A NEW SUBTYPE ("BREZOVÁ") OF TRIBEČ ORBIVIRUS (KEMEROVO GROUP) ISOLATED FROM *IXODES RICINUS* MALES IN CZECHOSLOVAKIA

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During an arbovirological survey in Slovak Karst (*1*), three antigenically identical virus strains were isolated from a total of 230 adult *Ixodes ricinus* ticks (179 ♀, 51 ♂) collected from domestic animals in 1983—85:RV-193 andRV-203 — pools of 12 ♂ and 12 partly engorged ♀, respectively, from cattle at Silická Brezová on June 23, 1983; RV-616 — a pool of 2 ♂ and 7 partly engorged ♀ from a sheep-dog at Kečovo on April 28, 1985. All strains were isolated (and re-isolated) by i.c. inoculation of suckling mice (SM; 1—3 d. old) and two also in Vero cell tube cultures. The original suspensions (SM_0) killed on average ca. a half (44 %) of the inoculated SM (within 9—13 days); in the first passage (SM_1), all inoculated SM died within 3—9 d., while SM_2 and SM_3 passages killed mice 3—6 and 2—4 d. p.i. The infectious titre was $10^{9.2}$ $SMicLD_{50}$/ /ml. In Vero cells, a marked cytopathic effect appeared at days 5—6 p.i. with SM_0 but after 1—2 d. with SM_1 or SM_2 passages. ICR mice 22—25 days old were insusceptible to all routes of infection; SM were partly susceptible (ca. 50 % lethality) to s.c. and i.p. inoculations. The agent passed through 220-nm membranes, was ether-sensitive, acid-labile (pH < 7), thermolabile (60 °C/30 min.), and devoid of haemagglutinin. The virus was identified as related to Lipovník (LIP) virus (*2*) by constant serum-varying virus dilution neutralization tests in Vero cell tube cultures: normal vs. immune (LIP) ovine serum gave log NI 2.4 with prototype LIP-91 strain, and 1.9 with RV-193 strain. Cross-neutralization test with 100 TCD_{50} and serial dilutions of antisera showed homologous titres to LIP and RV-193 viruses of 512 and 128, respectively, while the heterologous titres both were 32. Further tests (complement-fixation, CF; serum dilution-plaque reduction neutralization, PRN), confirmed that RV-193 (Brezová) is a member of the family *Reoviridae*, Kemerovo serogroup (NT = not tested):

Virus	Strain	Reciprocal titres (CF/PRN) of antisera: RV-193 (IVR-CZ)	RV-193 (CDC-US)	TRB	LIP	KEM
RV-193		NT/**320**	**1024/160**	64/320	128/40	32/< 20
Tribeč	orig.	NT/< 20	128/40	**64/320**	128/< 20	16/< 20
Lipovník	91	NT/< 20	256/40	64/40	**256/320**	32/< 20
Kemerovo	R-10	NT/< 20	64/< 20	32/< 20	64/20	**64/160**

PRN revealed antigenic similarity of RV-193 to Tribeč (TRB: *3*) and LIP viruses. However, immune mouse serum to RV-193 (3 i.p. doses) prepared in Czechoslovakia (IVR-CZ) did not neutralize TRB and LIP viruses as well as the hyperimmune mouse ascitic fluid (CDC-US). RV-193 appears to be a distinct subtype of Tribeč virus.

References

1. Hubálek, Z., Černý, V., Mittermayer, T., Kilík, J., Halouzka, J., Juřicová, Z., Kuhn, I., and Bárdoš, V., *J. Hyg. Epidem. (Praha)* **30**: 87, 1986.
2. Libíková, H., *Lek. Obzor* (Bratisl.) **13**: 607, 1964.
3. Grešíková, M., Kožuch, O., Ernek, E., and Nosek, J., in B. Rosický and K. Heyberger (Eds): *Theoretical Questions of Natural Foci of Diseases*, Prague, 1965, p. 439.