

# **Epidemiology Bulletin**

- 15 Scrub Typhus Among  
Kindergarten Children in  
Orchid Island  
20 Cases of Notifiable and  
Reportable Diseases,  
Taiwan-Fukien Area

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## **Scrub Typhus Among Kindergarten Children in Orchid Island**

### **1. Introduction**

Scrub typhus (tsutsugamushi disease) is an old disease in Taiwan. It has been particularly prevalent in offshore islands such as: Penghu, Kinmen, Matsu and the Orchid Island. In 1915, Hatori<sup>(1)</sup> first reported scrub typhus in Taiwan. Thereafter, more cases were reported from different parts of Taiwan<sup>(2)</sup>. Since the return of Taiwan to the Republic of China, cases of scrub typhus have often been reported from Penghu<sup>(3)</sup>. In 1970, an outbreak occurred in Fengping Township of Hualien County<sup>(4)</sup>. Of all the reported cases, however, none among the Orchid Island residents has ever been infected. Wu's<sup>(5)</sup> survey shows that in 1984 the serum antibody positive rate for scrub typhus of military draftees from the Orchid Island was the highest (80.6%). The survey of 1988 gave a serum antibody positive rate of 96% for residents of the Island<sup>(6)</sup>. The *Epidemiology Bulletin* of 1985<sup>(7)</sup> reported two infections of Taipei residents after visiting the Orchid Island. Why then have there been no reported cases of scrub typhus among residents of the Island? Is it because the pathogenicity of the rickettsia is lower there, or because cases are not identified and reported due to poorer medical care facilities? The question deserves further investigation.

### **2. Materials and Methods**

#### **(1) Collection of serum specimens and information**

In April 1990, 99 blood specimens were collected from kindergarten children of the Orchid Island. A year later in April 1991, blood specimens from these children, except seven who had then moved to Taiwan proper, were collected once again. These 92 children were interviewed as to whether they had been attacked by scrub typhus during the last year. Since the local health station is the only medical care resource on the island, its records of these children were also reviewed as to the treatment and antibiotics (such as tetracycline or chloramphenicol) for any illnesses, particularly for scrub typhus, that they had been given during the past year.

## (2) Serological testings

(a) Isolation of *Rickettsia tsutsugamushi*: Chiggers were first isolated from wild rats caught on the Island. Every five chiggers were grouped in one pool. They were grounded with tissue grinder, added to sucrose PG to become suspension, and then centrifugalized at 4°C 200 g for five minutes. The supernatant was injected into the abdominal cavity of 16-18 gram male mice of ICR strain. After the onset of disease in the mice in about two weeks, the abdominal fluid was taken and centrifugalized to remove the fluid. When the rickettsiae were identified through the micro-immunofluorescent-antibody test (MIFAT) method, the fluid cells were washed with Eagle's MEM twice, grounded again with tissue grinder, and centrifugalized at 4°C 200 g for five minutes. The supernatant was placed in L929 cells for culture for use as reference antigen in the MIFAT.

### (b) Preparation of antigen

The three proto antigens used for MIFAT were Karp, Gillan and Kato strains of *Rickettsia tsutsugamushi* bought from the American Type Culture Collection (ATCC). They were multiplied in L929 cells.

### (c) Preparation of antigen glass slides

The three local strains multiplied in the L929 cells and the three proto strains were diluted with PBS and dripped separately in the same well of the slide. The slide was dried under room temperature, fixed for 10 minutes with -20°C acetone, dried again under room temperature and then placed in a refrigerator below -70°C for subsequent use. The method follows that of Robinson et al.<sup>(8)</sup>

### (d) Preservation of serum specimens

Some serum specimens were diluted up to five times with serum dilution (containing 0.4% bovine serum albumin) and PBS (containing 0.05% sodium azide) and kept under 4°C for use. The rest of the specimens were kept under -20°C.

### (e) MIFAT:

1:5 serum antigens were diluted with PBS to 1:40—1:640 and kept on -70°C antigen glass slides. The antigen glass slides were taken out and dried. In each well, some diluted serum was added. The slides were then placed in an incubator for reaction at 37°C for 30 minutes, removed and washed with PBS and then immersed in PBS under room temperature for 10 minutes. The process was repeated for 10 minutes and the slides dried under room temperature. Then, some fluorescein conjugated goat anti-human IgG L+H chain, or IgM  $\mu$  chain specific affinity chromatography purified from Zymed was added to each well. The slides were placed in a 37°C incubator for 30 minutes, washed with PBS twice, dried and some 0.01M PBS containing glycerol at pH 8.0 were added. The slides were placed under a fluorescent microscope at 400 X for studying the florescent reaction.

### (f) Reading the findings

A case is identified as positive if the IgG or IgM antibody value is  $\geq$  1:40.

### 3. Results

#### Antibody positive rates for scrub typhus among kindergarten children

In April 1990, 99 serum specimens were collected from children 5-6 years old in the Orchid Island kindergarten. Among them, 43 were five years old and 56, six years old. When these specimens were tested with MIFAT for IgG and IgM antibodies, 64 were identified as positive, giving a positive rate of 64.64%. Of those in the five-year age group, 14 boys were identified as positive and 13 negative, and for the girls, 10 positive and six negative. Similarly, of those in the six-year age group, 19 boys were found to be positive and six negative, and for the girls, 21 positive and 10 negative. The infection rates by sex, when tested with X-square method, are found to be no significant difference ( $p > 0.05$ ) (see Tables 1-3). Of the 64 positive cases in 1990, one IgG and one IgM cases turned into negative in 1991. Some IgG antibody, in the absence of natural immunity, can decline to a level lower than the positive value in a few months to a few years; similarly, IgM antibody can also decline to lower than the positive value in three months. Thus the two cases turning from positive to negative in one year is not unlikely.

After a year of follow-up, seven of the 99 children could not be located, and blood specimens were collected again from the other 92 children in April 1991. The MIFAT for scrub typhus antibody showed that of the 37 children in the five-year age group, 30 were positive, giving a positive rate of 81.8%; and of the 55 in the six-year age group, all were positive, giving a positive rate of 100%. Table 4 shows that the positive rate of the five-year old children (born in 1985) increased from 58.14% in 1990 to 81.1% in 1991 while, for the six-year old children (born in 1984), from 71.43% to 100% in the same period. The infection rates of scrub typhus for children in this area thus are estimated to be 60% at age five, 70-80% at age six, and almost 100% at age seven. Of the 35 children whose IgG and IgM antibodies were both negative in the first year, six could not be located, while 24 of the other 29 had become positive, giving an infection rate of 82.76% per year. Records of the health station showed that 15 out of the 24 children, though having had fever in the past year, none had shown any of the three major symptoms of scrub typhus: eschar, lymphadenopathy and skin rash, nor had been treated with antibiotics such as tetracycline or chloramphenicol. The other nine children had not been sick in the past year. The health station is the only medical care institution on the island, consequently it becomes difficult to say that the fever is related to the infection of scrub typhus.

### 4. Limitations of study

Though the health and medical care conditions of the children could be obtained from interviews and from records of the health station, the interview was conducted only during the second collection of blood specimens where there could be recall biases of the parents. Records of the health station could also have imprecise information if the symptoms were mild or if they were treated elsewhere. The interview findings that the 24 children had not been sick in the past year were found to be untrue, as the

records of the health station showed that of the 24 children, 15 had visited the health station in the past year. This was probably due to the local Yami tribe's superstition; it is believed that if their children were answered on the questionnaire to have been sick they would be taken away by evil spirits. The present study has not been entirely successful in identifying reasons why there have been no cases of scrub typhus reported in this area, however, the high infection rate should not be ignored. To establish an effective monitoring system for scrub typhus is an important issue in the control of this disease.

## 5. Conclusions and Recommendations

The present findings show that the Orchid Island is an area of high infection of scrub typhus, and children of the area as well as visitors belong to high risk groups. Though constructions in recent years (such as the dock for the nuclear wastes, the new-highways, new apartments and expansion of the airport) have altered the natural environment, the antibody positive rate of scrub typhus in the area has not declined. The chiggers, vector of *Rickettsia tsutsugamushi*, still seem to be quite prevalent. Before the threat of scrub typhus has been effectively controlled, temporary residents such as soldiers, officials and visitors should be warned against the disease so as to minimize infection. Once infected, they should tell their physicians the possible source of infection. Physicians should also be trained in the detection and treatment of scrub typhus. An effective system of disease reporting should also be set up by the government for more effective prevention and control of the disease.

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**Table 1. Scrub Typhus Infection Rates among Kindergarten Children, in Orchid Island, 1990, by Sex**

Sex	Age (years)	
	5	6
Male	14/27 (51.85%)	19/25 (76%)
Female	10/16 (62.5%)	21/31 (67.74%)

**Table 2. Scrub Typhus Infection Rates by Sex, of 5-Year Age Children**

Antibody	Male	Female
Positive	14/27	10/16
Negative	13/27	6/16

$\chi^2 = 0.46, P > 0.05$

**Table 3. Scrub Typhus Infection Rates by Sex, of Six-Year Age Children**

Antibody	Male	Female
Positive	19/25	21/31
Negative	6/25	10/31

$\chi^2 = 0.46, P > 0.05$

**Table 4. Scrub Typhus Infection Rates Among Kindergarten Children in Orchid Island, 1990 and 1991**

Year of Birth	Age (years)		
	5	6	7
1985	25/43 (58.14%)	30/37 (81.1%)	
1984		40/56 (71.43%)	55/55 (100%)