TRANSMISSION OF SCRUB TYPHUS TO HUMAN VOLUNTEERS
BY LABORATORY-REARED CHIGGERS*

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SUMMARY: Laboratory-reared, Rickettsia tsutsugamushi-infected Leptotrombidium arenicola and L. fletcheri chiggers were fed on 1 and 2 human volunteers respectively. All subjects developed typical clinical signs and symptoms of scrub typhus beginning on days 8-10 post chigger attachment (PCA); these included fever, severe headache, myalgia, regional lymphadenopathy, and eschar. The two L. fletcheri subjects developed a transient generalized rash on days 3-4 after the onset of fever, and these two individuals also appeared to suffer a more severe clinical disease. Rickettsemias were detected in all three volunteers beginning on day 7 PCA, 1–3 days before the onset of clinical disease. Rises in indirect fluorescent antibody titers occurred starting on days 13–19 PCA (day 4–11 post fever) and in Weil-Felix OXK titers starting on days 16–22 PCA (days 7–14 post fever). These results strongly suggest that the use of laboratory-reared chiggers is a reliable means of transmitting scrub typhus infections to volunteers.

INTRODUCTION

A consistent and predictable method for infecting human volunteers with Rickettsia tsutsugamushi was required before a study on the chemoprophylaxis of scrub typhus could be approved by committees responsible for the review of research involving human subjects. The primary criterion for selection of a method was necessarily reliability, since we desired to expose the fewest number of volunteers to infection that was consistent with statistical considerations. Secondarily, we desired that the method be easily controlled, preferably in a laboratory setting, and mimic the natural transmission of scrub typhus.

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This study was approved by three Human Use Committees: at the Institute for Medical Research, Kuala Lumpur, Malaysia; at the Walter Reed Army Institute of Research; and the U.S. Army Human Subjects Research Review Board, Washington, D.C., USA.


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There is historical precedent for the inoculation of culture-grown rickettsiae (Smadel, Ley and Traub, 1950), but this method bears little resemblance to the transmission of scrub typhus in nature. Man normally acquires scrub typhus rickettsiae through the bite of any of several species of chiggers (larval mites) in the genus *Leptotrombidium*. These chiggers are typically found in the dense, low vegetation that emerges after the clearing of forests in endemic areas. Within this habitat type, small “mite islands” densely populated with infected chiggers can easily be identified (Philip, Traub and Smadel, 1949). However, the exposure of volunteers to these “hot spots” has led to very unpredictable infection rates (Philip, Traub and Smadel, 1949; Smadel et al., 1949) and therefore, would require a large study population to yield statistically meaningful results.

Colonies of *R. tsutsugamushi*-infected *Leptotrombidium arenicola* and *L. fletcheri* chiggers have been maintained in our laboratory for 6 and 15 years respectively. The chiggers in these colonies have been used to transmit infections to mice and monkeys (Shirai et al., in press), and the strains of *R. tsutsugamushi* carried by each species identified (Rapmund et al., 1969; Roberts and Robinson, 1977; Shirai et al., in press). Furthermore, the rickettsiae in these chiggers are passed transovarially from one generation to the next with extremely high efficiency (Rapmund et al., 1969; Roberts and Robinson, 1977), assuring a ready supply of infected chiggers. The bite of these chiggers appeared to us to be the ideal method for challenging volunteers in our planned chemoprophylaxis study if their ability to feed on and cause clinical scrub typhus in man could be established.

Consequently, approval for a small study to test the feasibility of rickettsial transmission from laboratory-reared chiggers to man was sought and obtained from appropriate review committees. In the study, three individuals were exposed to the bite of infected chiggers from our colonies. The result of this experiment forms the basis of this report.

**Materials and Methods**

**Volunteers:** Three senior scientists (two Americans, one British) in our laboratory served as volunteers. All were healthy men between the ages of 30 and 47 with no history of scrub typhus infections.

**Chiggers:** Laboratory-reared *L. arenicola* (17th generation) and *L. fletcheri* (32nd generation) chiggers were used. The colonies of both species were established using naturally infected chiggers, and these original infections have been perpetuated in subsequent generations by the transovarial passage of rickettsiae by the female mites (Rapmund et al., 1969; Roberts and Robinson, 1977). Siblings of the chiggers placed on the volunteers were examined for *R. tsutsugamushi* by direct fluorescent antibody (DFA) (Dohany et al., 1978) prior to the beginning of experiments to ensure that the generations used in the study still maintained the infection.

**Chigger feeding:** A small, plastic capsule was used to restrict the chiggers
during feeding (Dohany, Cromroy and Manikumaran, 1977). The capsule was attached to the anteromedial surface of each subject's left thigh 18 hr prior to the introduction of chiggers. Chiggers were placed in the capsules in groups of 10 until a minimum of 10 chiggers had attached. Day 0 was considered to be the first day that one or more attached chiggers were observed. Capsules were then checked after 7, 24, 31 and 48 hr to observe the progress of feeding. All attached chiggers engorged. Engorged chiggers were removed approximately 50 hr after application and checked for *R. tsutsugamushi* by DFA.

**Volunteer monitoring:** Subjects received a thorough physical examination prior to chigger application and were examined twice daily during the study period. They were judged to have scrub typhus and hospitalized if they developed persistent fevers of 37.6°C or greater that were accompanied by other signs and symptoms of the disease. Volunteers were treated with 200 mg doxycycline 72 hr after the onset of disease (Brown et al., 1978).

Blood was collected every third day. It was used for routine hematological and biochemical monitoring of the volunteers, rickettsemia determinations, and serological testing. Rickettsiae were isolated from blood by mouse inoculation (Brown et al., 1976), and these identified by DFA. Rickettsiae were quantified by inoculating mice with 10-fold serial dilutions of blood. The indirect fluorescent antibody test (IFA) was used to assay the sera for antibodies to eight prototype strains of *R. tsutsugamushi*: Karp, Kato, Gilliam, TA678, TA686, TA716, TA763, and TH1817 (Robinson et al., 1976; Shirai et al., 1979). Sera were also examined for Weil-Felix OXK (WF) agglutinins by the tube tests.

### Results

#### Chigger Feeding

All *L. fletcheri* chiggers attached rapidly to the volunteers, and only the initial applications of 10 chiggers each were required. In contrast, the *L. arenicola* chiggers either attached slowly or not at all. None of the first 10 chiggers applied to the volunteer were attached after 3 hr. An additional 20 chiggers were placed in the capsule at this time, and a total of 30 chiggers were left in place for 24 hr. Despite this large number, none attached. These chiggers were replaced with another group of 30. One chigger in this group attached over the next 24 hr. The unattached chiggers were again removed and replaced with 30 fresh chiggers. Of these, 12 attached during the next 24 hr. All engorged *L. fletcheri* and *L. arenicola* chiggers removed from the volunteers were confirmed to be infected with *R. tsutsugamushi* by DFA.

#### Clinical Course

All subjects developed typical clinical signs and symptoms of scrub typhus that included fever, eschar formation, regional lymphadenopathy, severe headache
TABLE I
Quantitation of rickettsemias*

<table>
<thead>
<tr>
<th>Day Post Chigger Attachment</th>
<th>L. fletcheri Subjects</th>
<th>L. arenicola Subject</th>
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<tbody>
<tr>
<td></td>
<td>T.K.</td>
<td>A.S.</td>
</tr>
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<td>1</td>
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<td>4</td>
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<td>7</td>
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<tr>
<td>10</td>
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<td>1.5</td>
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<tr>
<td>13</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>16</td>
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<tr>
<td>19</td>
<td>1.5</td>
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<tr>
<td>22</td>
<td>0.5</td>
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<td>25-42***</td>
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</table>

* Rickettsemias expressed as log_{10} of the 50% mouse infectious dose.
** Rickettsiae detected but were below quantifiable limits.
*** Blood was examined on days 25, 28, 31, 34, 37, 40 and 42 for subjects T.K. and A.S. and on days 25, 28, 31 and 34 for P.S.

and myalgia. The onset of scrub typhus was day 8 post chigger attachment (PCA) for one of the L. fletcheri subjects (T.K.), day 9 PCA for the other (A.S.), and day 10 PCA for the one L. arenicola subject (P.S.). A mild malaise was experienced by the volunteers 1 to 2 days prior to the onset of signs and symptoms that conformed to the study criteria established for a diagnosis of scrub typhus. The two L. fletcheri volunteers were admitted to the hospital one day after the onset of disease with fever, rigors, generalized lymphadenopathy, and photophobia. The clinical disease in the L. arenicola subject was milder; he was not hospitalized. Only the L. fletcheri subjects developed a transient generalized rash on days 3−4 after the onset of fever. Although eschars developed on all three subjects prior to the clinical disease, the lesions were more severe in the two L. fletcheri volunteers. All patients improved dramatically within 24 hr following antibiotic therapy.

Clinical Pathology

Most hematological and blood biochemical determinations were within the normal range. Two subjects (T.K. and P.S.) had moderate leukopenias and all had moderate thrombocytopenias as a result of infection. On day 13 PCA both L. fletcheri subjects showed slight rises in lactic dehydrogenase levels, and one (T.K.) had a rise in serum glutamic oxaloacetic transaminase.
TABLE II

Indirect fluorescent antibody and Weil-Felix OXK titers*

<table>
<thead>
<tr>
<th>Day Post Chigger Attachment</th>
<th>L. fletcheri Subjects</th>
<th>L. arenicola Subject-P.S.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T.K.</td>
<td>A.S.</td>
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<tr>
<td>IFA**</td>
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<td>IFA***</td>
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<td>800</td>
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<tr>
<td>34****</td>
<td>3200</td>
<td>100</td>
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* Titors expressed as the reciprocal of the last positive dilution.
** Highest IFA titer was to Kato antigen.
*** Highest IFA titer was to TA686.
**** Rises in antibody titers were not observed after day 34 PCA.

Rickettsemia

Rickettsemias were detected in all three volunteers beginning on day 7 PCA (Table I). The L. fletcheri subjects demonstrated a more consistent and higher rickettsemia than did the L. arenicola subject. The R. tsutsugamushi strains isolated from the L. fletcheri subjects were Karp, TA716, TA763, and Gilliam and from the L. arenicola subject were TA716 and TA763.

Serology

The WF titers and IFA titers to the most reactive R. tsutsugamushi antigens for each volunteer are presented in Table II. A broad cross-reactive IFA response to all antigens except TA678 was observed beginning on day 13 PCA for L. fletcheri subject A.S. and on day 19 for the other two volunteers. Significant WF titers developed over the corresponding period. The magnitude of IFA and WF antibody responses was much greater in the two L. fletcheri volunteers.

Discussion

A number of species in the genus Leptotrombidium are considered to be vectors of scrub typhus; however, definitive proof that these chiggers will feed on and transmit their infections to man is often lacking. Instead, strong cir-
cumstantial evidence has been used to incriminate these various species. Infected *L. fletcheri* chiggers have been removed from individuals who were exposed in an endemic area and subsequently developed scrub typhus (Philip, Traub and Smadel, 1949). However, *L. arenicola*, found principally on the sandy beaches of Peninsular Malaysia (Traub, 1960) and Indonesia (Hadi et al., 1979), has been considered only as a potential vector. Our findings provide conclusive evidence that both *L. fletcheri* and *L. arenicola* may serve as vectors of scrub typhus.

This study is the first report of the controlled experimental transmission of *R. tsutsugamushi* to man by the bite of chiggers. The development of clinical infections by all of the volunteers regardless of the vector species used is strong evidence that laboratory-reared chiggers can serve as an effective means of infecting volunteers in scrub typhus studies.

The use of chiggers for transmitting scrub typhus has a number of disadvantages, especially in studies demanding rigidly defined infections where dosage and strain of *R. tsutsugamushi* are of greatest concern. The dosages of rickettsiae administered by chiggers are unknown. The variable attachment of chiggers to volunteers further complicates the problem of delivering standardized inoculums using the vectors. Recent studies (Shirai et al., in press) have shown that chiggers infected with either multiple strains or, what appears to be, a single strain (as demonstrated by DFA) transmit multiple infections to animals. Therefore, infection with a single strain of *R. tsutsugamushi* may be difficult to produce by chiggers feeding. In addition, the rearing of chiggers is a technically demanding task that is beyond the capabilities of most laboratories.

However, we considered vector-transmitted *R. tsutsugamushi* infections to be the preferable methods of assessing scrub typhus protection in human subjects. Although Smadel, Ley and Traub (1950) were able to produce clinical scrub typhus, including eschars, in volunteers by the intradermal inoculation of rickettsiae, the known and unknown vagaries of natural transmission make its duplication by the inoculation of culture-grown *R. tsutsugamushi* impossible. Recent studies (Shirai et al., 1979) have shown that human infections with multiple antigenic types of *R. tsutsugamushi* may be more common than monotypic infections. Therefore, the transmission of scrub typhus by multiply-infected chiggers best simulates the dosages and number of strains acquired in natural infections. The safety of inoculating culture-grown rickettsiae must also be questioned. Most, if not all, laboratory strains of *R. tsutsugamushi* have been multiply passed in mice, embryonated hens’ eggs, and cell culture and could not be certified free of potentially harmful viruses.

Prophylaxis studies utilizing vector transmission can be approached in several ways. The study of selected high risk groups in endemic areas, such as oil palm workers (Brown et al., 1976), demands that very large populations be followed over long periods. This type of study is logistically cumbersome, hard to control, and in the case of a chemoprophylaxis study, difficult to assure compliance with drug treatment regimens. The exposure of volunteers to natural habitats containing high concentrations of infected vectors overcomes some of
these disadvantages. However, the low infection rates experienced by other investigators using this method indicate that a large volunteer population would be required to assure statistically meaningful data (Philip, Traub and Smadel, 1949; Smadel et al., 1949).

In contrast to the above methods of transmission, the use of infected laboratory-reared chiggers to transmit \textit{R. tsutsugamushi} infections to man appears to be highly reliable. Although there were only three individuals in our study group, both chigger lines have transmitted infections to mice and monkeys (Shirai et al., in press) with the same consistency in other studies. The high transovarial transmission rates of rickettsiae by these chiggers and the ability to test sibling chiggers for infections by DFA virtually assure that only \textit{R. tsutsugamushi}-infected chiggers will be selected for studies. The method also synchronizes infections, greatly simplifying observation and treatment of volunteers. This near complete control of variables reduces risks to the volunteers, yields high quality data, and enables one to use a minimum of volunteers.

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**REFERENCES**


