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## NATURAL FOCUS OF TSUTSUGAMUSHI FEVER

[Following is the translation of an article by I. V. Tarasevich, S. M. Kulagin, N. I. Kudryashova, I. M. Gopachenko and G. P. Somov, Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR and the Vladivostok Institute of Epidemiology and Microbiology, published in the Russian-language periodical Zhurnal Mikrobiologii i Immunologii (Journal of Microbiology, Epidemiology and Immunobiology), #5, 1964, pages 19-24. It was presented at a scientific conference of the Department of Naturally Focal Diseases of the Gamaleya Institute of Epidemiology and Microbiology on 23 Jan 1964 and submitted on 5 Feb 1964. Translation performed by Sp/7 Charles T. Ostertag Jr.]

During a study of unclear typhus fever morbidity in Primorye assumptions were often expressed concerning its affiliation to tropical typhus -- tsutsugamushi (Mill, 1936; Antonov and Nayshtat, 1936; Churilov, 1946; and citation by Filatov, 1959). Ye. N. Pavlovskiy, in analyzing materials on tick-borne typhus in Primorye, came to the conclusion that four forms of rickettsial diseases exist in the Far East, some of which could be related to tsutsugamushi (Pavlovskiy, 1947). However it was impossible to identify this disease with tsutsugamushi due to the lack of specific means of diagnosis.

For a long time tsutsugamushi fever was recorded only in Japan and in certain regions of Southeastern Asia. During the Second World War numerous outbreaks emerged in the Anglo-American contingents of forces, displaced in regions of the Pacific and Indian Oceans. During some of the outbreaks several hundred soldiers fell ill in a short period of time. In one of the British subunits following a 4-day military operation in the jungles of Ceylon, 756 men fell ill (Philip, 1947). All-told in the Anglo-American forces 18,000 cases of tsutsugamushi were noted, and of these 284 ended lethally (Elsom et al. 1961). This served as a stimulus for the development of a specific diagnosis and prophylaxis of the infection, after which many "new" descriptions appeared of foci of tsutsugamushi in regions where it had not been diagnosed previously.

In recent years data have been published concerning the distribution of tsutsugamushi in localities lying in direct contact with the borders of our country -- In Northern Japan (Shishido, 1958, 1963) and

South Korea (Jackson et al., 1957; Ley and Markelz, 1961). In these foci the natural carrying ability for the rickettsiae of tsutsugamushi was established in the mouse-like rodents Apodemus agrarius Pall., Micromys minutus Pall., Microtus fortis Buchn. and the trombiculid mites Leptotrombidium pallida Nag. In South Korea in particular the widespread distribution of the infection was noted among field mice. Eleven strains of tsutsugamushi rickettsiae were isolated from 77 individual biotests (177) (Jackson et al., 1957). The climatic-geographical and zoologo-parasitological peculiarities of these natural foci are analogous to those in certain regions of the Primorye Province.

What has been said above served as the grounds for the carrying out in Primorye of an examination of rodents and ticks as possible carriers and transmitters of tsutsugamushi rickettsiae. The work was conducted under the leadership of S. M. Kulagin.

The material was collected in September - October 1963 in the Khasanskiy region. The southern part of the Khasanskiy region is found within the limits of the North Korean natural province (Kolesnikov, 1956). The locality has a flat relief, now and then broken up by small knolls. A large portion of the area is very damp and is overgrown with diverse grass and beach grass meadows. The slopes of the knolls and the banks of the rivers are covered with sparse shrubbery and lone trees. There are many fresh and salt lakes and small streams and tributaries.

Over the period of work from 24 Sep through 7 Oct 1963, 305 small mammals were investigated. They made up 6 species (table 1). The animals were caught in animal traps. Their numerical strength was calculated with Gero traps [snap traps].

From all the animals more than 60,000 trombiculid mites were collected. The most numerous turned out to be the species Leptotrombidium pallida, L. pavlovskiy and Neotrombicula japonica. L. orientalis, N. tamiyai, N. mitamura were encountered in small numbers (table 2).

Around 8,000 larvae were investigated in 41 biological tests in white mice. The method for carrying out biological tests was the generally used one. In a biotest 90-700 (an average of 200-300) larvae were used that were taken from animals of the same species, captured in the same locality. Part of the mites were left for computation.

Blood was taken from live animals for investigation. The sera from 170 animals were investigated in the complement fixation reaction with dissolved antigen from R. tsutsugamushi according to the method of Golinevich (Zdrodovskiy and Golinevich, 1956). The antigen was prepared from the Gilliam strain<sup>1</sup> according to the modified method of Topping and

Shepard (Tarasevich, 1963). [1. In considering the well-known heterogeneity of the strains of *tsutsugamushi rickettsiae* (Smeydl, 1956) we note that antibodies to the Gilliam strain (Shishido, 1963) are isolated in the sera from rodents in Northern Japan.] In setting up the complement fixation reaction three positive results in the titer of 1:4 were obtained from 128 investigated sera of field mice (table 3). Due to the small quantity, sera in further dilutions were not investigated.

The greatest amount of attention in the work was given to an investigation of the animals for natural *rickettsiae* carriage. The biotests from the organs of the animals were set up in the following manner: The spleen and liver of from 1 - 10 animals were pulverized in a mortar with silica sand and diluted with a physiological solution in a calculation of 2 ml for the stated organs of one animal. The prepared suspension was administered intraperitoneally in volumes of 0.5 ml to 2-3 white mice weighing 15-20 g. Animals of the same species, the same age and captured at the same station were assembled in the same biotest. Observations were conducted of the infected mice and in the absence of clinical symptoms or death were killed for passaging on the 12-15th day following infection. As a rule, 2-3 "blank" passages were made. During the appearance of the clinical symptoms of the disease (dyspnea, dishevelled wool, sluggishness), noted after the 5th day following infection, the mice were killed and their spleen and liver were extracted for a subsequent passage. All told 153 animals and 29 biotests were investigated (table 4).

As a result of setting up the biotests on white mice, strains of the causative agent were isolated from the organs of the field mouse, rat-like hamster, Far-Eastern vole and the great Ussuri shrew. Some of the strains from the organs of the animals and ticks were adapted to guinea pigs and chick embryos. The morphological, tinctorial, biological and serological properties of the isolated causative agent turned out to be identical to the properties of *rickettsiae tsutsugamushi*, studied at length and described in detail by a number of investigators (Tarasevich, 1960, 1964; Nagayo, 1930; Lewthweithe and Savoors, 1936; Bengston, 1945; Blake et al., 1946; Philip, 1946; Smeydl, 1956, ct. Rivers; Jackson, 1957; Shishido, 1958).

We will cite the characteristics of strain No. 15, isolated from 104 mite larvae, among which were species of *L. pavlovskyi* and *N. japonica*. The mites were collected from field mice captured in the Tumyntszyan River Valley. A suspension of the mites in 3 ml of physiological solution was introduced in a volume of 0.5 ml intraperitoneally to three white mice weighing 12-15 g. On the 10th day one of these died and upon autopsy peritoneal exudate and the appearance of autolysate were found in it. In the other two mice, killed in the same period of time for passaging, an

enlargement of the spleen was noted and the formation of a small exudate in the abdominal cavity. In smear-scrappings from the peritoneum, stained according to Zdrovovskiy and Romanovskiy -- Giemsa, rickettsial formations were noted. In the course of the following passages we observed a pathological picture in the mice which was characteristic for tsutsugamushi according to the description of the above named authors; some of the animals died on the 8-10th day. During dissection of animals that survived up to these same periods, the formation of a mucous or hyaline peritoneal, less seldom hyaline pleural, exudate was established, the enlargement of the spleen, the formation of a fibrinous film on its surface, considerable hyperemia of the adrenal glands and injection of the vessels of the peritoneum. In smears from the peritoneal exudate, spleen and liver we found small, polarly stained, short diplobacillary or ovoid forms of rickettsiae, staining azure-blue according to Zdrovovskiy, and blue-violet according to Romanovskiy-Giemsa. They were located in the cytoplasm, grouped mainly around the nucleus, or extracellularly. An abundant accumulation of rickettsiae were noted from the 3rd passage. In seedings, made from the spleens of mice on bacterial media, there was no growth.

Guinea pigs were infected with a suspension from the spleen and liver of white mice. In animals of the 1-6th passage following 5-10 days of incubation (an average of 7) a febrile disease was observed which lasted 5-11 days (an average of 8). The disease ended in recovery and the formation in the sera of complement fixing antibodies to the rickettsiae of tsutsugamushi (Gilliam strain) in titers of 1:10 on the 25th day and up to 1:160 on the 33-45th day. With the help of antigen, prepared from the isolated causative agent, it was possible to expose complement fixing antibodies to the Gilliam strain of tsutsugamushi rickettsiae up to the extreme titer.

In pigs, dissected on the 2nd-3rd day of fever, the formation of an abundant (sometimes up to 10-12 ml) peritoneal exudate was observed, along with a small enlargement of the spleen, hyperemia of the adrenal glands, a sharp injection of the vessels of the peritoneum, hemorrhage in the subcutaneous cellular tissue of the thoracic area and the abdomen. Rickettsiae were detected (see picture) in smears from the spleen, liver, peritoneal exudate and membranes of the testes.

Strain No. 15 was adapted to chick embryos which died on the 7-8th day without any pathological changes. In smears from the yolk sacks of the embryos, rickettsiae were detected in the form of short diplobacilli or sometimes chains. The properties of the remaining strains were not different from those described above. The study is continuing of the immunogenic, toxigenic and other properties of the isolated strains.

#### Conclusions

1. As a result of zoologo-parasitological investigations it was established that the fauna of small mammals and trombiculid mites of Southern

Primorye is analogous to the fauna of carriers and vectors of R. tsutsugamushi in North Korea and Japan. It is made up mainly by the following species: Apodemus agrarius, Micromys minutus, Rattus norvegicus caraco, Cricetulus triton, Microtus fortis, Crocidura lasiura and Leptotrombidium pallida, L. orientalis, L. pavlovskyi, Neotrombicula japonica, N. tamiyai, N. mitamura.

2. Three positive results were obtained during the investigation of 128 sera of A. agrarius in the reaction of complement fixation with specific antigen from R. tsutsugamushi (Gilliam strain).

3. Strains of rickettsia were isolated in 6 out of 17 biotests on Ap. agrarius, in 1 out of 7 biotests from M. fortis, in 1 test out of 1 investigation from C. triton, in 1 test out of 2 in C. lasiura. All told 9 strains of rickettsia were isolated from the stated animals.

4. An analogous causative agent was isolated in 6 biotests out of 41 from the larvae of trombiculid mites, represented by species of Leptotrombidium pallida, L. pavlovskyi, L. orientalis, Neotrombicula japonica.

5. Based on morphological, tinctorial, biological and serological properties, the isolated strains were identical to R. tsutsugamushi.

6. On the basis of the data obtained, it is apparent that the region studied is a potential natural focus of infection, identical to tsutsugamushi.

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Photo caption: R. tsutsugamushi (strain No. 15) in a smear from the peritoneal exudate of a guinea pig.

Table 1

Place of capture	Number of animals captured, by species							All told	Percentage of hits
	<i>Rattus norvegicus caraco</i>	<i>Apodemus agrarius</i>	<i>Micromys minutus</i>	<i>Cricetus triton</i>	<i>Microtus fortus</i>	<i>Crocidura lasiura</i>			
Shore of Lake Doretsini	7	129	2	1	6	11	156	14.4	
Shore of Lake Talmi	-	5	-	-	-	1	6	-	
Tumrytsyan River Valley	1	66	1	-	10	5	83	26	
Yanchikhe River Valley	-	44	-	-	-	-	44	19	
Cherukhe River Valley	-	6	-	-	10	-	16	16	
All told	8	250	3	1	26	17	305		

Species composition and number of small mammals in the Khasansk region.



Table 2

Results of investigating the larvae of trombiculid mites in biological tests on white mice.

Place of capture	Number of biotests		Species of trombiculid mites in the positive biotests	Species of host for the trombiculid mites
	All told	No. Pos.		
Shore of Lake Doretsin	9	1	<u>L. pallida</u> , <u>L. pavlovskyi</u> , <u>N. japonica</u>	Field mouse
	1	1	<u>N. japonica</u> , <u>L. pallida</u> , <u>L. pavlovskyi</u> , <u>L. orientalis</u>	Rat-like hamster
	3	1	<u>L. pallida</u> , <u>L. orientalis</u> , <u>N. japonica</u>	Far-Eastern Vole
Shore of Lake Talmi	1	-		Field mouse
Tumyntsyian River Valley	5	2	<u>L. pavlovskyi</u> , <u>N. japonica</u>	Field mouse
	8	1	<u>L. pavlovskyi</u> , <u>N. japonica</u>	Far-Eastern Vole
Yanchikhe River Valley	5	-		Field mouse
Cherukhe River Valley	1	-		Field mouse
	8	-		Far-Eastern Vole

Table 3

Place of capture	Number of animals investigated, by species													
	Rattus norvegicus caraco		Apodemus agrarius		Micromys minutus		Cricetulus triton		Microtus fortus		Crociodura lasiura			
	Total	Positive	Total	Positive	Total	Positive	Total	Positive	Total	Positive	Total	Positive	Total	Positive
Shore of Lake Doretsini	6	-	65	3	1	-	1	-	5	-	7	-		
Shore of Lake Talmi	-	-	3	-	-	-	-	-	-	-	1	-		
Pumyntsyran River Valley	1	-	40	-	1	-	-	-	7	-	2	-		
Yanchikhe River Valley	-	-	17	-	-	-	-	-	-	-	-	-		
Cherukhe River Valley	-	-	3	-	-	-	-	-	10	-	-	-		
All told	7	-	128	3	2	-	1	-	22	-	10	-		

Results of investigating the complement fixation reaction of sera from small mammals.

Table 4

Place of capture	Results of investigating mammals of various species													
	Apodemus agrarius		Microtus fortus		Cricetulus triton		Rattus norvegicus caraco		Crocidura lasiura					
	No inv.	No pos.	No inv.	No pos.	No inv.	No pos.	No inv.	No pos.	No inv.	No pos.				
Shore of Lake Doretsini	75/8	2	5/3	1	1/1	1	6/2	-	4/2	1				
Shore of Lake Talmi	3/1	1												
Tumyntsyan River Valley	28/5	2	4/2											
Yanchikhe River Valley	19/3	1	8/2											
All told	125/17	6	17/7	1	1/1	1	6/2	-	4/2	1				

Numerator: Number of animals investigated; denominator: Number of biotests.  
 No inv. = Number investigated. No pos. = Number positive.