

# BHANJA VIRUS (BUNYAVIRIDAE) ISOLATED FROM *DERMACENTOR MARGINATUS* TICKS IN CZECHOSLOVAKIA

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Adult ixodid ticks (total 516: *Dermacentor marginatus* 430, *Ixodes ricinus* 55, *Haemaphysalis punctata* 31) were collected from sheep in S. parts of Silica plateau (Rožňava district, E. Slovakia) during May 1987; 56 suspensions were prepared (3 to 7 engorged females and 10 to 20 unengorged females or males per pool) in cooled Eagle's MEM with 10% calf serum and antibiotics, centrifuged for 10 min. at 1500 × g, and inoculated intracerebrally (i.e.) into ICR mice 2–3 days old (SM).

A pathogenic agent was isolated from one sample (RV-760: 15 males of *D. marginatus*; locality Kečovo) that killed 6 of 10 inoculated SM in the days 7 to 11 post infection (p. i.). In the first passage (SM<sub>1</sub>) of the bacterially sterile 10 % suspension of SM brains, all i. c. inoculated mice died on days 6 or 7 p.i., and the passages SM<sub>2</sub> and SM<sub>3</sub> killed SM on days 4 or 5 p.i. Infectious titre (per ml) of the agent RV-760 at its SM<sub>3</sub> passage was 10<sup>9</sup> SMicLD<sub>50</sub>, and 10<sup>9.3</sup> TCD<sub>50</sub> in Vero cells, where the agent produced a complete cytopathic effect with a characteristic morphology: small clusters of rounded cells were formed first in an otherwise unchanged monolayer. Standard procedures (1) were used to identify RV-760. The agent passes through a 220 nm Millipore membrane, it is sensitive to diethyl ether and sodium deoxycholate (infectious titre decreased by 5.9 and 3.1 log, resp.). Suckling mice were killed by all routes of infection (i.e., i.p., s.c.), while 24-day-old ICR mice only after i.e. inoculation but not after i.p. inoculation. Neutralization test with the serially diluted virus and a constant dilution of diagnostic serum demonstrated in Vero cells that RV-760 is a strain of Bhanja virus: immune ovine serum with a neutralizing titre of 1 : 410 against Bhanja virus decreased the infectious titre of RV-760 suspension (after incubation for 90 min. at 37 °C) significantly compared to normal ovine serum (log NI was 5.3). The virus was reisolated from the original SM<sub>0</sub> suspension after its 4-month preservation at –60 °C, both in SM and Vero cells. We did not work with Bhanja virus during the isolation experiments in our laboratory.

The isolation represents the most northern (2) demonstration of Bhanja virus, and its first recovery from *D. marginatus* in Europe (similar record in Armenia: Ref: 3). Circulation of Bhanja virus in the Silica plateau area was indicated by our preceding serological surveys (4–6).

## References

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