Susceptibility of some species of rodents to rickettsiae

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Abstract. The present study was designed to test the susceptibility of free-living rodents, viz. Apodemus flavicollis, Microtus arvalis, Clethrionomys glareolus, Mus musculus, and outbred white mice from Dobrá Voda farm, CSFR, to Coxiella burnetii, rickettsiae of the spotted fever group (Rickettsia sibirica, R. conori, R. slovaca and R. akari) and rickettsiae of typhus group (R. typhi and R. prowazekii) by various routes of administration. The highest levels of antibodies to C. burnetii were found in A. flavicolli and M. arvalis inoculated intraperitoneally and intracerebrally. Antibodies to C. burnetii exerted peak levels between days 13 and 16 in contrast to white mice which showed maximum levels on day 28. When 10^6.5 and 10^6.05 EID₅₀/0.25 ml of C. burnetii was administered intraperitoneally to A. flavicollis, M. arvalis and white mice, the agent was detected only in organs of wild animals. In addition to spleen, the bone marrow appeared as a predilective tissue for the detection of this agent. R. akari at a dose of 10^4.5 EID₅₀/0.25 ml caused overt illness and death in rodents. Antibody levels to R. sibirica and R. conori were dependent on dosage, route of inoculation and duration of infection, but were not dependent on animal species. Antibodies to R. slovaca and R. akari were dependent on dosage, infection duration and animal species but were not dependent on the route of infection. For R. conori, R. sibirica and R. slovaca a sharp increase of antibody levels with high titres on days 4–6 and peak levels about day 11 post intraperitoneal infection was characteristic. Antibody level to R. akari increased up to day 21. Spotted fever group rickettsiae in rodents inoculated intraperitoneally were observed in various organs, particularly in tunica vaginalis and spleen at days 2–8 post infection. R. typhi at a dose of 10^4.3 EID₅₀/0.25 ml inoculated intracerebrally or intraperitoneally killed white mice and inoculated intraperitoneally killed C. glareolus and M. musculus. The antibody response of white mice to intraperitoneal, subcutaneous or intranasal inoculation of this rickettsia was low and no antibody was detected following peroral administration. M. musculus did not develop antibodies after intracerebral, intranasal, subcutaneous or peroral inoculation of R. typhi. The target organs for this rickettsia were the spleen and tunica vaginalis. R. prowazekii inoculated intraperitoneally into white mice at a dose of 10^6.2 EID₅₀/0.25 ml and at a dose of 10^4.5 EID₅₀ into C. glareolus was fatal for these rodents. Following intraperitoneal inoculation of 10^2.3 and 10^4.2 EID₅₀, this agent was not detected in both rodent species and the difference in antibody response between them was not significant in the infection duration but was evident with various doses of inoculated rickettsiae. Titres of rickettsiae measured by antibody response of intraperitoneally inoculated rodents and by the presence of agents in yolk sacs of chicken embryos showed 1 log unit higher values in rodents inoculated with C. burnetii and about 1–3 log units higher levels in rodents inoculated with R. sibirica and R. conori. The titres of R. slovaca were almost the same in both substrates while R. akari reached the highest titres in white mice, lower in wild rodents and the lowest in yolk sacs. No significant difference was found in the titration of R. typhi and R. prowazekii between small rodents and yolk sacs of chicken embryos with the exception of M. musculus in which the titres of R. typhi were much lower. We suggest that A. flavicollis, C. glareolus and M. arvalis could be used for the isolation of rickettsiae from diverse field samples as well as for laboratory investigations, e.g. to acquire sera with high levels of antibodies to rickettsiae. However, white mouse, outbred line of Dobrá Voda, is suggested as the animal of choice for work with tested members of typhus group of rickettsiae.
In general, small rodents are considered as important hosts and in many cases also reservoirs of rickettsiae in their natural foci. High susceptibility has been found for various species of small mammals living in Australia and in the U.S.A. to Coxella burnetii (Derrick et al. 1939, 1940, Sidwell and Gebhardt 1962, 1963) and for some species of rodents living in Europe to Rickettsia typhi and R. prowazekii (Řeháček et al. 1985). The probable role of some European species of rodents in the circulation of C. burnetii and Rickettsia slovaca in their natural foci, based upon rickettsemia, agent presence and antibody response post subcutaneous infection has been demonstrated by Řeháček et al. (1976a, b). The present study was designed to compare the susceptibility of most common European species of free-living rodents to some species of rickettsiae occurring in Europe. The study also aimed to determine the eventual replacing of laboratory bred white mice with wild rodents in some special experiments with these agents.

MATERIALS AND METHODS

Four species of free-living rodents, i.e. Apodemus flavicollis (Melch), Clethrionomys glareolus Schreber, Microtus arvalis (Pall.), Mus musculus L. and to provide a comparison to laboratory animals the outbred strain of the white mouse from Dobrá Voda farm, CSFR, were included in the experiments. Wild rodents were captured in localities near Bratislava. Some experiments also employed M. arvalis reared in our laboratory. Wild rodents were pretested for rickettsial antibodies after 4 weeks keeping at quarantine; only seronegative specimens were included in the experiments.


Rodents were inoculated with rickettsiae either by the intracerebral (ic), intraperitoneal (ip), subcutaneous (sc), intranasal (in) or peroral (po) routes. The presence of rickettsiae in mammals was detected directly using smears of organs stained by the Gimenez method and indirectly by antibody response using the complement fixation. The latter method employed corpuscular antigen of C. burnetii and soluble antigens of the rickettsiae prepared from the same strains which were experimentally inoculated into rodents. The antigens were prepared by centrifugation and ether purification of agents cultivated in yolk sacs of chick embryos (Ormsbee 1962). This “classic” serological method in comparison to modern methods, i.e. indirect immunofluorescence and ELISA, was shown with regard to rodent sera as the most specific (our unpublished data). Sera of animals were prepared from blood taken by puncture of sinus orbitalis.

Experiments with one species of rickettsiae always employed 5–12 adult specimens of each rodent species. Lethality, morbidity, presence of the agent in the organs (evaluated visually by crosses: + – single, ++ – tenths, +++ – hundreds and +++++ – thousands particles seen in a microscope field) and of antibody in blood, were chosen as criteria indicating infection with rickettsiae. The results were evaluated using one and multiway analyses of variance, Student–Newman–Keuls test and the Fisher–Snedecor test.

RESULTS

Antibody response of rodents 21 days after the infection with rickettsiae administered by various routes C. burnetii

No animal tested died or developed overt signs of illness following inoculation
with $10^7 \text{EID}_{50}$ (egg infection dose) /0.25 ml of rickettsiae. Highly significant differences in antibody response among species of hosts ($F = 8.16$, $P < 0.0001$) were established (Fig. 1). In general, the lowest response was found in white mice and in increasing order of antibody levels were *A. flavicollis*, *M. musculus*, *C. glareolus* and *M. arvalis*. The highest levels of antibody (1:8192) were detected in *A. flavicollis* after ic and in *M. arvalis* and *M. musculus* after ip administration of *C. burnetii*.

![Graph showing antibody response](image)

**Fig. 1.** Antibody response of four species of rodents after exposure to *Coxiella burnetii* by various routes: IC – intracerebral, IP – intraperitoneal, SC – subcutaneous, IN – intranasal, PO – peroral administration.

**Rickettsiae of the spotted fever group**

Following sc administration of $10^3 \text{EID}_{50}$/0.25 ml of *R. sibirica*, *A. flavicollis*, *C. glareolus* and white mouse showed a low level antibody response: *A. flavicollis*, 1.808; *C. glareolus* and white mouse, 1.866 log EID$_{50}$/0.25 ml. None of the animals exhibited overt signs of illness. Also no clinical signs of the infection were apparent in rodents injected with $10^{3.5} \text{EID}_{50}$/0.25 ml of *R. conorii*. Multiway analyses revealed that antibody levels to this rickettsia were dependent on the route of administration ($F = 7.03$, $P < 0.01$) but were not dependent on host species ($F = 1.05\text{ns}$). The highest antibody levels were seen in animals inoculated by ip or in routes (Fig. 2A).

*R. slovaca* administered at dose of $10^{3.5} \text{EID}_{50}$/0.25 ml did not cause death or overt illness in small mammals. As determined by multiway analysis, the antibody level was independent on the route of the administration of the agent ($F = 3.46\text{ns}$) but was affected by animal species ($F = 15.55$, $P = 0.02$) which contrasted with the data obtained for *R. conorii*. The lowest levels of antibodies were detected in the white mouse (average log titre 1.2) comparated to *A. flavicollis* and *C. glareolus*.
Fig. 2. Antibodies in rodents 21 days after inoculation with rickettsiae (dose $10^{2.3}$ EID$_{50}$/0.25 ml) by various routes: A – Rickettsia conorii; B – R. slovaca; C – R. akari.
where the antibodies were significantly higher (average log titre 2.4) (Fig. 2B). *R. akari* was lethal when administered ip at a dose of $10^4$ EID$_{50}$/0.25 ml to white mice, and in or po at a dose of $10^{2.5}$ EID$_{50}$ to *M. musculus*, contrasting with other examined species of rickettsiae. By two way analysis the differences in antibody levels were shown to be independent on the route of administration (F = 0.357ns) but dependent on the animal species (F = 50.88, P = 0.002), as with *R. slovaca* (Fig. 2C).

**Rickettsiae of the typhus group**

The dose of $10^{4.3}$ EID$_{50}$/0.25 ml of *R. typhi* caused death of white mice after ic and ip administration and of *C. glareolus* and *M. musculus* after ip inoculation. *M. musculus* did not develop antibody following ic, sc, in or po inoculation of this rickettsia. White mice reacted to sc and in administration of *R. typhi* by developing low antibody levels but po administration of rickettsiae did not result in antibody response. The remaining species of wild animals examined, i.e. *A. flavicollis*, *M. arvalis* and *C. glareolus* developed relatively high levels of antibodies, primarily after ip and sc inoculation of these rickettsiae (Table 1).

**Table 1. Antibody response of small rodents following inoculation with *R. typhi* or *R. prowazekii***

<table>
<thead>
<tr>
<th>Rickettsia</th>
<th>Dose</th>
<th>Route of inoculation</th>
<th>White mouse</th>
<th>A. flavicollis</th>
<th>M. arvalis</th>
<th>C. glareolus</th>
<th>M. musculus</th>
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</thead>
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<tr>
<td><em>R. typhi</em></td>
<td>$4.3^+$</td>
<td>ic</td>
<td>death</td>
<td>$45^{+}$</td>
<td>223</td>
<td>223</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ip</td>
<td>death</td>
<td>119</td>
<td>294</td>
<td>death</td>
<td>death</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sc</td>
<td>3</td>
<td>69</td>
<td>128</td>
<td>137</td>
<td>death</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in</td>
<td>4</td>
<td>32</td>
<td>–</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>po</td>
<td>0</td>
<td>56</td>
<td>169</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td><em>R. prowazekii</em></td>
<td>$3.3^+$</td>
<td>sc</td>
<td>60</td>
<td>274</td>
<td>169</td>
<td>69</td>
<td>–</td>
</tr>
</tbody>
</table>

$^+$ log EID$_{50}$/0.25 ml  
$^{++}$ titre expressed by antibody response (geometric mean from 10–12 specimens)  
– not tested

Higher levels of antibodies were demonstrated in *A. flavicollis* and *M. arvalis* than in *C. glareolus* and white mice following sc inoculation of $10^{3.3}$ EID$_{50}$/0.25 ml of *R. prowazekii* (Table 1). None of the inoculated animals died.

**Detection of rickettsiae and antibody responses in small rodents during 20–28 days following ip inoculation – *C. burnetii***

First of all the susceptibility of white mice and *A. flavicollis* indicated by antibody response and by the presence of the agent in organs of rodents in 2–5 days
intervals during 28 days following ip inoculation with $10^{0.5}$, $10^{2.5}$ and $10^{4.5}$ EID$_{50}$/0.25 ml of C. burnetii was investigated (Figs. 3A–C). Significant differences in antibody response between these species were observed (F = 9.85, P = 0.002). With an infection dose of $10^{4.5}$ or $10^{2.5}$ EID$_{50}$ the highest levels of antibodies in white mice were detected on day 28 (at which time the experiment was terminated); in A. flavicollis the same dose of agent resulted in peak levels of antibodies on day 13–16 after the infection. C. burnetii antibodies were detected in both animal species inoculated ip with C. burnetii at a dose of $10^{4.5}$ on day 4, a dose of $10^{2.5}$ on day 6 and a dosage of $10^{0.5}$ EID$_{50}$ on day 8. After inoculation with $10^{2.5}$ or $10^{0.5}$ EID$_{50}$ the antibody level in sera of A. flavicollis was significantly higher than that in white mice (F > $10^6$, P < 0.0001) (Figs. 3A–B). C. burnetii antibodies were also detected in white mice inoculated ip with $10^{0.05}$ EID$_{50}$ (A. flavicollis not tested) in mean antibody levels 2, 15 and 24 on day 16, 21 and 28 post infection.

Two way analysis indicated that the difference in the presence of C. burnetii in organs between white mice and A. flavicollis was significant (F = 11.85, P = 0.001)

![Graphs A, B, C](image)

Fig. 3. Antibody response of white mouse and Apodemus flavicollis to Coxiella burnetii inoculated in three different doses: A – $10^{0.5}$; B – $10^{2.5}$; C – $10^{4.5}$ EID$_{50}$/0.25 ml.
Figs. 4–6. Inclusion of Coxiella burnetii in different organs of white mouse 7 days after intraperitoneal infection. Fig. 4. Bone marrow; Fig. 5. Kidney; Fig. 6. Spleen.
Fig. 7. Detection of *Coxiella burnetii* in organs of *Apodemus flavicollis* and white mouse during 28 days after intraperitoneal inoculation with the agent.
(Figs. 4–7). The numbers of infectious particles in relation to the time period changed markedly ($F = 17.68, P < 0.001$), increasing during the first 13 days and decreasing afterwards, depending on animal species ($F = 2.69, P = 0.007$). C. burnetii was clearly visible on day 2 and 4 post inoculation and the highest numbers were seen in the cells of spleen and bone marrow. After inoculation of $10^{0.5}$ EID$_{50}$/0.25 ml dose, C. burnetii was detected only in the organs of A. flavicollis from day 6 to day 8 post infection, mostly in the spleen. Together with C. burnetii particles seen in single organs of tested animals, the formation of inclusions was observed which were most expressive in bone marrow and in spleen of both rodent species inoculated ip with the dose of $10^{4.5}$ and $10^{2.5}$ EID$_{50}$ and with dosage of $10^{0.5}$ in organs only of A. flavicollis (Figs. 4–7). The development of C. burnetii inclusions in the organs of infected rodents was very demonstrative and characteristic for this rickettsial species.

In a separate experiment comparing the susceptibility of white mice with M. arvalis following ip administration of $10^{0.5}$ EID$_{50}$, no antibodies were found in sera of either species until day 10 post infection. However, the agent was detected between day 4 and 10 in organs of only M. arvalis (Fig. 8). There was a significant difference in detection of the agent in M. arvalis among organs ($F = 12.46, P < 0.0001$) as well as the time periods after inoculation of C. burnetii ($F = 10.36, P < 0.001$). The prevalence of the agent was greatest in the cells of the spleen and bone marrow.

![Graph showing the amount of rickettsiae in different organs over time](image)

Fig. 8. Detection of Coxiella burnetii in organs of Microtus arvalis during 10 days after intraperitoneal infection.

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Spotted fever group rickettsiae

Several animals died on day 3–7 following ip administration of $10^4 \text{EID}_{50}/0.25 \text{ml}$ of *R. sibirica*. Rickettsiae were seen in dead animals most often in the *tunica vaginalis* and spleen and least in the liver and kidney. Comparing the antibody response to $10^{4.0}$, $10^{2.0}$, and $10^{0.5} \text{EID}_{50}/0.25 \text{ml}$ of *R. sibirica* in white mice and *M. musculus* the results showed that the differences were highly dependent on the dose inoculated ($F = 12.37, P < 0.0001$) and were not dependent on animal species ($F = 0.19ns$). The duration of infection in this case was insignificant ($F = 0.80ns$) (Figs. 9A–B). The differences in antibody response of other animal species, as evaluated by multiway analysis, were highly significant with regard to animal species ($F = 8.51, P < 10^{-4}$) and to the infection length ($F = 14.73, P 10^{-4}$). *R. sibirica* provoked the highest levels of antibodies (1:256) in *C. glareolus* and *A. flavicollis* between days 8–16 with a maximum on the 11th day after infection (Fig. 9C).

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

**Fig. 9.** Antibody response of rodents to intraperitoneal infection with *Rickettsia sibirica*. A – white mouse (doses $10^{0.5}$, $10^{2.0}$ and $10^{4.0} \text{EID}_{50}/0.25 \text{ml}$); B – *Mus musculus* (doses the same as in A); C – four rodents (white mouse, *M. musculus*, Clethrionomys glareolus, Apodemus flavicollis) (dose $10^{4.0} \text{EID}_{50}/0.25 \text{ml}$).
The death or overt signs of illness in mammals following the inoculation of *R. conorii* in dose of $10^3$ EID$_{50}$/0.25 ml were not demonstrated. Rickettsiae were seen in white mice and *M. arvalis* on day 2, 4 and 6 in lymphatic nodes, on day 4 and 6 in the *tunica vaginalis*, and between days 6–13 in the spleen. The most demonstrative finding of rickettsiae was in the *tunica vaginalis* on day 6 post infection. By one and multiway analyses the differences in the development of antibodies among species examined were not significant (*F* = 1.90ns). However, highly significant differences were related to the time following the inoculation (*F* = 15.72, *P* < $10^{-4}$). The peak levels of antibodies were on day 11, then the antibody levels decreased slightly and were maintained until the termination of the experiment, i.e. for 20 days (Figs. 10A, B).

**Fig. 10.** Antibody response of rodents to intraperitoneal infection with *Rickettsia conorii*. A – white mouse, *Clethrionomys glareolus*, *Apodemus flavicollis* (dose $10^3$ EID$_{50}$/0.25 ml); B – white mouse and *Microtus arvalis* (doses $10^4$ and $10^5$ EID$_{50}$/0.25 ml).

Occasional death after ip administration of $10^{4.5}$ EID$_{50}$/0.25 ml of *R. sylvatica* were observed in white mice, *M. arvalis* and *C. glareolus* on day 6–8 after the infection. After ip inoculation with a dose of $10^5$ EID$_{50}$ of rickettsiae, the agent was seldom seen on day 2 in lymphatic nodes, on day 2 and 4 in the *tunica vaginalis* (the quantity scored ++ ) but on days 6 and 8 it was seen in *tunica vaginalis* only rarely as well as in spleen on day 6. The highest numbers of rickettsiae appeared in the *tunica vaginalis* of *A. flavicollis* injected ip with a dose of $10^{2.5}$–$10^{4.5}$ EID$_{50}$ on day 6 after the inoculation (the amount scored ++ to +++). The development of antibodies was influenced significantly by animal species (*F* = 8.53, *P* = 0.049), dosage (*F* = 4.68, *P* = 0.003) and the time following the inoculation (*F* = 3.20, *P* = 0.013). The investigations of antibody response in small rodents to ip administration of various doses of rickettsiae during a 20 day period showed significantly
higher titres in free-living mammals than in white mice (Fig. 11A, B). Three different strains of *R. slovaca* were included in the present experiments and resulted in a markedly different antibody response of white mice (Fig. 11C). It is remarkable that relatively high levels of antibodies were established in free-living animals as well as in white mice on days 4–6 after the infection.

![Graphs A, B, and C showing antibody response of rodents to intraperitoneal infection with *Rickettsia slovaca*.](image)

Fig. 11. Antibody response of rodents to intraperitoneal infection with *Rickettsia slovaca*. A – strain B (dose $10^{1.5} \text{ EID}_{50}/0.25 \text{ ml}$); B – strain B (dose $10^{3.5} \text{ EID}_{50}/0.25 \text{ ml}$); C – strain Pravica 85 (dose $10^{4.0} \text{ EID}_{50}/0.25 \text{ ml}$).

Compared to other tested spotted fever group rickettsiae, the examined rodents appeared as the most susceptible for *R. akari*. Overt signs of illness and death developed in white mice on day 5 after ip inoculation of $10^{4.5} \text{ EID}_{50}/0.25 \text{ ml}$ of *R. akari*. The same amount of this rickettsia caused the death of *A. flavicollis* between days 6–10. Rickettsiae were found in organs of free-living rodents and white mice between days 4–10 after ip infection with $10^{4.5} \text{ EID}_{50}$; in *tunica vaginalis* and spleen in amounts scored + to +++ and in liver, lung, kidney and heart in the amount scored +. Evaluation by multiway analysis showed a highly significant
effect of dosage \((F = 16.36, P < 10^{-4})\) and the infection length \((F = 37.67, P < 10^{-4})\) on antibody level in white mice (Fig. 12A). In comparing two doses, i.e. \(10^{2.5}\) and \(10^{4.5}\) EID\(_{50}\) of rickettsiae administered ip to \(M.\ musculus\) and \(A.\ flavicollis\), a significant difference between the animal species \((F = 4.38\text{ns}, P = 0.019)\) and an insignificant effect of the dosage \((F = 1.89\text{ns})\) was evident. All examined animals reacted similarly to the same doses of the agent (Figs. 12B, C). Antibody levels were very high in white mice as well as in other rodent species examined. The highest values were found in \(A.\ flavicollis\) and \(C.\ glareolus\); levels higher than 1:1024 were detected on day 6 following ip infection with a dose of \(10^{4.5}\) EID\(_{50}\). The early antibody response of rodents to \(R.\ akari\) is remarkable; e.g. by infection with \(10^{2.5}\) EID\(_{50}\) the antibodies were detected as early as 48 hours after the infection. Comparing the development and maintenance of antibody to \(R.\ akari\) with those of other spotted fever group rickettsiae, the principal difference is quite evident. The curve of antibody level exhibits a gradual increase (regardless to the dose of \(R.\ akari\) inoculated) until day 21 at which time the experiment was not yet terminated, while other rickettsial species examined reached their peak of antibody level at about day 11 after the infection.

![Graphs A, B, C](image-url)

**Fig. 12.** Antibody response of rodents to intraperitoneal infection with two doses \((10^{2.5} \text{ and } 10^{4.5} \text{ EID}_{50}/0.25 \text{ ml})\) of *Rickettsia akari*. A – white mouse; B – *Apodemus flavicollis*; C – *Mus musculus*. 277
Typhus group rickettsiae

The susceptibility of white mice and *M. musculus* to *R. typhi* was compared. Rickettsiae were seen in dead and surviving animals of both species inoculated with $10^{4.3} \text{ EID}_{50}/0.25 \text{ ml}$. Forty eight hours after inoculation, the level of infection in sexual glands and spleen scored +++, on day 3 in liver, lungs and peritoneal cells scored + to +++, on day 4–7 isolated rickettsiae occurred in spleen and sexual glands. Following inoculation with $10^{3.5}$ of *R. typhi*, the rickettsial agent was observed 48 hours later only in the *tunica vaginalis* and in the spleen. The rickettsiae were not detected when lower doses were used. Antibody levels for the 21 day period following inoculation were relatively low, ranging mostly between 1:16–32. A value of 1:128–256 was detected 13–21 days post infection. The length of infection is statistically significant ($F = 6.51, P < 0.01$) for antibody development, that is also dependent on the inoculated dose of the agent ($F = 6.91, P = 0.039$) and on the animal species ($F = 20.27, P = 0.0004$) (Fig. 13). The duration of infection was not the same in examined rodents even if the difference among them were only on borderline significance ($F = 3.83, P = 0.063$).

![Graph A](image)

**Fig. 13.** Antibody response of white mouse and *Mus musculus* to intraperitoneal inoculation with two doses of rickettsiae. A – *Rickettsia typhi*; B – *R. prowazekii*.

The death of white mice was observed after ip inoculation of *R. prowazekii* at a dose of $10^{6.5} \text{ EID}_{50}$ and of *C. glareolus* after inoculation of $10^{4.5} \text{ EID}_{50}/0.25 \text{ ml}$. The presence of the agent and development of antibodies was followed after ip inoculation of $10^{4.2}$ and $10^{2.3} \text{ EID}_{50}/0.25 \text{ ml}$ of rickettsiae in white mice and in *M. musculus*. Although rickettsiae were not detected, the animals developed an antibody with titres of 1:8–32. The difference between the two hosts was statisti-
cally insignificant (F = 1.14ns) and infection length (F = 1.38ns) had no influence on the antibody level. However, the antibody level was dependent on dose (F = 11.65, P = 0.011), which contrasts with the results for *R. typhi* (Fig. 14).

![Graph showing antibody levels in different species](image)

**Fig. 14.** Antibodies in five species of rodents 21 days after intraperitoneal inoculation of *Rickettsia typhi*.

**Titration of rickettsiae in rodents by antibody response C. burnetii**

In three experiments *C. burnetii* titres established by antibody response in small mammals on day 21 post ip infection were 1–4 log units higher than in yolk sacs of chick embryos. The values in white mice and free-living rodents did not reveal any significant difference (Table 2).

**Spotted fever group rickettsiae**

A comparison of the titres of these rickettsiae in yolk sacs of chicken embryos and in small rodents demonstrated highly significant differences among rickettsial species (F = 20.10, P < 10^{-4}) and among host species (F = 6.99, P = 0.004) (Table 2). Titres of *R. sibirica* in rodents were about 1–2.5 dilutions higher than those in yolk sacs of chick embryos. Titres of rickettsiae in white mice did not significantly differ from those in wild rodents (F = 0.185ns). *R. conorii* does not differ much from *R. sibirica*: its titres determined by antibody response of small rodents were mostly about 1–3 dilutions higher than in yolk sacs of chick embryos. Titres of *R. slovaca* in small rodents and in yolk sacs of chick embryos were
Table 2. Comparison of the titration of rickettsiae in small rodents and yolk sacs of chick embryos

<table>
<thead>
<tr>
<th>Rickettsia</th>
<th>Exp. No.</th>
<th>Yolk sac of chick embryo</th>
<th>White mouse</th>
<th>A. flavicollis</th>
<th>M. arvalis</th>
<th>C. glareolus</th>
<th>M. musculus</th>
</tr>
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<tbody>
<tr>
<td>Coxiella burnetii</td>
<td>1</td>
<td>5.5</td>
<td>6.5</td>
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Yolk sac of chick embryos – log EID_{50}/0.25 ml
Rodents – log ID_{50}/0.25 ml (expressed by antibody presence)
– not tested

especially the same, in free-living animals about one dilution higher. In antibody levels established after the inoculation of particular dilutions of rickettsiae, the difference of susceptibility among white mice, A. flavicollis and C. glareolus were always highly significant (F = 8.54, P = 0.0049). The antibody levels varied in white mice between 1:16–32 while the levels of 1:128 were frequent in free-living animals. Titres of R. akari in white mice and A. flavicollis were significantly higher than those noted in yolk sacs of chick embryos. In contrast to other members of spotted fever group rickettsiae examined in present experiments, the highest titres of this rickettsia were observed in white mice. On day 21 after ip administration of 10^{1–7} EID_{50}/0.25 ml of rickettsiae surviving animals of all species tested demon-
strated high antibody levels ranging from 1:128 to 1:256 or even higher in most dilutions inoculated; however, in the first three dilutions the values obtained surpassed 1:4096.

**Typhus group rickettsiae**

Titres of *R. typhi* in white mice were slightly higher than those obtained in free-living rodents except *M. musculus* in which they were about 2.5 log units lower (Table 2). Statistical analysis showed that *A. flavicollis, C. glareolus*, white mice and yolk sacs of chick embryos did not differ significantly in titres (*F* = 2.24*ns*). After the inoculation of single dilutions of rickettsial suspensions, the antibody levels in white mice ranged mainly from 1:32 to 1:128 in individual dilutions contrary to *A. flavicollis, C. glareolus* and *M. arvalis* in which titres 1:64–1:256 in concentrated suspensions were prevalent. Mean values of antibody levels are presented in Fig. 14. Based upon analysis of variance, the differences among animal species and doses were highly significant (*F* = 6.41, *P* = 0.0002) and (*F* = 17.55, *P* < 10<sup>-5</sup>), respectively.

Titration of *R. prowazekii* in rodents and yolk sacs of chick embryos revealed about the same results in both substrates or slightly lower values in rodents examined except *M. musculus* in which titres obtained were approximately 2–3 log units lower than in yolk sacs (Table 2). No significant differences appeared between yolk sacs of chick embryos and small rodents, white mice, *A. flavicollis* and *C. glareolus* while *M. musculus* exhibited irregularities in the development of antibodies, e.g. many mice did not respond by antibody formation to an inoculated dose of 10<sup>4.5</sup> EID<sub>50</sub> of rickettsiae.

**DISCUSSION**

In the present study we did not always have the required number of animal species and necessary individual specimens. The number of animals for an experiment varied with seasons. *A. flavicollis* was always abundant whereas other species, especially *M. arvalis*, demonstrated quantitative differences in capture success.

Factors exerting an influence on the course of a rickettsiosis as well as of other infections in mice and other animals are based in the first line upon a genetical base of a host (Anderson and Osterman 1980a, b, Nacy and Meltzer 1982, Eisenman et al. 1984. O’Brien et al. 1986). Genetic factors determine a cell factor, i.e. macrophages which first come into contact during the infection with rickettsiae (Chyong-din-Keyt 1977, Kokorin et al. 1978). We can state that the resistance of mice to rickettsiae is determined by a rickettsiocidal capacity of mouse macrophages (Kokorin et al. 1978). Other important variables include rickettsial species, its strain, the route of infection and the amount of agent administered. The importance of the rickettsial strain used was proved experimentally, for instance with *R. conorii* in mice, strain C3H/HeJ which following ip infection with
R. conorii, strain Malish die, while they survive infection with the strains Casablanca and Moroccan (Eiseman et al. 1984).

Experimental studies on the pathogenesis in mammals in addition to lethality, illness, presence of agents in host organism, antibody response, skin lesions and erythema often include splenomegaly as one of the criteria of infection. We also observed this phenomenon in our experimental animals, however, because animals trapped in nature and kept in the laboratory before experimental use sometimes became ill or died exhibiting enlarged spleens (this was also many times seen when healthy rodents trapped in nature were screened for antibodies against rickettsiae) this was not evaluated. The present study also did not evaluate pathological changes in cells and tissues of rodents infected with rickettsiae. These have been reported to include for instance in C. burnetii hyperplasia and necrotic lesions in the liver (Kokorin et al. 1985) and an interstitial pneumonia in animals infected by the respiratory route (Hall et al. 1981).

It is evident that both the agent and antibody would be detectable in experimental animals beyond the 3–4 weeks observation period as the acute illness usually transforms to a latent infection: for example, C. burnetii was detected in the spleen of white mouse at 190 days post infection (Tokarevich 1979).

In contrast to our findings, laboratory animals, i.e. white mice and guinea pigs were shown to be more susceptible to ip inoculation of C. burnetii than certain species of wild rodents (Sidwell and Gebhardt 1963). Because different strains of white mice vary in susceptibility to C. burnetii, from resistance to high sensitivity as demonstrated in inbred white mice (Scott et al. 1978, 1987), our results comparing susceptibility of free-living rodents with that of white mice should be related only to the outbred strain used. This may also explain the discrepancy between the results of Sidwell and Gebhardt (1962, 1963) and our observations. The question of possible variability in susceptibility of animals to C. burnetii also applies to rodents living in nature even though the animals employed in our experiments were captured in an area of about 1 km². The results of laboratory investigations on antibody levels and presence of the agent in animals did not exhibit any distinct variability compared with outbred white mice with the exception of M. musculus.

Our recent experiments demonstrated a relatively low sensitivity of rodents to some members of spotted fever group rickettsiae as indicated by antibody response on the 21st day after the administration by different routes with R. sibirica and R. conorii, a higher one with R. slovaca and the highest one with R. akari. There was found a highly significant difference in antibody level following different routes of administration of R. slovaca in white mice and free-living rodents what could be used as a helpful biological marker in the differentiation of this rickettsia from R. conorii and R. sibirica. In spotted fever group rickettsiae, the course of the development of antibodies following ip administration of the agent may serve as another marker in case of infections with R. conorii, R. sibirica and R. slovaca where the antibodies reach their peak levels about the 11th day after the infection, as
contrasted to *R. akari* where antibodies increase gradually until day 21 at which
time the experiment was not yet terminated.

In experiments comparing the titration of rickettsiae in small mammals and yolk
sacs of chick embryos, *R. akari* significantly differed from the other rickettsiae
examined. White mice, outbred strain of Dobrá Voda farm, which was employed
in our experiments, could be considered as the experimental animal of choice not
only from the reason of the formation of the highest levels of antibodies following
administration of low amount of rickettsiae but also because the rickettsia is lethal
for this mouse strain.

In comparing the advantages and disadvantages of using white mice or free-living
rodents in various studies with above rickettsiae or yolk sacs of chick embryos,
it is evident that the use of mammals is necessary in some cases. Peak levels of
antibodies, e.g. 1:8192 obtained by ip or ic administration of *C. burnetii* in
*A. flavicollis*, *M. arvalis* and also white mice, Dobrá Voda strain, shows that these
animals can be used for the preparation of sera with high antibody levels. In
addition, the use of small free-living rodents seems to be highly effective for the
detection of small numbers of rickettsiae from nature. However, it is evident that
one could raise objections against this statement as wild caught animals may
frequently harbour infections and that it might be rather desirable to screen all
readily available laboratory strains of laboratory reared animals and to select the
most sensitive one for the work with rickettsiae. This sounds well but the infestation
of rodents with undesirable infections including rickettsiae in European conditions
is low and then is possible to eliminate it during quarantine and by antibody
screening. The advantages of the use of wild rodents for the work with rickettsiae
would include their high sensitivity and also economical reasons since they are
simply provided in the field (namely in autumn) and easily reared in the laboratory.

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