CONTRIBUTION TO THE NATURAL FOCALITY OF
RICKETTSIAE BELONGING TO THE ROCKY MOUNTAIN
SPOTTED FEVER (RMSF) GROUP IN SLOVAKIA

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Abstract. The infestation of ticks, small mammals, bigger wild animals and men with rickettsiae of
the RMSF group was studied in Slovakia from 1968 to 1970.

The infestation with the rickettsiae of ticks as assayed by the haemocytometer test was highest
(45 %) in D. marginatus ticks collected on May 5, 1970 in the vicinity of the village S. of area No. 3.
Altogether, 36 strains of rickettsiae were recovered from ticks.

The rodents tested in various localities (in area No. 3) for antibodies against rickettsiae of the
RMSF group were positive in 1968 in 7 %, in 1969 in 30 % and in 1970 in 21 %. The total incidence
of antibody in small rodents tested in various areas covering almost the whole territory of Slovakia
was 36 %.

The sera of bigger wild animals tested for antibody against rickettsiae of the RMSF group in area
No. 3 in Slovakia in 1968 and 1970 were positive in 29 and 16 %; the human sera collected in this
area in 1969 and 1970 were positive in 3.4 and 12 %, respectively.

In 1968, a new rickettsiosis for Czechoslovakia was proved by recovery of two strains
from Dermacentor marginatus ticks collected in Central Slovakia (Brezina et al. 1969).

It is known that primary reservoirs of tick typhus in the U.S.S.R. are ticks and
small rodents (see review by Piontkovskaya and Korshunova 1960). Because
preliminary identification of the rickettsiae isolated in Slovakia showed their close
relationship to the causative agent of tick typhus, we assumed that also under our
conditions, small rodents and ticks would play a similar role. To confirm this assumption,
we have examined ticks and small rodents for rickettsial infestation in both the area
where the rickettsiae had originally been isolated from ticks (see map-area No. 3) and
in various other localities in Slovakia. Furthermore, we attempted to elucidate as to
whether also wild bigger animals are involved in the circulation of rickettsiae in
nature, and the importance of this rickettsiosis for man.

MATERIAL AND METHODS

Ticks were collected by skidding a white woollen blanket over pasture land bushes and their infestation
with rickettsiae was assayed by the haemocytometer test (Řeháček et al. 1971). A part of ticks,
found positive by this test, was used in isolation experiments.

Small mammals were captured into traps, exsanguinated from the orbital sinus, and the sera ob-
tained were tested for antibodies. The spleen and brain taken from serologically positive animals
were used in isolation experiments. The sera from bigger animals (game, etc.) were obtained by
cardiac puncture. Human sera were collected by courtesy of the staff of the Regional Hygiene Epidemiological Station in Banská Bystrica (Dr. Pálenová) and the District Hygiene Epidemiological Station in Komárno (Dr. Helbichová).

Isolation experiments from suspensions of ticks, and spleens and brains of small rodents were carried out in 7-day-old chick embryos (Brezina et al. 1969). Serological examinations were done by the warm method of the complement fixation (CF) test. To 0.2 ml of serum dilution were added 0.2 ml of antigen (2 units) and 0.4 ml of complement (2 units), and after incubation for 90 minutes at 37°C, 0.4 ml of haemolytic system (2 units of amboceptor and 1% sheep erythrocytes). Soluble antigens prepared from the Bitterroot strain of *Rickettsia rickettsii* and from strains D and B originally isolated in Central Slovakia were used. Sera fixing complement starting from a dilution of 1:8 or 1:10 were considered as positive.

**RESULTS**

Our investigations were carried out from 1968—1970. During this period, representative numbers of ticks and of sera from wild animals and men were collected mostly in the area under study, e.g. the area situated at the border of the districts Z., L. and V. K. (area No. 3), which before the discovery of the rickettsiosis of the RMSF group, had been considered to be only Q-fever foci.

Fig. 1. Map of Slovakia. Area 1 = Lowland localities along the southern branch of the Lesser Carpathian Mts.; area 2 = Stiavnica Mts.; area 3 = Krupina Hills; area 4 = South Slovak Karst; area 5 = Northern part of the Ondava Hills; S = Locality in the Zahorie Lowland; V. B. = The Danube Lowland; LU = Choč Mts.; P = Vihorlat; V. Ka. = Tisa Lowland.

1. Ticks. In 1968, a considerable number of ticks were tested for infection with rickettsiae of the RMSF group. They were collected mainly in the area mentioned above.

The chosen localities in the vicinity of the villages S. and V. L. (both in area 3) were examined at approximately one-month-intervals, with the aim to study the seasonal infestation of ticks with the rickettsiae. Both localities were selected because they offered the most suitable conditions for the persistence of the rickettsiosis due to high numbers of infected ticks and of host animals, and a convenient habitat. The ticks obtained from other areas while studying the distribution of Q-fever foci in Slovakia were examined occasionally for the presence of rickettsiae of the RMSF group. The infestation with rickettsiae of ticks, especially of *Dermacentor marginatus*, was high mainly in the selected areas, reaching up to 45% in ticks collected on May 21, 1970 in V. L. From the data presented in Table 1 it is obvious that, with the exception of ticks collected in May 1970, the tick infestation with rickettsiae was variable, being the highest in early spring and in autumn. The infestation with rickettsiae of *Ixodes ricinus* and *Haemaphysalis punctata* ticks was very low as compared with that of *Dermacentor marginatus*.

Similar results were also obtained in other localities in and outside the study area. In the latter case, especially *Dermacentor marginatus* ticks collected in D. (area No. 4) were highly infected with rickettsiae (Table 2). The finding of only one *D. marginatus* ♀ in Zc. (area No. 2) positive for rickettsiae and yielding a rickettsial strain, was interesting (Tables 2 and 3). In addition to ticks quoted in the tables, 30 ♀ *Haemaphysalis inermis* collected on October 20, 1970, in Pl. (area No. 2) and 138 ♀ and 42 ♂ of
### Table 1. The infestation of ticks, collected in the vicinity of the villages V. L. and S. (area No. 3) in 1968—1970 with rickettsiae belonging to the RMSF group

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date</th>
<th>D. marginatus</th>
<th>I. ricinus</th>
<th>H. punctata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>females (%)</td>
<td>males (%)</td>
<td>females (%)</td>
</tr>
<tr>
<td>S.</td>
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<td>202/39*</td>
<td>248/30</td>
<td>15</td>
</tr>
<tr>
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<td>April 2</td>
<td>240/36</td>
<td>190/40</td>
<td>18</td>
</tr>
<tr>
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<td>April 25</td>
<td>51/4</td>
<td>44/3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>May 28</td>
<td>9/0</td>
<td>0/0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>June 20</td>
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</tr>
<tr>
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<td>18/4</td>
<td>18/5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Sept. 24</td>
<td>8/2</td>
<td>8/2</td>
<td>25</td>
</tr>
<tr>
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<td>April 5, 69</td>
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<td>120/18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>May 21, 70</td>
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<td>3/1</td>
<td>16</td>
</tr>
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<td></td>
<td>June 16</td>
<td>6/1</td>
<td>1/0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>July 10</td>
<td></td>
<td>1/0</td>
<td>4/1</td>
</tr>
<tr>
<td></td>
<td>Sept. 17</td>
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</tr>
<tr>
<td></td>
<td>Oct. 8</td>
<td>12/5</td>
<td>7/4</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Nov. 18</td>
<td>4/1</td>
<td>2/1</td>
<td>1</td>
</tr>
<tr>
<td>V. L.</td>
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<td>35/15</td>
<td>43</td>
</tr>
<tr>
<td></td>
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<td>310/28</td>
<td>280/28</td>
<td>9</td>
</tr>
<tr>
<td></td>
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<td>160/30</td>
<td>150/14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>May 28</td>
<td>21/3</td>
<td>7/1</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>June 20</td>
<td>20/3</td>
<td>8/1</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>October 11</td>
<td>18/6</td>
<td>27/11</td>
<td>38</td>
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<tr>
<td></td>
<td>October 25</td>
<td>42/10</td>
<td>36/6</td>
<td>21</td>
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<td>260/73</td>
<td>19</td>
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<td>60/3</td>
<td>60/7</td>
<td>8</td>
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<td>3/1</td>
<td>45</td>
</tr>
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<td></td>
<td>July 10</td>
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<td></td>
</tr>
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<td>13/4</td>
<td>20/1</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Oct. 8</td>
<td>48/12</td>
<td>46/12</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Nov. 18</td>
<td>12/4</td>
<td>7/1</td>
<td>26</td>
</tr>
</tbody>
</table>

*) No. of ticks tested/No. of ticks positive in the haemocyte test.

*Deracantor pictus* collected on November 11, 1970, in P. B. (area No. 1) were tested for rickettsiae with negative results.

The first two strains of rickettsiae belonging to the RMSF group were recovered from *D. marginatus* ticks collected in the spring of 1968 (Brezina et al. 1969), and additional 5 strains were isolated from ticks of the same species collected in autumn of the same year (Table 3) in V. L. (area 3).

In 1968 we succeeded to isolate additional 13 strains of rickettsiae from *D. marginatus* ticks. Five strains were isolated from suspensions prepared from 1—3 ticks and the remaining strains from suspensions prepared from groups of 20 ticks each (Table 3).

In 1970, a representative number of ticks positive by the haemocyte test were each separately half-engorged for 4—5 days on white mice to establish the possibility of transmission of rickettsiae to host animals. These ticks were further investigated in isolation experiments. Thereafter the sera of the bitten mice were tested for the presence of antibodies against the rickettsiae mentioned above. Unfortunately most of these
mice died, probably due to toxic substances present in tick saliva. Out of the four surviving mice, two bitten by *D. marginatus* females infected with rickettsiae of the RMSF group (as revealed by the haemocyte test) developed specific antibodies. The remaining two mice, bitten by *Ixodes ricinus* females infected with the same rickettsiae failed to develop antibodies. All ticks found positive in the haemocyte test were also positive in isolation experiments (Table 3). A total of 16 rickettsial strains were isolated. However, only some ticks were used in the isolation experiments, because our first goal was the study of tick infestation with rickettsiae and the haemocyte test proved to be sufficient for this purpose.

One strain of rickettsiae was isolated from faeces of 9 ♀♂ *D. marginatus* (collected in S. (area No. 3), October 10, 1970) feeding on healthy guinea pigs for 6 days. From these faeces a suspension was prepared in saline and inoculated intraperitoneally into 8 g white mice. The injected animals manifested signs of a nonspecific illness during the subsequent days — they refused food, were humpbacked, had ruffled hair and died. Smears prepared from their spleen contained rickettsiae which were identified by immunofluorescence as belonging to the RMSF group.

All the strains isolated multiplied poorly in yolk sacs, killing the embryos on the 3rd-4th day after infection. Guinea pigs reacted to intraperitoneal infection with the

**Table 2.** The infestation of ticks, collected in various parts of Slovakia from 1968—1970, with rickettsiae belonging to the RMSF group

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date</th>
<th><em>D. marginatus</em></th>
<th><em>I. ricinus</em></th>
<th><em>H. punctata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>females</td>
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<td>Area No. 1</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.</td>
<td>April 4, 68</td>
<td>1/1</td>
<td>52/0</td>
<td>22/0</td>
</tr>
<tr>
<td>Z.</td>
<td>June 3, 68</td>
<td>66/5</td>
<td>58/8</td>
<td>10</td>
</tr>
<tr>
<td>Sept. 23, 70</td>
<td></td>
<td>213/20</td>
<td>141/17</td>
<td>10</td>
</tr>
<tr>
<td>Area No. 2</td>
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<td>1/1</td>
<td>52/0</td>
<td>22/0</td>
</tr>
<tr>
<td>Ps</td>
<td>May 18, 70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ze</td>
<td>May 21, 70</td>
<td>24/1</td>
<td>9/1</td>
<td>6</td>
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<td>Area No. 3</td>
<td></td>
<td>257/24*</td>
<td>232/10</td>
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</tr>
<tr>
<td>H. S.</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.</td>
<td>April 2, 68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N.</td>
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<td>Po.</td>
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<td>9/1</td>
<td>6</td>
</tr>
<tr>
<td>Pr.</td>
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<td>232/10</td>
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<tr>
<td>Area No. 4</td>
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<td></td>
</tr>
<tr>
<td>D.</td>
<td>April 3, 68</td>
<td>23/4</td>
<td>9/0</td>
<td>13</td>
</tr>
<tr>
<td>R.</td>
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<td>8/0</td>
<td>0</td>
</tr>
<tr>
<td>S.</td>
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<td>5/0</td>
<td>6/1</td>
<td>9</td>
</tr>
<tr>
<td>St.</td>
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<td></td>
<td></td>
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<tr>
<td>Pl.</td>
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<tr>
<td>Sv.</td>
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<td></td>
<td></td>
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<tr>
<td>K.</td>
<td>June 29, 68</td>
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*) No. tested/No. positive in the haemocyte test.
Table 3. List of strains of rickettsiae belonging to the RMFS group recovered from ticks collected in 1968—1970 in Slovakia

<table>
<thead>
<tr>
<th>Strain</th>
<th>Locality</th>
<th>Tick</th>
<th>Sex</th>
<th>Date of collection</th>
<th>No. of ticks in isolation experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>S.</td>
<td></td>
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<td>M.</td>
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<td></td>
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<td></td>
<td>♂♂</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Sept. 11, 68</td>
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</tr>
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<tr>
<td>95</td>
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<td>24</td>
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<td>18</td>
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<td>14</td>
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<td>April 5, 69</td>
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<td>51</td>
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<td>69</td>
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<td>D. marginatus</td>
<td>♀♀</td>
<td>April 2, 70</td>
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</tbody>
</table>

agent only exceptionally by a short and weak febrile response. The intensity of scrotal
reaction attained scarcely a value of ♂♂, although this phenomenon occurred also
in animals showing no febrile response.

According to the results obtained we consider ticks, especially Dermacentor marginata-
tus, to serve as a primary vector and probably also as a reservoir of rickettsiae belonging
to the RMSF group occurring in Slovakia. Ticks are not only a very convenient substrate
for the discovery of the rickettsiosis in nature but they offer also excellent material for
isolation of the agent.

2. Small mammals. Out of 159 small rodents examined in 1968 in localities of
area No. 3 of Central Slovakia investigated, 11, i.e., 7 %, contained antibodies against
rickettsiae of the RMSF group. The highest percentage of animals with antibodies was
found in the species Microtus arvalis — 17 %, Apodemus flavicollis and A. sylvaticus —
9 % and Clethrionomys glareolus — 7 %. The sera of Mus musculus, Rattus norvegicus
and Erinaceus roumanicus were negative (Table 4).
Table 4. Serological survey for rickettsiae of the RMSF group in sera of small mammals collected in area No. 3 in Central Slovakia in 1968

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date</th>
<th>A. flavicollis</th>
<th>Mus musculus</th>
<th>C. glareolus</th>
<th>M. arvalis</th>
<th>Rattus norvegicus</th>
<th>Erinaceus roumanicus</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>April 3</td>
<td>1/0*</td>
<td>2/0</td>
<td>4/0</td>
<td>5/0</td>
<td>3/0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.</td>
<td>Sept. 11</td>
<td>7/0</td>
<td>3/1</td>
<td>6/1</td>
<td>2/0</td>
<td>16/2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.</td>
<td>June 20</td>
<td></td>
<td>4/0</td>
<td>4/0</td>
<td>5/0</td>
<td>13/0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N.</td>
<td>June 20</td>
<td>5/1</td>
<td>1/0</td>
<td>2/0</td>
<td>7/1</td>
<td>14/0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr.</td>
<td>April 3</td>
<td>11/3</td>
<td>1/1</td>
<td>12/4</td>
<td>33/0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se.</td>
<td>April 3</td>
<td>11/0</td>
<td>5/0</td>
<td>16/0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.</td>
<td>April 3</td>
<td>6/0</td>
<td>10/0</td>
<td>2/0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. L.</td>
<td>June 29</td>
<td>3/0</td>
<td>10/0</td>
<td>23/0</td>
<td>159/11</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*) No. captured/No. positive.

To obtain the highest possible number of mammals occurring in this area, the following localities were selected for studies in further years: the valley of the small rivers M. and T., the vicinity of the villages S. and V. L. — all places offering the most convenient conditions for the occurrence of rodents. In 1969 and 1970, our studies were practically confined to these localities. The animals were captured especially in the spring and autumn.

Out of 217 rodents and insectivores captured in 1969 in this area, 66, i.e. 30%, had antibodies against rickettsiae of the RMSF group. The highest incidence was found in the species *Apodemus flavicollis* and *A. sylvaticus* — 34%, and in *Clethrionomys glareolus* — 17%. Sera of *Micromys minutus较* and *Erinaceus roumanicus* were negative, but only very few *M. arvalis* were tested.

Out of the 262 small mammals tested in 1970, 56, i.e. 21%, were found serologically positive. The highest percentage of positive animals occurred in the summer of 1970 in V. L. (55%) (Table 5). The most frequently infested species were *C. glareolus* — 26%, and again *A. flavicollis* and *A. sylvaticus* — 21%.

These results indicated that the infestation of small rodents with rickettsiae of the RMSF group in nature varied. The highest incidence of antibodies against these rickettsiae in the examined area was found in *A. flavicollis, A. sylvaticus* and *C. glareolus*. These species belong to the most common ones in this area and their habitats overlap with those of the ticks. All hedgehogs examined contained no antibodies against the above rickettsiae.

To obtain information as to whether this rickettsiosis occurs also in other areas of the country, small rodents in various parts of Slovakia were examined for antibodies against rickettsiae of the RMSF group (see map). All the examined species captured in localities covering almost the whole territory of Slovakia were serologically positive (Table 6). The highest percentage (48%) of infestation with rickettsiae of small rodents occurred in the vicinity of G. (area No. 4), which is also the habitat of *Dermacentor*
### Table 5. Serological survey for rickettsiae of the RMSF group in sera of small rodents collected in 1969 and 1970 in area No. 3 in Central Slovakia

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date</th>
<th>A. flaccilis</th>
<th>A. sylicaticus</th>
<th>C. glareolus</th>
<th>M. arvalis</th>
<th>E. roumanicus</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>juvenile</td>
<td>sub-adult</td>
<td>adult</td>
<td>Total</td>
<td>juvenile</td>
<td>sub-adult</td>
</tr>
<tr>
<td>S.</td>
<td>1969</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>March 18</td>
<td>84/36</td>
<td>45/12</td>
<td>15/13</td>
<td>24/11</td>
<td>3/1</td>
<td>3/1</td>
<td>1/0</td>
</tr>
<tr>
<td></td>
<td>April 16</td>
<td>103/22</td>
<td>13/10</td>
<td>25/2</td>
<td>62/16</td>
<td>9/1</td>
<td>2/0</td>
<td>1/0</td>
</tr>
<tr>
<td></td>
<td>April 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sept. 17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct. 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>187/64</td>
<td>58/22</td>
<td>43/15</td>
<td>86/27</td>
<td>12/2</td>
<td>2/0</td>
<td>1/0</td>
</tr>
<tr>
<td>S.</td>
<td>1970</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>July 7</td>
<td>86/21</td>
<td>14/0</td>
<td>45/10</td>
<td>27/11</td>
<td>9/2</td>
<td>8/1</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>July 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. L.</td>
<td>Nov. 20</td>
<td>17/5**</td>
<td>1/0</td>
<td>2/0</td>
<td>14/5</td>
<td>11/2</td>
<td>1/0</td>
<td>10/2</td>
</tr>
<tr>
<td></td>
<td>Nov. 9</td>
<td>133</td>
<td>1/0</td>
<td>1/0</td>
<td>11/0</td>
<td>11/0</td>
<td>1/0</td>
<td>1/0</td>
</tr>
<tr>
<td></td>
<td>Nov. 20</td>
<td>21/3</td>
<td>1/0</td>
<td>2/1</td>
<td>12/2</td>
<td>12/5</td>
<td>3/3</td>
<td>11/0</td>
</tr>
<tr>
<td>S.</td>
<td>June 16</td>
<td>60/4</td>
<td>4/1</td>
<td>33/1</td>
<td>23/2</td>
<td>6/1</td>
<td>1/0</td>
<td>1/0</td>
</tr>
<tr>
<td>L.</td>
<td>June 16</td>
<td>8/2</td>
<td>1/0</td>
<td>2/0</td>
<td>2/0</td>
<td>2/1</td>
<td>2/0</td>
<td>1/0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>256/43</td>
<td>21/1</td>
<td>94/17</td>
<td>91/25</td>
<td>46/12</td>
<td>1/0</td>
<td>17/6</td>
</tr>
</tbody>
</table>

†) No. captured/No. positive.  *) titres ranging from 1:20—40 in one case 1:80  **) titres ranging from 1:20—40

### Table 6. Serological survey for rickettsiae of the RMSF group in sera of small rodents collected in 1970 in various areas of Slovakia

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date</th>
<th>A. flaccilis</th>
<th>A. sylicaticus</th>
<th>A. agrarius</th>
<th>M. arvalis</th>
<th>C. glareolus</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>juvenile</td>
<td>sub-adult</td>
<td>adult</td>
<td>Total</td>
<td>juvenile</td>
<td>sub-adult</td>
</tr>
<tr>
<td>B.</td>
<td>Aug. 27</td>
<td>55/19*</td>
<td>6/1</td>
<td>25/7</td>
<td>24/11</td>
<td>1/0</td>
<td>1/0</td>
<td>4/4</td>
</tr>
<tr>
<td>G. (area</td>
<td>Oct. 24</td>
<td>24/6</td>
<td>3/1</td>
<td>13/3</td>
<td>8/2</td>
<td>5/4</td>
<td>1/0</td>
<td>4/4</td>
</tr>
<tr>
<td>No. 4)</td>
<td>Aug. 27</td>
<td>68/32</td>
<td>39/16</td>
<td>29/16</td>
<td>1/0</td>
<td>1/0</td>
<td>4/4</td>
<td>3/2</td>
</tr>
<tr>
<td>Lu.</td>
<td>Oct. 23</td>
<td>18/3</td>
<td>8/1</td>
<td>3/1</td>
<td>7/1</td>
<td>1/0</td>
<td>1/0</td>
<td>3/2</td>
</tr>
<tr>
<td>T.</td>
<td>Oct. 22</td>
<td>6/2</td>
<td>2/0</td>
<td>6/2</td>
<td>1/0</td>
<td>2/0</td>
<td>1/0</td>
<td>2/0</td>
</tr>
<tr>
<td>Sc.</td>
<td>Aug. 24</td>
<td>51/17</td>
<td>11/1</td>
<td>17/2</td>
<td>23/14</td>
<td>2/1</td>
<td>10/2</td>
<td>3/2</td>
</tr>
<tr>
<td>V. Ka.</td>
<td>Oct. 20</td>
<td>3/0</td>
<td>1/0</td>
<td>2/0</td>
<td>19/5</td>
<td>8/2</td>
<td>11/3</td>
<td>2/0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>225/79</td>
<td>28/4</td>
<td>98/29</td>
<td>99/46</td>
<td>10/3</td>
<td>21/5</td>
<td>15/0</td>
</tr>
</tbody>
</table>

*) No. tested/No. positive
marginatus. But also in the vicinity of S., where this tick species had never been found, we demonstrated serologically a high infestation (35 %) of rodents with rickettsiae of the RMSF group. In this and similar types of localities, such as for example in the vicinity of Lu. and B. B., the tick *Ixodes ricinus* can probably be taken into consideration as a vector of rickettsiae of the RMSF group. The total infestation with rickettsiae of the RMSF group of rodents captured in these localities as revealed by serological examination was 36 %; *A. flavicollis* and *A. sylvaticus* participated in 35 %, *A. agrarius* in 26 %, *Microtus arvalis* in 60 % and *C. glareolus* in 37 % (Table 6).

Table 7. Serological survey for rickettsiae of the RMSF group in bigger wild animal sera in area No. 3 of Central Slovakia in 1968 and 1970

<table>
<thead>
<tr>
<th>Species</th>
<th>1968</th>
<th>1970</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested/</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>No. positive</td>
<td></td>
</tr>
<tr>
<td>Cerfus elaphus</td>
<td>10/1</td>
<td>10</td>
</tr>
<tr>
<td>Capreolus capreolus</td>
<td>5/5</td>
<td>100</td>
</tr>
<tr>
<td>Sus scrofa</td>
<td>7/3</td>
<td>43</td>
</tr>
<tr>
<td>Lepus europaenus</td>
<td>93/29</td>
<td>31</td>
</tr>
<tr>
<td>Phasianus colchicus</td>
<td>16/0</td>
<td>0</td>
</tr>
<tr>
<td>Vulpes vulpes</td>
<td>3/0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>134/38</td>
<td>29</td>
</tr>
</tbody>
</table>

*) Titres of 1 : 16, 1 : 32, in two cases 1 : 64

The highest infestation among all the species tested in Slovakia occurred in adults, followed by subadults; but also juvenile individuals had antibodies against the rickettsiae of the RMSF group, which demonstrates that the rickettsiae circulate at present in all parts of Slovakia. The titres of antibodies were 1 : 20 or 1 : 40 and exceptionally 1 : 80.

Attempts to isolate the agent from the spleen and brain of serologically positive animals were unsuccessful despite the presence of the agent in the spleen of several animals revealed by Gimenez staining.

3. Bigger wild animals. Because area No. 3 under investigation in Central Slovakia is very rich for game, we attempted to find out whether also these animals are involved in the circulation of rickettsiae of the RMSF group in nature.

In 1968 and 1970 we collected 134 and 177 serum samples, respectively. The highest number of sera positive with rickettsiae of the RMSF group were found in *Capreolus capreolus* (5 out of 5 animals tested), *Sus scrofa* (43 and 36 %) and *Lepus europaenus* (31 and 18 %). The sera of pheasants and foxes tested were all negative (Table 7). The sera of 16 hares hunted in the Small Carpathians in the vicinity of M. were also negative.

The incidence of rickettsiae of the RMSF group in game, involving red deer, roe deer, wild swine, hare, fox and pheasant in the area of Central Slovakia investigated amounted to 28 and 16 per cent in 1969 and 1 : 70, respectively. These figures varied according to localities, which were not identical in both years, but the animals originated from one territory of about 900 km².
4. Man. Our attention has also been focused to the question of whether the rickettsiae discovered in Slovakia are pathogenic for man. So far, no cases of disease resembling that caused by rickettsiae of the RMSF group have been reported by local physicians. But, as mentioned previously (Brezina et al. 1969), the strains isolated manifest a very low virulence. This fact could influence the clinical course of an infection so that the patients do not develop a clinically distinct disease and, therefore, do not consult a physician.

During studies on Q-fever in the village V. in 1968, several human sera were tested also for antibodies against rickettsiae of the RMSF group. Two out of 26 employees of a cooperative farm and 6 out of 7 professional wool-shearers working temporarily at the same farm were serologically positive against the above rickettsiae. This fact demonstrated a certain connection of the infection in man and a contact with domestic animals and their ectoparasites.

In 1969, 501 human sera were collected from various localities in the districts L. and V. K. (area No. 3). Seventeen sera, i.e. 3.4 %, contained antibodies in titres from 1 : 10—1 : 40. The CF test was performed with corpuscular and soluble antigen of strain B. The specificity of results was confirmed by negative results of tests with corpuscular and soluble antigen prepared from other rickettsiae (R. prowazeki, R. mooseri and C. burneti). All positive persons had come into contact with herds of grazing domestic animals or had worked in forests. Two persons reported having had exanthema in 1967.

In 1970, 258 human sera were obtained from the districts Z. and L. (area No. 3) and 12 % of them reacted in the CF test with the antigen of the strain B of rickettsiae of the RMSF group.

These results proved that, in the areas investigated, man had come into contact with rickettsiae of the RMSF group. An analysis of the reaction of man to infection with this agent is required.

DISCUSSION

According to data available on other members of the RMSF group of rickettsiae, into which also the rickettsiae isolated in Slovakia are included, it may be assumed that ticks and small rodents serve probably as primary reservoirs of this rickettsiosis in Slovakia as they do with Rickettsia rickettsii in the U.S.A. (Lundgreen and Thorpe 1966, Hoogstraal 1967, Burgdorfer 1970), R. sibirica in the U.S.S.R. (Korshunova 1967) and R. conorii var. pjiyperi in Africa (Gear 1954, Heisach et al. 1962).

Therefore, we studied the ecology of rickettsiae of the RMSF group occurring in Slovakia. So far, 36 strains of rickettsiae were isolated from ticks collected in Central Slovakia. Further material of ticks found positive for rickettsiae by the haemocyte test has been stored at -70 °C and will soon be used in isolation experiments. Findings of rickettsiae in ticks collected in early spring — February and March — confirm our suggestion that the rickettsiae hibernate in ticks. The transmission of these rickettsiae by tick bite has been proved also in our experiments. All these data indicate, therefore, that ticks serve as vector and reservoir of the rickettsiae under consideration. However, it may be anticipated that also other ectoparasites could be involved in the circulation of these rickettsiae in nature. The high infestation of small rodents with rickettsiae, as evidenced by the presence of antibody, in North Slovakia, where the number of ticks is low as compared with that in other parts of Slovakia, supports this hypothesis. R. sibirica was recovered in the U.S.S.R. from mites and fleas, and, therefore, we think that also the rickettsiae occurring in Slovakia may have a wider spectrum of vectors.
The finding of antibodies against rickettsiae of the RMSF group in sera of small rodents and bigger wild animals supports our assumption that small rodents and bigger animals may serve as reservoirs of these rickettsiae.

To characterize the ecology of rickettsiae of the RMSF group in the territory of Slovakia in more detail, additional data on the role of domestic animals, birds and reptiles in the circulation of these rickettsiae in nature are required. Of these three groups of animals, grazing domestic animals and also several species of birds and reptiles, as for example blackbirds and lizards, are heavily infested with ticks and could, therefore, be actively involved in the circulation of the rickettsiae in nature. However, the presence of antibodies in sera of animals does not solve definitely the question of their role as reservoirs of infection; such conclusions must be based on experimental data concerning susceptibility of the animals to the agent, rickettsiaeemia, persistence of the agent in the host organism, etc. In any case, these animals serving as hosts of ticks are thus involved in the circulation of the rickettsiae in nature.

As yet we have no explanation of the fact that we failed to isolate the agent from serologically positive animals although rickettsiae were found in smears prepared from their spleens. On the other hand, the isolation of rickettsiae from ticks was easy, including cases in which the number of rickettsiae in ticks, as estimated by the haemocyte test, was very low.

The infection of man with these rickettsiae is still obscure. The infection could be transmitted either by bite of *Ixodes ricinus* ticks (*Dermacentor marginatus* ticks attack men only exceptionally) or contact with ticks, domestic and wild living animals and their excrements. However, many problems are not yet clear as, for example, the elimination of agent by animals, the susceptibility of man to the agent, etc.

**CONCLUSIONS**

The infestation of ticks, small mammals, bigger wild animals and men with rickettsiae of the RMSF group was studied in Slovakia in 1968—1970.

The infestation with these rickettsiae of ticks, especially of *Dermacentor marginatus*, was very high, mainly in localities of the area No. 3 in Central Slovakia. It was the highest (45 %) in the vicinity of the village S. in ticks collected on May 5, 1970. *Ixodes ricinus* and *Haemaphysalis punctata* ticks were infested at a lower rate.

Altogether, 36 strains of rickettsiae were recovered from ticks, 33 from *Dermacentor marginatus* and 3 from *Ixodes ricinus*. One strain of rickettsiae was isolated from faeces of *D. marginatus* females while these were feeding on healthy guinea pigs. The infestation of ticks with rickettsiae was studied during three years at monthly intervals in the vicinity of S. and V. L. (area No. 3), it was highest in early spring and autumn.

Eleven out of 159 small rodents tested in 1968 in various localities in area No. 3 for antibodies against rickettsiae of the RMSF group, i.e., 7 %, were positive. The positive species included *Microtus arvalis* (17 %), *Apodemus flavicollis* and *A. sylvaticus* (9 %), and *Clethrionomys glareolus* (7 %). The sera of *Mus musculus*, *Rattus norvegicus* and *Erinaceus roumanicus* were negative. In 1969, 66 out of 217 small mammals examined, i.e., 30 %, had antibodies against rickettsiae of the RMSF group (*A. flavicollis* and *A. sylvaticus* — 34 % and *C. glareolus* — 17 %). In 1970, 56 out of 262 sera from small mammals, i.e., 21 %, were positive in the CF test for antibodies against the rickettsiae under consideration. The highest incidence of antibodies was found in *C. glareolus* (26 %) and *A. flavicollis* and *A. sylvaticus* (21 %).

Antibodies against rickettsiae of the RMSF group were found in the sera of all small rodent species captured in various areas covering almost the whole territory of Slo-
vacia. The total incidence of antibody in small rodents tested was 36%; in Apodemus flavicollis and A. sylvaticus it was 35, in A. agrarius 26, in Microtus arvalis 60 and in C. glareolus 37%. Attempts to isolate the agent from the spleen and brain of serologically positive animals were unsuccessful.

Out of 134 and 177 sera from bigger wild animals tested for antibody against rickettsiae of the RMSF group in area No. 3 in Slovakia in 1968 and 1970, 38 and 28 (i.e. 29 and 16%) respectively, were positive. This number included 5 out of 5 Capreolus capreolus, 43 and 36% (in 1968 and 1970) of Sus scrofa and 31 and 18% (in 1968 and 1970) of Lepus europaeus. The sera of three foxes and 53 pheasants were serologically negative.

Serological examination of men in 1968 in the village V. (see map) showed that two out of 26 employees of a cooperative farm and 6 out of 7 professional wool shearers had antibodies against rickettsiae of the RMSF group. In 1969, 501 human sera were collected in the districts L. and V. K. (area No. 3) and 17, i.e. 3.4%, were positive. In 1970, 258 human sera were obtained from the districts Z. and L. (area No. 3) and 12% had antibodies against rickettsiae of the RMSF group.

Based on the results obtained we consider ticks, especially Dermacentor marginatus, to be primary vectors, and ticks, small rodents and bigger wild animals to be reservoirs of rickettsiae of the RMSF group occurring in Slovakia, but the latter assumption needs further experimental confirmation. The relation of the rickettsiae under consideration to man, despite the finding of specific antibodies in human sera, requires further investigations.

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Zagadnienia akarologii — Problems of Acarology (Documents from the conference organized by the Plant Protection Committee of the Polish Academy of Sciences) Zeszyty problemowy postępów nauk rolniczych 109, 1970. Price zl. 54,—.

Polish acarology has been lately developing on a very remarkable scale; especially the works devoted to the agricultural acarology attract considerable attention even abroad. The recently published volume has proved it again. Here, too, the basic part is due to the school of Prof. J. Bocek, Agricultural College in Warsaw. Although the first part of the volume is devoted exclusively to phytophagous mites and to their predators, the second half of the book includes works on mites of stored products, i.e. the species living close to man and arousing the interest of parasitologists from many aspects. First of all, there is an important finding of W. Chmielewski who ascertained, that up to 7 per cent of mites Carpoglyphus lactis L. and Thyropsphaerus entomophagus Lab. could survive a passage through the digestive tract of some vertebrates. Other papers deal with the susceptibility of mites to the frequency of high voltage field and heat convection (Golebiowska, Biedroń), to X-rays (Chmielewski, Czaplicki, Głogowski), as well as to various chemical substances (Bednarek, Kuzitowicz, Lewandowski). J. Jakubowska’s paper on the importance of preliminary grain purification is worth attention, too, as well as that of B. Czajkowska, concerning the applicability of fungi as food for mites and the changes in the developmental cycle which are caused by fungi as the only kind of food. All papers are published in Polish with short abstracts in Russian and English.

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