

TWO STRAINS OF RICKETTSIAE OF ROCKY MOUNTAIN SPOTTED
FEVER GROUP RECOVERED FROM *DERMACENTOR*
MARGINATUS TICKS IN CZECHOSLOVAKIA.
RESULTS OF PRELIMINARY SEROLOGICAL IDENTIFICATION

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Summary. — Two strains of rickettsiae belonging according to their biological and antigenic properties indisputably to the Rocky Mountain spotted fever (RMSF) group were recovered from *Dermacentor marginatus* Sulz. ticks collected in the Lučenec district in central Slovakia. Preliminary classification based on the results of the complement fixation (CF) test has shown both strains very closely related to *Rickettsia siberica*.

The ecology of Q fever has been extensively studied in central Slovakia since April, 1967. The natural nidality of this disease has been proved in 1967 by recovering *Coxiella burneti* from *Haemaphysalis punctata* and *Dermacentor marginatus* ticks (Řeháček *et al.*, 1968) and confirmed in 1968 by another two findings of the agent from *D. marginatus* ticks (to be published). In isolation attempts carried out in guinea pigs we often observed signs of scrotal reaction; the accompanying febrile responses were either inexpressive or failed to develop at all. Sera taken from several of these animals reacted positively in microscopic agglutination reaction with *Rickettsia conori* antigen. These findings suggested the possibility to recover rickettsiae other than *C. burneti* in the isolation experiments. With regard to the inexpressive reaction in guinea pigs which could reflect a low virulence of the strain, we completed the isolation experiments in guinea pigs by inoculation of the tick suspensions directly into the yolk sacs of chick embryos. In this way we succeeded to isolate two strains of rickettsiae whose basic properties and preliminary serological identification are described in this paper.

Isolation procedures. The exterior surfaces of the ticks were decontaminated by immersing them into 70% ethanol. After subsequent careful washing in saline, pools of 20 ticks were ground in a mortar in 3 ml of buffered saline diluent (pH 7.2). Thereafter 100 units of penicillin and 100 μ g of streptomycin per ml were added to the suspension. Seven days old embryonated hen's eggs were inoculated into yolk sacs with 0.25 ml of this suspension. For further passages, 10% suspensions of yolk sacs were inoculated into 5 days old chick embryos, based on experiences of Stoenner *et al.* (1962).

Preparations from yolk sacs were stained by the method of Gimenez (1964).

Infection of guinea pigs. Guinea pigs were inoculated intraperitoneally with 1 ml of a 10% suspension of yolk sacs infected with 1) strains recently isolated; 2) other strains of the RMSF group and 3) strains of *C. burneti* and *R. prowazeki*. The blood samples for obtaining immune sera were taken 24 days after infection.

Preparation of antigens. The two strains isolated by us and Soviet strains of *R. conori* (M 1) and *R. akari* (Toger) were used. The antigen of *R. siberica* (lot 13—675) together with the res-

pective immune serum was kindly supplied by Dr. Tarasevich, Gamaleya Institute of Epidemiology and Microbiology, Moscow. Dr. R. A. Ormsbee, Rocky Mountain Laboratory, Hamilton, U.S.A., kindly supplied the antigen of *R. conori* (SF 40 Simko), which we compared with our antigen prepared from the M 1 strain.

From infected yolk sacs, 20% suspensions were prepared in saline with the addition of 0.2% formalin. The rickettsiae were sedimented at 10 000 rev/min for 20 minutes, resuspended in 1/3 of the original volume used and then purified by shaking with 1.5 volumes of ether. The water phase freed of ether represented the antigen for CF tests.

CF test. The warm method with 0.2 ml serum dilutions, 0.2 ml antigen, 0.4 ml complement (2 units) and 0.4 ml of 1% sheep erythrocytes sensitized with 2 units of haemolysin was employed.

Isolation of B and D strains of rickettsiae

The "B" strain was isolated from a pool of male and female *Dermacentor marginatus* ticks collected in the surroundings of the villages Šula and Príboj in the district Lučenec (central Slovakia). The "D" strain was isolated from males of the same ticks species collected in the area of Madačka vilage in the same region. The presence of rickettsiae in yolk sacs was demonstrated 6—7 days after inoculation of test materials by staining smears by Gimenez method. Already in the first passage most of the embryos died on the 4th day after inoculation. In further passages (up to the 7th) the survival period was stabilized at 3—4 days. Both strains multiplied poorly in yolk sacs; the multiplication of "B" strain, as shown in Fig. 1, was an exceptional case. The titres of both strains in the 4th passage in yolk sacs did not exceed the value of 4×10^4 /ml. The intranuclear localization of rickettsiae was clearly demonstrated in yolk sac smears.

Experimental pathogenicity

The pathogenicity and further biological properties of the newly isolated agents are being studied in detail and the results will be published later. So far, it is possible to judge on a low virulence of these strains. Guinea pigs reacted only exceptionally in no more than 25% of cases on intraperitoneal injection of 1 ml of a 10% yolk sac suspension by a short and weak febrile response. The intensity of scrotal reaction attained scarcely a value scored ++; however, this phenomenon also occurred in guinea pigs showing no febrile reaction. The intraperitoneal injection of rickettsiae in mice did not kill these animals. The anatomical findings in mice were rather scarce, being limited to slightly enlarged spleen only. The rickettsiae were found

Table 1. CF antibody responses of guinea pigs inoculated with strains "B" and "D"

| Guinea pig No. | Strain inoculated | CF antibody titre with antigen | | |
|----------------|-------------------|--------------------------------|--------------------|------------------|
| | | B | <i>R. siberica</i> | <i>R. conori</i> |
| 1 | B | 20 | 20 | < 10 |
| 2 | B | 80 | 80 | 10 |
| 3 | B | 20 | 20 | < 10 |
| 4 | D | 20 | 20 | < 10 |
| 5 | D | 40 | 40 | 10 |

only in isolated cells after a very careful examination of the preparations. Fig. 2 illustrates the presence of "D" strain rickettsiae in the spleen of a mouse killed on the 6th day after infection.

Serological relationships between "B" and "D" strains and the other rickettsiae of the RMSF group

Table 1 summarizes the results of CF tests with sera of guinea pigs infected with "B" and "D" strains and antigens prepared from the "B" strain, *R. siberica* and *R. conori*. While the titres of antibodies with "B" strain and *R. siberica* antigens were practically equal, antibodies reacting with *R.*

Table 2. Cross reactions of strains B and D, *R. siberica* and *R. conori* in CF tests

| Antigen | CF antibody titres with antisera to | | | | | |
|---------------------|-------------------------------------|----|--------------------|------------------|-------------------|-------------------|
| | B | D | <i>R. siberica</i> | <i>R. conori</i> | <i>R. prowaz.</i> | <i>C. burneti</i> |
| B | 160 | 40 | 320 | — | 0 | 0 |
| D | 160 | 40 | 320 | 80 | 0 | 0 |
| <i>R. siberica</i> | 80 | 20 | 640 | 20 | 0 | 0 |
| <i>R. conori</i> | 40 | 10 | 160 | 160 | 0 | 0 |
| <i>R. prowazeki</i> | 0 | 0 | 0 | 0 | 320 | 0 |
| <i>C. burneti</i> | 0 | 0 | 0 | 0 | 0 | 640 |

means < 10; — = not done.

conori antigen were present in lower titres in two of six guinea pigs. On the other hand, the "B" and *R. siberica* antigens detected antibodies against *R. conori* similarly to the homologous antigen.

Table 2 illustrates the results of cross CF reactions between "B" and "D" strains, *R. siberica* and *R. conori*. The results with control *R. prowazeki* and *C. burneti* antigens and sera against them clearly showed that "B" and "D" strains belong to the group of RMSF rickettsiae and that they are antigenically related. The results of serological cross reactions between the "B" and "D" strains and *R. siberica* and *R. conori* put them in a row closer to agent of Asian tick typhus.

These data are also supported by the results of CF tests with sera of forest mouse (*Apodemus flavicollis*) which indisputably represents a reservoir animal of this rickettsiosis newly reported for Czechoslovakia. Although with "D" and "B" antigens the serum was positive in a titre of 1 : 40, the reactions with *R. conori* and *R. akari* antigens were negative.

Discussion

A natural focus of a new rickettsiosis for Czechoslovakia has been established in the Lučenec district in central Slovakia. Two strains, designated "B" and "D", were isolated from *D. marginatus* ticks. According to their behaviour in chick embryos, propagation in cell nuclei and antigenic properties both strains belong indisputably to rickettsiae of the RMSF group. Based on results of CF tests we consider these strains as closely related to *R.*

siberica. Nevertheless, their exact taxonomic classification will be possible only after testing them in more exact reactions, like the CF test with washed antigens, cross-immunity tests, and the most important toxin neutralization test in mice (Bell and Pickens, 1953; Bell and Stoenner, 1960) and the CF test with mouse species specific sera (Pickens *et al.*, 1960). Because even our preliminary antigenic comparison suggested a significant difference of our strains from *R. conori* and since also some ecological data, especially as concerns the reservoir animals, point to a relationship to *R. siberica*, we are of the opinion that we have discovered the first focus of this rickettsiosis in Europe. According to Zdrodovsky and Golinevich (1966) this rickettsiosis does not occur in the European part of the U.S.S.R., the western border of its distribution area being the Ural.

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Explanation of Photomicrographs:

Fig. 1. Strain "B" rickettsiae in the yolk sac of chick embryo. Smear preparation stained according to Gimenez. $\times 1500$.

Fig. 2. Strain "B" rickettsiae in mouse spleen. Stained according to Gimenez. $\times 1500$.