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A TEN YEARS EXPERIENCE ON DIAGNOSIS OF RICKETTSIAL DISEASES USING THE INDIRECT IMMUNOPEROXIDASE METHODS

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Summary. - An accurate diagnosis of Tsutsugamushi disease (TD) can be made within a few hours after receiving serum specimens by detecting the specific IgG and IgM antibodies in the patient's serum. Using the indirect immunoperoxidase test we found a total of 730 cases of TD in 32 out of 47 prefectures when serum samples from 2,224 cases of suspected disease or fever of unknown origin or from patients with febrile exanthema of unknown cause were examined during a 10 years period (from May 1980 till December 1989). In addition, 27 cases of spotted fever group rickettsial infection were confirmed and one case of African spotted fever group infection was also detected in 1988. Furthermore, possibly the first case of TD was found in People's Republic of Congo (Africa) in 1989.

Key words: Tsutsugamushi disease; spotted fever group rickettsial disease, immunoperoxidase test

Introduction

Tsutsugamushi disease (TD) had been recognized as a serious disease in Japan more than several hundred years ago; however, the number of cases has been steadily declining with only a few severe cases being reported during the years 1965 to 1975. The disease seemed to have been nearly eliminated. Since about 1976, however, the reported cases of the disease began to increase in many parts of the country even where it had never been identified previously.

This significant increase in the number of reported cases of TD in Japan has occurred concurrently with the wide use of β-lactam antibiotics which are not effective in treating TD. The use of these antibiotics led to the occurrence of several fatal cases of TD which were confirmed by a specific serological examination following death (Suto, 1985). This unfavourable development served as a stimulus to develop a rapid and accurate serologic diagnostic procedure employing the indirect immunoperoxidase (IP) test as presented in the IIIrd

International Symposium held in Smolenice in 1984 (Suto, 1985).

Using this procedure we also have confirmed 27 cases of spotted fever group rickettsial (SFGR) infections which had never been recognized in Japan before 1984 (Suto, 1985; Uchida *et al.,* 1985). Among them one case of African SFGR infection (Ishii *et al.,* 1989) was also detected in 1988. Furthermore, we have detected possibly the first case of TD probably infected in Africa, the People's Republic of Congo, in 1989.

Materials and Methods

Rickettsial antigen. Three strains of *Rickettsia tsutsugamushi (RT),* namely Gilliam, Karp, and Kato strains which were kindly supplied by Dr. Tamura of Niigata College of Pharmacy, Japan, in 1980, were passaged in our laboratory in monolayer of L-cell cultures. Two strains of SFG rickettsia, i.e. a strain of tick borne rickettsia „Thai tick typhus (Robertson and Wisseman, 1973), TT-118" (TTT) and „Siberian tick typhus", *Rickettsia sibirica* (STT), were supplied by Dr. Kelly of the US Army Medical Research Unit in Kuala Lumpur, Malaysia in 1984, and were passaged in our laboratory in monolayer of L-cell cultures.

A strain of SFGR "Katayama" was isolated by us in a Green Monkey Kidney Cell line from a patient suffered from Japanese Spotted Fever in Tokushima, Japan, in October 1987 (Suto *et al.,* 1988).

Serum specimens. Serum specimens taken from a total of 2,224 patients from May 1980 through 1989 who were suspected for TD or fever and/or exanthema of unknown origin were sent to our laboratory for the specific diagnosis of TD from almost all over Japan, especially in Akita prefecture. The sera were usually tested on the day of arrival, and the rest of specimens was stored at -20 °C.

Antibody titration. The IP test (Suto, 1985) was performed according to the Suto's procedure as presented in the IIIrd International Symposium on Rickettsia and Rickettsial Diseases at Smolenice.

Twelve sets of rickettsial antigens were spotted by a micropipette on the single frosted slide glass (Matsunami Glass industries, Japan). Minute dots of each 3 strains of antigen in one circle were spotted in fixed order. Aceton fixed antigen slides were stored at -20 °C for several months, and used at any times desired.

Serially diluted sera of the patients were first placed on the antigen spots and the slides were incubated for 20 min at 37 °C. After washing with PBS, peroxidase conjugated anti-human IgG or anti-human IgM rabbit serum (DAKO, Inc.) was allowed to react for 20 min at 37 °C. After a second washing, the slides were treated with a substrate solution (Diamino-benzidin 4HCl and peroxide) for 10 min at room temperature. Finally, the slides were faintly stained with dilute methylene blue solution for a few seconds. After final washing with running tap water, air-dried slides were mounted with Diatex (AB-wilh, Sweden) for observation with an ordinary light microscope with the magnification of 400 x.

The reaction was determined as positive when the rickettsial particles were stained light brown. The reciprocal of the highset serum dilution with a positive reaction was expressed as the IP antibody titre.

Results and Discussion

During these 10 years, from 1980 to 1989, we have checked a total of 2,224 cases of suspicious TD, fever of unknown origin, or febrile exanthematous diseases, from all over the country.

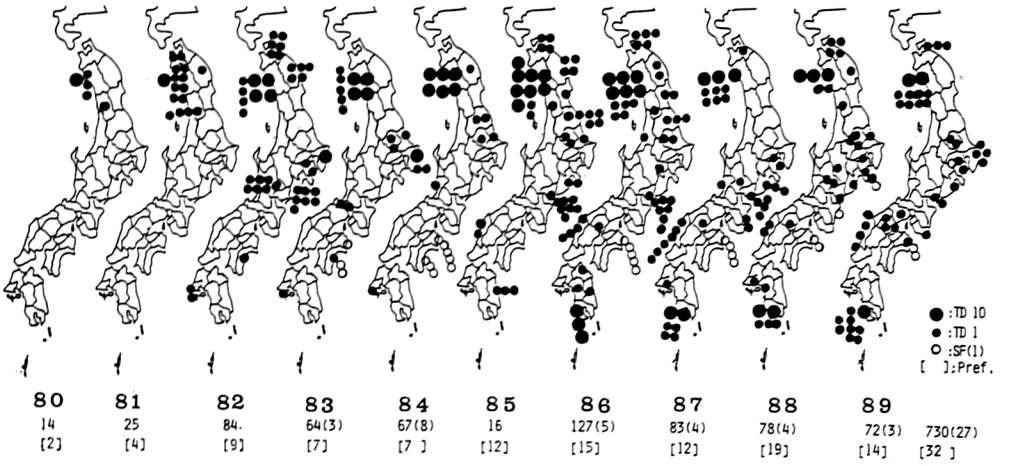

Fig. 1
Regional distribution of the definite infections of Tsutsugamushi disease and SFGR infection diagnosed by IP method from 1980 to 1989

The normal course of the determination of the disease and the announcement for TD in our Akita prefecture is illustrated in Fig. 2.

In our prefecture, if there is any suspicious patient, the doctor will send us the serum of the patient for the determination of TD. Initially, the doctor will call on us and talk about the symptom of the patient. We talk about the treatment of the patient or how to send the serum to us and wait the arrival of the serum. Soon after the arrival of the serum, we check the antibody of the patient. If we confirm the patient as TD, immediately tell the results to the doctor by telephone, and ask to report the patient to the local health centre by telephone, because legal statistical number of the TD depends on the doctor's report to the local health centre. Then the health centre will check the epidemiological data of the patient, and reports to the prefectural office by telephone. The prefectural office will announce on the same day about the patient to the mass communication for calling the further attention. The serial numbers of the cases of the year appears in the TV news and in the newspapers of the next morning. Such a prompt style of the TD control system may not exist in other prefectures in Japan.

More than 200 doctors have the experience of reporting the TD in Akita prefecture. By this system, almost all of the TD could be detected in Akita prefecture. Furthermore, we were asked for serologic diagnosis of TD from many doctors in all over the country. We performed the confirmation of the disease with the serum in the same manner at any time.

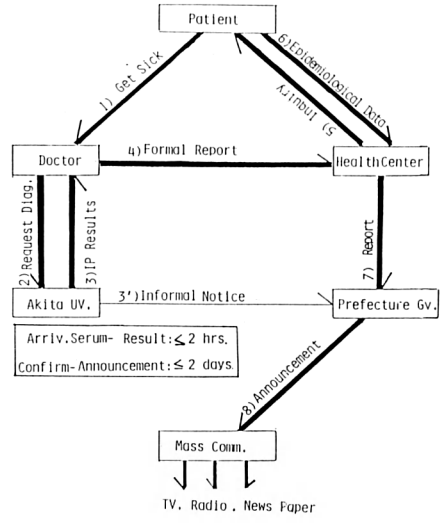

Fig. 2
The normal course of announcement for Tsutsugamushi disease in Akita prefecture Japan

During these 10 years, from 1980 to 1989, we have checked a total of 2,224 cases of suspicious TD, fever of unknown origin, or febrile exanthematous diseases, from all over the country, and finally 730 cases of TD and 27 SFGR infection were confirmed from 32 prefectures in Japan (Table and Fig. 1). The age distribution of the 730 cases of TD revealed that the most of the patients aged 40 to 70 years and 56 cases of children under 15 years suffering from this disease were detected. The seasonal distribution was also clearly demonstrated. From May to June is the most remarkable season for TD in Akita prefecture and in North-eastern Japan or most of the areas in front of Japan Sea. While in southern areas or in the Pacific coast, more patients appeared from late autumn to early winter. Such a difference will depend on the kinds of chiggers in the area. The patients transmitted in the summer by a mite named Akamushi (*Leptotrombidium akamushi)* which was believed to be the only vector of the disease have been very rare in recent years. However, the cases which were probably transmitted by *Leptotrombidium pallidum* in autumn and in spring or by *Leptotrombidium scutellare* in late autumn to winter were detected in many parts of the country even where the disease had never been identified before. And the ecology of the chiggers (Takahashi *et al.,* 1988) became more clear during the last few years.

The analysis of the antigenic type of Rickettsial disease cases confirmed during these 10 years is illustrated in Fig. 3. In North-eastern (Tohoku) Japan, Karp type was most common and Kato type was only detected in Akita during the summer season as "Classical Tsutsugamushi Disease". In southern Japan, Gilliam and "Unknown" increased. "Unknown" means that no difference was

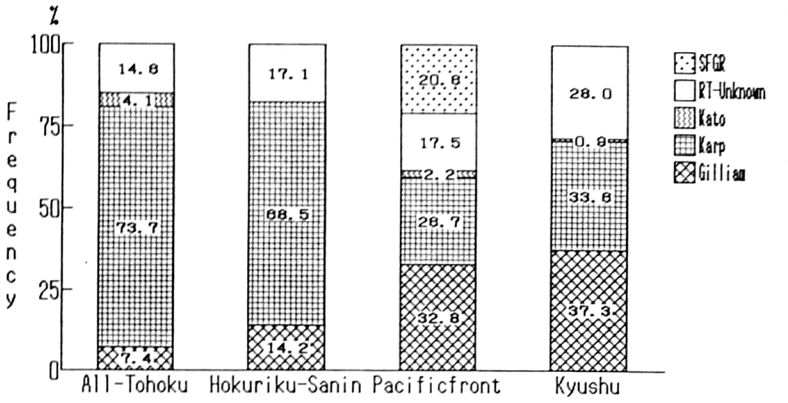

Fig. 3
The analysis of the antigenic type of Rickettsial disease cases confirmed by IP method

observed between the IP titres against Gilliam, Karp, and Kato antigens. We did not perform any absorption test. The patients might be infected by newly recognized antigenic strains.

The existence of RT in most of the prefectures in Japan had been already confirmed during the years 1959–1964 by US-Japan cooperating work (Tamiya, 1968). Thus the increase of the reported cases of TD in Japan might be supported by the regulation of Chloramphenicol and the intensive use of β-lactum antibiotics which are not effective in treating TD but used as the first choice for most febrile infectious diseases. The use of the latter antibiotics led to the occurence of several fatal cases, and this unfavourable occurence had served as a stimulus to develop an enlightenment with rapid diagnosis and rapid treatment.

Now, many prefecture have the authorized diagnostic laboratory using IP or IF technique. And the number of doctors who can identify the TD is much increased, and resulted in the recent increase of the reported cases of TD in Japan. This will be rather valuable for the people who may get this disease during every incidental season, because the patients will be easily cured by administration of the tetracycline group of antibiotics according to the correct diagnosis.

A total of 27 cases of SFGR infection was confirmed by our IP system. The location of the most patients is Shikoku Island. The detection of the patient was from 1983 to 1989. Mahara presented about the clinical findings in this symposium. In addition, we found a case of African SFGR infection (Ishii *et al.*,

Table 1. Number of definite Tsutsugamushi disease infections diagnosed by IP method from 1980 to 1989

Year	Akita Pref Conf/Test	Other Pref Conf/Test	Total Conf/Test
1980	13/ 29	1/ 11	14/ 40
1981	18/ 60	7/ 12	25/ 72
1982	44/ 152	40/ 79	84/ 231
1983	45/ 247	19/ 91	64/ 338
1984	60/ 202	7/ 27	67/ 229
1985	82/ 247	34/ 54	116/ 301
1986	65/ 208	62/ 111	127/ 319
1987	36/ 153	47/ 148	83/ 301
1988	32/ 110	46/ 101	78/ 211
1989	28/ 89	44/ 93	72/ 182
Total	423/ 1497 (28.3 %)	307/ 727 (42.2 %)	730/ 2224 (32.8 %)

(From 1984, a total of 27 SFGR infections was also confirmed)

1989), looked like Rickettsia Pox, infected in South Africa in 1988. So far there has been no report of TD from Africa. Recently we detected a case of TD probably infected in Africa (in the People's Republic of Congo) as presented in the Poster session of this symposium.

Long distance international travel is common and rapid transportation results in such a situation, therefore it is important to consider those imported infectious diseases at any time. And we believe that the early diagnosis and early treatment is most important and most effective way to control the rickettsial disease at present time in Japan.

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Reference

Ishii, N., Komatsu, H., Nakajima, H., Tani, K., and Suto, T. (1989): Imported rickettsial African spotted fever in Japan. *British J. Dermatology* **210**, 701-704.

Robertson, R. G., and Wisseman, C. L. Jr. (1973): Tick-borne Rickettsiae of the Spotted Fever Group in West Pakistan. II. Serological Classification of Isolates from West Pakistan and Thailand; Evidence for Two New Species. *Amer. J. Epidemiol.* **97**, 55-64.

Suto, T. (1985): Rapid and accurate serologic diagnosis of Tsutsugamushi disease in Japan employing the immunoperoxidase reaction. pp. 444-452. In *Rickettsiae and Rickettsial Diseases,* Proceedings of the IIIrd International Symposium, Bratislava.

Suto, T. (1985): Evidence of Spotted fever Rickettsial infection in Japan as demonstrated by the indirect immunoperoxidase test. *Microbiol. Immunol.* **29**, 1243–1246.

Suto, T., Hatakeyama, H., Ito, R., Nakamura, Y., and Mahara, F. (1988): *In vitro* susceptibility of a strain of rickettsia recently isolated from a case of Japanese spott ed fever to chemotherapeutic agents. *J. Japan Assoc. inf. Dis.* **63**, 35–38 (in Japanese).

Takahashi, M., Murata, M., Nogami, S., Hori, E., Kawamura, A., Jr., and Tanaka, H. (1988): Transovarial transmission of *Rickettsia tsutsugamushi* in *Leptotrombidium pallidum* successively reared in the laboratory. *Japan J. exp. Med.* **58**, 213–218.

Tamiya, T. (1968): Recent advances in studies of Tsutsugamushi disease in Japan, Tokyo, pp. 42–79.

Uchida, T., Mahara, F., Tsuboi, Y., and Oya, A. (1985): Spotted fever group rickettsiosis in Japan. *Japan J. Med. Sci. Biol.* **38**, 151–153.