, P. RÖDL and Z. JUŘICOVÁ
Institute of Vertebrate Zoology, Czechoslovak
Academy of Sciences, Brno, and Institute of
Hygiene and Epidemiology, Prague