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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, OFROVA 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 (POHOMOV, 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 zoonoses, natural foci of encephalitis may be discovered in areas where the disease is not observed in humans.

This phenomenon of enzooticity without endemicty 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 (especially among rodents) the 
channel which would divert it into the human organism may be lacking. The best 
illustration of this thesis is the discovery of the rickettsia 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; OLSUFYEV, 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 
[the whole of] 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 Nelidovo District (Nelidovski 
Raion) of Velikye 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 near the forest. It was at this spot that I.M. OLOGH in the summer of 1942 collected ixodid ticks, from which encephalitis virus was extracted in M.P. CHUMAKOV'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, Mikhailov 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 Mikhailov District by him and Assistant A.N. NAIDENNOVA.

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 collected, then, at the Niva Village travelling laboratory 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 50% 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. ZAKHVAKIN. 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 1943 and January 1945, 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 Nellidovo Hospital at the beginning of June 1944, was not given a virological or epidemiological examination, since it was 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: 11th 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 Pankhika 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, 1943-1944.

<table>
<thead>
<tr>
<th>Survey data</th>
<th>1943</th>
<th>1943</th>
<th>1943</th>
<th>1943</th>
<th>1943</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>
</tr>
<tr>
<td>Number of animals caught</td>
<td>24</td>
<td>21</td>
<td>26</td>
<td>94</td>
<td>24</td>
<td>205</td>
</tr>
<tr>
<td>Breakdown:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrew (Sorex araneus)</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Red vole (Evotomys glareolus)</td>
<td>11</td>
<td>13</td>
<td>25</td>
<td>59</td>
<td>14</td>
<td>122</td>
</tr>
<tr>
<td>Dark vole (Microtus agrestis)</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Common vole (Microtus arvalis)</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Wood-mouse (Apodemus sylvaticus)</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>26</td>
<td>8</td>
<td>56</td>
</tr>
<tr>
<td>Yellow-neck mouse (Apodemus flavicolli)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* 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, Zhiglički Kural Soviet, Nelidovo District, Velikiye Luki Region

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<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>43</td>
<td>75</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>11</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>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Red vole (Eutomys glareolus Schr.)</td>
<td>10</td>
<td>16</td>
<td>19</td>
<td>21</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>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Yellow-neck mouse (Apodemus flavicollis Welch.)</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>5</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 1st, 1944 and Sept. 11, 1944) and one hare (Lepus europaeus Pall.) shot at the same place on Jan. 22nd, 1944.
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. 1914 and Jan, 1915</th>
<th>May-July-Sept, 1914</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>
<tr>
<td>Breakdown:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domest;i mouse (Mus musculus L.)</td>
<td>5</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Yellow-neck mouse (A. flavicollis Melch.)</td>
<td>6</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Grey rat (Rattus norvegicus Berkenh.)</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Black rat (Rattus rattus rattus L. and R. r. alexandrinus Geoffr.)</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

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.
The results of our survey of mice and shrews at different seasons of the year and our findings on the extent of their infestation with ecto-parasites are given in Table 4 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 all the ixodid tick. Ixodidae collected from rodents are almost exclusively in the larval or nymph stage (POMERANCEV, 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. Found on humans.
3) *Ixodes trianguliceps* Birul. One female on *Sylvilagus floridanus*, 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; CHUKAKOVA, MIRONOV, OROVA, PETROVA and SHLUGER, 1944), and finally *Ixodes trianguliceps* Birul., infecting 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
Table 4. Ectoparasites collected from small mammals

<table>
<thead>
<tr>
<th>Ectoparasites</th>
<th>Date of collection</th>
<th>At focus</th>
<th>In control area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jan-Mar 1944</td>
<td>Jan-Mar 1944</td>
<td>Dec, 1943</td>
</tr>
<tr>
<td></td>
<td>1944</td>
<td>May-Sept 1944</td>
<td>Feb-Sept 1944</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July-Sept 1944</td>
<td>July-Sept 1944</td>
</tr>
<tr>
<td>Tick's (Ixodidae)</td>
<td>1 (larva)</td>
<td>132</td>
<td>2 (larvae)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>including 5 imagos</td>
<td></td>
</tr>
<tr>
<td>Gamasoidea</td>
<td>155</td>
<td>170</td>
<td>173</td>
</tr>
<tr>
<td>Chiggers (Trombiculidae)</td>
<td>579</td>
<td>1974</td>
<td>435</td>
</tr>
<tr>
<td>Fleas (Aphaniptera)</td>
<td>70</td>
<td>82</td>
<td>72</td>
</tr>
</tbody>
</table>

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.

- 9 -
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 ayztes.* These are the principal species
2) *Ctenophthalmus uncinnatus.*
3) *Ceratophyllus penicilliger.*
4) *Ceratophyllus turbidus.*
5) *Ctenopsyllus segnis.* Isolated specimens of these
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 4028 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 wood-mice (*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 (*Eutamys 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 type-specific 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 equine encephalomyelitis, and sheep serum immune to the virus of "louping 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.
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 louping ill. Legend in Table 27:

- 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 Eutomys 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 epi-
demiology 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.)