ON THE PROBLEM OF THE FOCAL CHARACTER OF
SPRING-AND-SUMMER (TICK) ENCEPHALITIS

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1. INTRODUCTION

Spring-and-summer (tick) encephalitis, first detected in the Far
Eastern Province, is an infection which is considerably more widespread than
was at first supposed. The Cis-Ural and Volga areas, Vologda, Leningrad and
Velikiye Luki Regions /oblasts/ are likewise districts where during the
last five years cases of encephalitis have been detected, and the virus has
been extracted both from ixodid ticks and from rodent wild-life (SOLOV'YEV,
1944; CHUMAKOV, MIRONOV, PETROVA, ORLOVA and SHLUGER, 1944).

Naturally the question has come up of the wide distribution of the
natural foci of this infection and of the necessity of studying the causes
which determine its focal character. The tendency of some of these foci
to be concentrated toward areas where ixodid ticks are present in large
numbers (POCHANKOV, 1935), the extraction of the virus from ticks in cases
where the outbreaks have been studied, and finally the indubitable connection
between tick-infestation and the incidence of the disease in humans; all
these facts have confirmed the idea of the extremely important role of these
arthropods in the propagation of encephalitis among mankind. In some
quarters, the close connection has given rise to a belief that the natural-
focus character of spring-and-summer encephalitis is completely a function
of the presence of infected ixodid ticks. Thus GRASHCHENKOV in his address
(1943) declared flatly that "the endemic extent of encephalitis is connected
with the extent of infection in ticks".

No doubt this statement of the case is correct for understanding
the laws of the spreading of encephalitis in humans, but it is hardly
adequate for a more profound analysis of the whole problem of the focality
of this infection. One must suppose that, as in the case of certain other
zooses, natural foci of encephalitis may be discovered in areas where the
disease is not observed in humans.

This phenomenon of endozoticity without endemicity is to the
highest degree characteristic of a whole list of other infections which are
spread among mankind by ticks and rodents (bubonic plague, tularemia, the leptospiroses, the rickettsioses, tick-borne relapsing fever, cutaneous leishmaniasis); that is, even when there exist channels ensuring continued circulation of the infection among wild creatures (Leptospiroses, Rickettsia prowazekii) the channel which would divert it into the human organism may be lacking. The best illustration of this thesis is the discovery of the rickettsiosis of Rocky Mountain Fever in the ticks infesting hares and rabbits (Haemaphysalis leporispalustris Say.) in many parts of North America where, thanks to the absence of the characteristic vector Dermacentor andersoni Stiles (Parker, 1935, 1938), the disease has not been observed in humans.

This lends support to the idea that foci of encephalitis may be more widespread in our country than was supposed a few years ago, and we are very definitely confronted with the task of studying the locality of encephalitis. Here it is evident that the solution of the problem must be undertaken by methods somewhat different from those hitherto used. First of all, it is essential that the survey of a focus be regular and unceasing; we must not be satisfied with a short-term expeditionary inspection, as has usually been the case. Indeed it is important to maintain the surveillance of a focus for a number of years, so as to make due allowance for the existence of fluctuations in the numbers of the virus hosts (wild-life and ixodid ticks). No less is it important not to limit ourselves to the survey of any one group of ectoparasites playing an important part in the transmission of the infection to man (the ixodid ticks), but to pay attention to other arthropods capable of carrying the infection from one wild creature to another, thus promoting the circulation of the virus within the focus.

Thus it is particularly important to investigate the gamasid mites, which obviously play a considerable part in transmitting both tularemia and the rickettsial diseases from rodent to rodent (SIMMONS, 1941).

Likewise chiggers (Trombiculidae), which transmit acute infections like tsutsugamushi fever to humans, may also play a part in spreading virus infections among the wild-life of a focus. Finally we might also follow up the matter of whether one link of the chain of propagation might not be the fleas, which plays such an important role in the epidemiology and epizootology of bubonic plague, tularemia and the rickettsial diseases (IOFF, 1944; SIMMONS, 1941; OLSUF'EV, 1940).

In carrying out a detailed survey of this kind at any one focus, we should also at the same time investigate the whole of whatever area is known to have an encephalitis tendency, both in order to establish whether it may not constitute a potential focus of infection, and also to chart the movement of the rodent population and its ectoparasites, within the focus and outside it.

2. PLACE AND METHOD OF SURVEY

A focus was investigated in the Nefidovo District (Nefidovskii Raion) of Velikiye Luki Region (Belast). Here, in the triangle formed by
Faniklia Siding and the villages of Niva and Gora, cases of encephalitis
were observed in the summer of 1942 among military units stationed in the
forest. It was at this spot that I.M.OLIGER in the summer of 1942 collected
ixodid ticks, from which encephalitis virus was extracted in M.P.CHIRKOV's
laboratory. No cases of encephalitis had been observed among the civilian
population before the war, nor were any observed in 1942, 1943 and 1944.

As a control area we selected the vicinity of Avdotino Village of
Semenov Rural Soviet, Nikheev District, Moscow Region. Our choice of this
place was based on the fact that the existence of a hospital here for the
past 40-odd years, and of a Tularemia Station since 1938, would have shown
up any cases of encephalitis during recent years if any had occurred.

The collecting and counting of small mammals and
their ectoparasites in the focus-area was carried out
by N.I.KALABUKHOV in the Nikheev District by him and
Assistant A.N.NAIDENOV.

For capturing the animals we used the "Hero"
mouse-trap and other non-killing traps. The count in
each survey was handled by setting usually 40 to 50
traps and keeping the reckoning for a four to five
day period, so as to have not less than 200 trap-
hours. The traps were patrolled (and bait changed)
twice a day, A.M. and P.M. The animals caught were
put in bags to ensure all ectoparasites being col-
lected, then, at the Niva Village travelling labora-
tory and at the Tularemia Station in the town of
Semenovskoye, the animals were looked over and a count
made of ectoparasites found, which we either collected
alive (ixodid ticks, gamasids and fleas) or preserved
in 50% glycerine (chiggers.)

Here too we dissected the animals, excising the
brain and preserving it in 80% glycerine. From then on
the brains were kept in insulated containers on ice.
Upon arrival of the specimens at Moscow, the parasites
were identified. The species-classification of the
ticks was undertaken by A.B.LANGE, a graduate worker
in the Faculty of Entomology of Moscow State University,
under the direction of Professor A.A.ZAKHVTAKIN. The
fleas were identified by Doctor I.O.IOFF and V.A.
TIFLOV (Stavropol' Anti-bubonic Station).

Then the collected specimens were examined for
virus content according to the usual technique
(SOLOV'YEV, 1944) under the direction of A.K.SHUBLADZE
at the Virus Division of the Headquarters of the
Institute of Experimental Medicine.

Between December 1942 and January 1943, eleven surveys were carried
out, six of them in the focus-area and five in the control area.

* One case, clinically diagnosed as encephalitis at the Nifiedo hospital at the beginning of
June 1942, was not given a virological or epidemiological examination, since we were not
on the spot at the time.
A) In the focus-area: 19th to 30th January; 20 to 29th March; 20th to 30th May; 18th to 27th July; 21st to 29th September; 23rd to 31st January, 1945.

B) Outside the focus: 14th to 18th December, 1943; 21st to 26th February; 6th to 12th July; 6th to 12th September; 17th to 22nd November, 1944.

Thus the survey covered the whole annual cycle of the life of these mammals and their ectoparasites. However, we were unable, for a number of reasons, to carry out the survey in the control area at the beginning of spring, the period when the ixodid ticks come out of hibernation, and at the focus we could not carry out the survey at the beginning of winter. Curves of the species-composition and population-figures for the mammals and the ectoparasites, in the focus-area and in the control area, are given below.

3. SPECIES-COMPOSITION AND POPULATION, SMALL MAMMALS

Our findings on the species-composition of the small mammalian fauna, in the forest at the focus and in the control area, are presented in tables 1, 2, and 3 and in figure 1.

Figure 1 gives the census for the forest near Avdotino Village, shown in percent of the number of trap-hours. In Table 2 we give figures for the catch in the focus-area; that is, in a portion of the forest in the shape of a triangle formed by the villages of Gora and Niva and the road running to Paniklia Siding on the railroad.

Data on the relative numbers of the basic types of animals are given in figure 1 (as percentages of the number of trap-hours).

Assuming that the yellow-necked mouse Apodemus flavicollis Lelch. must exist in the forest, a species which is a virus-vector in the Volga Country (SOLOVYEV, 1944), and knowing that in winter this species of rodent usually moves into settlements, we simultaneously undertook to trap the animals in the houses of Niva Village too.

The figures for this census, which are of indubitable interest, are given in table 3.

From figure 1 it will be seen that the cycles of rodent population in the two areas coincide.

Obviously, the steady growth of the rodent population towards autumn is of real epizootological significance; it was from specimens obtained in September that we extracted two strains of the virus (vide infra).
### Table 1. Census of small mammals in the forest near Avdotino Village, Semenov Rural Soviet, Mikhnev District, Moscow Region, 1943-44.

<table>
<thead>
<tr>
<th>Survey data</th>
<th>11th to 18th Jan 1943</th>
<th>21st to 26th Feb.</th>
<th>6th to 1st July</th>
<th>6th to 12th July</th>
<th>17th to 6th Sept.</th>
<th>22nd to 12th Oct.</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of trap-hours</td>
<td>216</td>
<td>208</td>
<td>284</td>
<td>237</td>
<td>213</td>
<td>1158</td>
<td></td>
</tr>
<tr>
<td>Number of animals caught</td>
<td>24</td>
<td>21</td>
<td>42</td>
<td>94</td>
<td>24</td>
<td>205</td>
<td></td>
</tr>
</tbody>
</table>

**Breakdown:**

- **Shrew** *(Sorex araneus)*
  - 3
- **Red vole** *(Evotomys glareolus)*
  - 11
  - 13
  - 25
  - 59
  - 12
  - 122
- **Dark vole** *(Microtus agrestis)*
  - 1
  - 1
  - 6
  - 2
  - 10
- **Common vole** *(Microtus arvalis)*
  - 1
  - 3
  - 2
  - 6
- **Wood-mouse** *(Apodemus sylvaticus)*
  - 9
  - 6
  - 7
  - 26
  - 8
  - 56
- **Yellow-neck mouse** *(Apodemus flavicollis)*
  - 1

*In addition, ectoparasite and brains were collected from 161 common voles *(Microtus arvalis)* caught in hay-ricks and on the fields near Avdotino Village, Dec., 1943, 85 specimens; Feb., 1944, 61 specimens; Nov., 1944, 12 specimens.*
Table 2. Small rodent population in forest in focal area, Žhiglički Kural Soviet, Nelidovo District, Velikiye Luki Region

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of trap-hours</td>
<td>324</td>
<td>282</td>
<td>130</td>
<td>263</td>
<td>203</td>
<td>228</td>
<td>1730</td>
</tr>
<tr>
<td>Number of animals caught</td>
<td>17</td>
<td>24</td>
<td>21</td>
<td>13</td>
<td>19</td>
<td>13</td>
<td>193</td>
</tr>
<tr>
<td>Breakdown:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common shrew (Sorex araneus L.)</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>14</td>
<td>5</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td>Middle-sized shrew (S. macropygmaeus Pall.)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Red vole (Evotomys glareolus Schr.)</td>
<td>10</td>
<td>16</td>
<td>19</td>
<td>24</td>
<td>61</td>
<td>8</td>
<td>138</td>
</tr>
<tr>
<td>Dark vole (Microtus agrestis Pall.)</td>
<td>3</td>
<td>4</td>
<td>-</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Common vole (Microtus arvalis Pall.)</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>5*</td>
</tr>
<tr>
<td>Field-mouse (Apodemus agrarius Pall.)</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Yellow-neck mouse (Apodemus flavicollis Welch.)</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>

* Also investigated were 15 specimens of Microtus arvalis Pall. caught in the fields near Niva Village (July 14th, 1941 and Sept. 11, 1941) and one hare (Lepus europaeus Pall.) shot at the same place on Jan. 22nd, 1941.
Table 3. Catch of rodents in buildings of Niva Village (Nelidovo District, Velikiye Luki Region)

<table>
<thead>
<tr>
<th>Period of Census</th>
<th>Jan.-Mar 1945</th>
<th>May-July-Sept, 1945</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of trap-hours</td>
<td>120</td>
<td>83</td>
<td>203</td>
</tr>
<tr>
<td>Total of animals caught</td>
<td>16</td>
<td>13</td>
<td>29</td>
</tr>
</tbody>
</table>

Breakdown:
- Domestic mouse (Mus musculus L.)
  - Jan., 1945: 5
  - May-July-Sept, 1945: 9
  - Totals: 14
- Yellow-neck mouse (A. flavicollis Melch.)
  - Jan., 1945: 6
  - May-July-Sept, 1945: 0
  - Totals: 6
- Grey rat (Rattus norvegicus Berkenh.)
  - Jan., 1945: 3
  - May-July-Sept, 1945: 0
  - Totals: 3
- Black rat (Rattus rattus rattus L. and R. r. alexandrinus Geoffr.)
  - Jan., 1945: 2
  - May-July-Sept, 1945: 4
  - Totals: 6

Fig. 1. Seasonal population-curves of basic species of small mammals.

Legend: 1 - All species. 2 - Evotomys. 3 - Apodemus sylvaticus. 4 - Sorex.

Above: encephalitis-free district.
Below: focus-area.
4. SPECIES-COMPOSITION AND POPULATION, ECTOPARASITES

The results of our survey of mice and small animals at different seasons of the year and our findings re the extent of their infestation with ectoparasites are given in Table 1 and Figure 2. If we now turn to the details of the species-composition of the parasites of the small mammals in the areas surveyed, we find first of all the ixodid tick. Ixodidae collected from rodents are almost exclusively in the larval or nymph stage (POJERANCEV, 1935; OLSUF'YEV, 1940). This makes it in practice almost impossible to determine their species. However, from isolated specimens of males and females found on rodents, cattle and plants, it was possible to establish that four species of tick are found in the focus-area.

1) *Ixodes ricinus* L. Found on vegetation, on cattle and on humans.  
2) *Ixodes persulcatus* Sch.  
3) *Ixodes trianguliceps* Birul. (One female on Svtoryx glareolus, May 29, 1944)  
4) *Dermacentor* sp. (imago not found).

In Mikhnev District, where the ixodid tick population was generally speaking extremely small, we found:—

1) *Ixodes ricinus* L.  
2) *Dermacentor pictus* Herm.

For the solution of the problem with which we are concerned, a very interesting point is the discovery of three specimens of *Ixodes* coexisting in the focus-area: the widespread *Ixodes ricinus* L., then *Ixodes persulcatus* Sch., known to be a carrier of encephalitis (SOLOV'YEV, 1944; CHUKAKOV, MIRONOV, ORLOVA, PETROVA and SULUGER, 1944), and finally *Ixodes trianguliceps* Birul., infesting rodents and likewise carrying the encephalitis virus (SOLOV'YEV, 1944). The last-named species was discovered in earlier collections from Mikhnev District too, made by N.G. OLSUF'YEV (1940), although we did not find it in our surveys.

There was a very regular infestation of the rodents with chiggers, the * Trombiculidae.* Particularly numerous (as many as 50 to 100 individuals per head) on the red voles (Figure 2), these chiggers were found only as isolated individuals on Microtus agrestis and only occasionally on other species of forest mammals. We also note that the degree of tick-infestation of mammals of all species was higher in the focus-area than in the "free area (Figure 2).

The degree of infestation of the animals with fleas was for us an exceedingly important piece of information, because it was from these ectoparasites of the focus that one of the strains of the virus was extracted, in September 1944. The virus was discovered in fleas collected from red voles (as determined by Ye.A. SULUGER).
**Table 4. Ectoparasites collected from small mammals**

<table>
<thead>
<tr>
<th>Ectoparasites</th>
<th>At focus</th>
<th>In control area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jan-March</td>
<td>May-Sept</td>
</tr>
<tr>
<td><strong>Tick</strong> (Ixodidae)</td>
<td>1 (larva)</td>
<td>132 (including 5 imagos)</td>
</tr>
<tr>
<td>Gamasoidea</td>
<td>155</td>
<td>170</td>
</tr>
<tr>
<td>Chiggers (Trombiculidae)</td>
<td>579</td>
<td>1974</td>
</tr>
<tr>
<td>Fleas (Aphaniptera)</td>
<td>70</td>
<td>82</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Degree of infestation with ticks, voles *Evotomys glareolus* Schr.

**Legend:** Vertically, mean number of ectoparasites per vole; 1 - fleas, 2 - gasamids, 3 - chiggers, 4 - ixodidae

Above - encephalitis-free area.
Below - focus-area.
voles (count on 16 individuals). We should mention that on the rodents in the focus-area the following eight species of fleas were found:

1) *Ctenophthalmus aytites.*
2) *Ctenophthalmus uncinatus.*
3) *Ceratophyllus penicilliger.*
4) *Ceratophyllus turbidus.*
5) *Ctenopsyllus segnis.*
6) *Ctenopsyllus bidentatus.*
7) *Rhadinopsylla intellega.*
8) *Hystrichopsylla talpae.*

In the control area, we found the three first-mentioned and the two last-mentioned species of flea, also isolated specimens of *Ceratophyllus sciurorum, Amphipsylla rossica, Ctenopsyllus sylvaticus, Ctenophthalmus assimilis* and *Doratopsylla dasycnemus.*

5. RESULTS OF VIRUSOLOGICAL TESTS

427 brains of different rodents were tested, in batches of three or four, and 4,028 specimens of the ectoparasites, in batches of 4 to 20. The test-material from each batch was passaged intracerebrally through white mice not less than three times. In one case, a strain of virus was extracted on the third passage from the brain of a wood-mouse (*Apodemus sylvaticus*) caught outside the encephalitis focus. The other strain of the virus was extracted, likewise on the third passage, from fleas collected from voles (*Eomys glareolus*) caught inside the focus. Both the strains thus obtained were passaged ten times through white mice, with 100% positive results. This intracerebral infection induced in the mice symptoms of injury to the nervous system of varying degrees of severity, in the form of tremors and convulsions. In the animals infected with the virus extracted from the wood-mice, various paralyses of the hind limbs were the predominant symptom. On the other hand the virus extracted from fleas did not as a rule cause paralysis in mice on the first passages. Assuming different typespecific immune sera, we made serological identification tests with other neuroviruses to identify the strains we had obtained. For tests on the neutralization of the wood-mouse and flea viruses, we used the following sera: horse and rabbit sera hyperimmune to the virus of spring-and-summer encephalitis; serum from a person suffering from a chronic form of diffuse sclerosis; dog serum hyperimmune to the virus of silver fox encephalitis; rabbit serum hyperimmune to the virus of oquine encephalomyelitis, and sheep serum immune to the virus of "loping ill". In all cases the virus extracted from the wood-mice was neutralized only with sera specific for the virus of spring-and-summer encephalitis.
C S, (-2, t -1) 

The virus extracted from fleas was only feebly neutralized by serum from rabbits immunized with the virus of equine encephalomyelitis and by serum from sheep immunized to lopping ill. Legend in Table 57: 

- Complete neutralization of the virus.

- Weak neutralization of the virus.

- No neutralization.

Summing up our experiments on the serological identification of the viruses under study, it is in order to suggest that the virus extracted from wood-mice is a strain of spring-and-summer encephalitis virus; the nature of the second virus, extracted from fleas, remains uncertain that study thereof will be continued in further, more broadly based researches.

CONCLUSIONS

The data we have obtained are of real significance for the study of the problem of the focality of tick encephalitis. The first of the facts which are of inescapable interest to us is the detection of the tick encephalitis virus in the brains of wood-mice from the control area, where
cases of encephalitis had not been observed. This fact once more demonstrates that the reason for the "freedom" of such an area is the absence of contact of the local population with the source of the infection (perhaps because of the absence here of that tick-species which is prone to attack man, namely *Ixodes persulcatus* or the existence of a natural immunity in the local population (SOLOV'YEV, 1944).

The extraction of the virus from wood-mice, animals which SOLOV'YEV (1944) did not include in his list of spontaneous virus-vectors, is nevertheless not so unexpected, since in the reports of the work of the Academy of Sciences' Kazakhstan Branch there was recently published a paper by GALUZO (1943) in which he mentions that he was successful in extracting the virus of Alma-Ata encephalitis from the brain of this species. It seems to us that these findings do not really contradict SOLOV'YEV's conclusion (1944) that wood-mice are not susceptible to encephalitis virus; on the contrary these findings impress upon us that a low susceptibility of some species to this infection in no way prevents the maintenance of the infection in the focus.

Of great interest is the discovery of a virus (to be sure, one which has not yet been identified with the virus of tick encephalitis) in fleas from the red vole *Evotomys glareolus* Schr. caught in the focal area. This finding, this enlargement of the circle of possible vectors of the virus, is not only of interest for the solution of a number of problems in the epidemiology of this infection; it also points out the possible propagation-route of the disease in wild creatures which are not infested with ixodid ticks, for instance the propagation of silver fox encephalitis in animal-breeding nurseries or of marmot encephalitis in the high mountain regions of Kazakhstan (GALUZO, 1943). The discovery of *Ixodes trianguliceps* Birula on rodents inside and outside the focus also compels us to agree with the thesis that the propagation of encephalitis among the smaller animals is perhaps closely connected with the presence of this species of tick, as suggested by SOLOV'YEV (1944).

The survey sets us a large number of other interesting problems, the answers to which it will be possible to give only after the researches here begun have been continued on a more thorough-going and persistent basis.
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* Name here given as ORLOV, but it appears twice in text as ORLOVA. (Tr.)