

TOXICITY OF *RICKETTSIA SLOVACA*

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Summary. — Suspensions of *Rickettsia slovaca* proved to be toxic for intravenously inoculated mice provided that their infectivity was $\geq 10^7$ EID₅₀/ml. Suspensions of *R. slovaca* were less toxic than those of *R. sibirica*.

Key words: *Rickettsia slovaca*; toxicity

Introduction

Toxicity for mice was described in several rickettsial species (Cooke, 1961). The purpose of our study was to show whether *Rickettsia slovaca*, a rickettsial agent isolated in Central Slovakia (Brezina *et al.*, 1969) and included into the spotted fever group of rickettsiae (Úrvölgyi and Brezina, 1978), possesses also toxic properties for mice after intravenous administration.

Materials and Methods

Rickettsiae. The following strains were used: strain B of *R. slovaca*, isolated from *Dermacentor marginatus* ticks in Central Slovakia (Brezina *et al.*, 1969), in its 9th egg passage (EP 9); strain R 42 (EP4), isolated from *D. marginatus* ticks in Austria (Bázliková *et al.*, 1977), apparently identical with *R. slovaca* based on the antigenic and biological properties (Kaaserer, pers. comm.); and strain Netsvetayev of *R. sibirica* (EP 28), kindly supplied by Dr. V. F. Ignatovich, The Gamaleya Institute of Epidemiology and Microbiology, Moscow, U.S.S.R.

To obtain high yields of rickettsiae, 7 days old chick embryos were inoculated into the yolk sacs with doses *R. slovaca* strains that killed the embryos as late as 10—11 days post infection (p.i.) (approx. 10^4 EID₅₀/ml). Strain Netsvetayev of *R. sibirica* (approx. 10^3 EID₅₀/ml) killed chick embryos usually from day 4 to 8 with the highest rickettsial yield 6—8 days p.i. which, however, was lower than the yield of the former rickettsiae. Toxic suspensions were prepared only from yolk sacs heavily infected with rickettsiae as determined by Gimenez staining. All procedures were performed on ice. The harvested yolk sacs were immediately (without freezing) homogenized with equal volumes of brain-heart infusion (BHI). The suspensions were centrifuged for 10 min at 700 × g, and the supernatants without the top fat layers were collected and stored at -70 °C. The infectivities of the resulting suspensions were titrated in chick embryo yolk sacs and expressed in log EID₅₀/ml values.

Toxicity of the rickettsiae was assayed in 10 g mice (Dobrá Voda breed) which were inoculated intravenously (i.v.) with 0.5 ml of undiluted or diluted rickettsial suspensions. The suspensions were considered toxic when they caused death of at least 50 % of the inoculated mice within the observation period of 24 hr. Suspensions prepared from uninfected yolk sacs of 10 days old chick embryos served as control.

Toxicity neutralization. Sera collected from guinea pigs 3 weeks after intraperitoneal infection with a 10 % yolk sac suspension of the given rickettsial strain were used. The complement fixing antibody titres in these sera varied from 256 to 1024 as determined by the micromethod. Serial

twofold dilutions of the sera in BHI were mixed with equal volumes of such a dilution of toxic suspension, which contained the resulting toxic dose of 2 MLD₅₀ in 0.5 ml. The mixtures were incubated at room temperature for 1 hr, then cooled in an ice bath and inoculated into mice. The toxicity neutralization titre was expressed as reciprocal of that dilution of serum which prevented death of at least 50 % of the inoculated mice.

Results

Data in Table 1 indicate that undiluted and diluted (1 : 2) suspensions of *R. slovaca* strains B and R 42 proved to be toxic provided that their egg infectivity titre was about 10⁷ EID₅₀/ml. Undiluted suspension of strain B with a titre of 10⁴ EID₅₀/ml did not kill the mice. By contrast, a suspension of strain Netsvetayev of *R. sibirica* with a similar titre (10^{4.1} EID₅₀/ml) was toxic for mice also in a 1 : 2 dilution.

Table 1. Toxicity of some rickettsial strains of the spotted fever group

Rickettsial species and strain	Titre log EID ₅₀ /ml	undiluted	Toxicity of suspension*		
			1 : 2	1 : 4	1 : 6
<i>R. slovaca</i> , B					
suspension 1	7.6	6	6	2	0
suspension 2	7.2	6	5	0	0
suspension 3	4	0	0	0	0
<i>R. slovaca</i> , R 42	7	6	5	0	0
<i>R. sibirica</i> , Netsvetayev	4.1	6	5	0	0
Uninfected yolk sacs		0	0	0	0

*No. of mice dead out of 6 mice inoculated.

Specificity of the toxicity observed was proved by its neutralization with guinea pig sera containing complement-fixing antibodies against *R. slovaca* strains (Table 2).

Table 2. Neutralization of toxicity of *R. slovaca* strains by homologous sera

<i>R. slovaca</i> strain	Toxin control*	No. of mice dead out of 6 mice inoculated							
		2	4	Reciprocals of serum dilution				128	256
				8	16	32	64		
B	6	0	0	0	0	0	4	6	6
R 42	6	0	0	0	0	1	6	6	6

*2 MLD₅₀/0.5 ml.

Discussion

Our previous failure to demonstrate toxicity of *R. slovaca* can be explained by the use of rickettsial suspensions of lower infectivity. In the present experiments suspensions of *R. slovaca* were toxic only if their in-

fectious titre was $\geq 10^7$ EID₅₀/ml. To prepare such highly infective rickettsial suspensions, it was necessary to use such dose of *R. slovaca* strains which killed chick embryos as late as 10–11 days p.i. and resulted in abundant growth of rickettsiae in the yolk sacs. The same applies also to other strains of *R. slovaca* isolated in Central Europe (unpublished results), but not to *R. sibirica*, which killed chick embryos earlier and the yield of which was lower. However, toxicity of *R. sibirica* could be demonstrated also with suspensions of lower infectivity (10^4 EID₅₀/ml). The titre of *R. slovaca* in field-collected *Dermacentor marginatus* ticks in Central Slovakia does not exceed 10^4 EID₅₀/ml (Župančičová, 1974), and the low virulence is a common feature of all strains of this rickettsia isolated so far. We assume that a concentration of *R. slovaca* necessary for demonstrating its toxicity can be achieved only under laboratory conditions. Toxicity of *R. slovaca* suspensions along with other properties justifies the classification of this rickettsiae with other pathogenic rickettsiae of the spotted fever group.

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