363.
2. Tada, J., Ohashi, T., Nishimura, N., Shirasaki, Y., Ozaki, H., Fukushima, S., Takano, J., Nishibuchi, M. and Takeda, Y. (1992): Detection of the thermostable direct hemolysin gene (*tdh*) and the thermostable direct hemolysin-related hemolysin gene (*trh*) of *Vibrio parahaemolyticus* by polymerase chain reaction. Mol. Cell. Probes, 6, 477-487.
3. Sugiyama, A., Nakano, Y., Iwade, Y., Yamauchi, A., Nakayama, O., Matsumoto, T., Kou, Y., Ito, T., Nishinaka, T. and Shoji, T. (1999): Isolation of thermostable direct hemolysin-producing *Vibrio parahaemolyticus* from food implicated in food poisoning in Mie Prefecture. Infect. Agents Surveillance Rep., 20, 189-192 (in Japanese).
4. Arakawa, E., Murase, T., Shimada, T., Okitsu, T., Yamai, S. and Watanabe, H. (1999): Emergence and prevalence of a novel *Vibrio parahaemolyticus* O3:K6 clone in Japan. Jpn. J. Infect. Dis., 52, 246-247.

Laboratory and Epidemiology Communications

Occurrence of Scrub Typhus (Tsutsugamushi) in Kanagawa Prefecture and Types of *Orientia tsutsugamushi* Involved

Yumiko Furuya*, Takashi Katayama, Miyuki Hara, Yoshiya Yoshida, Mitsunobu Imai and Toshikatsu Hagiwara[1]

Kanagawa Prefectural Public Health Laboratory, Nakao 1-1-1, Asahi-ku, Yokohama 241-0815 Kanagawa and [1]National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku 162-8640 Tokyo

Communicated by Shigetou Suzuki

(Accepted May 9, 2000)

Scrub typhus, or Tsutsugamushi disease, is caused by *Orientia tsutsugamushi*. Kanagawa Prefecture near Tokyo experienced a large epidemic of scrub typhus in 1988-1989. It then wanned: in 1996 only 9 cases were reported. However, the epidemic now appears to be in resurgence. From April 1997 to March 1998, 17 clinical cases were reported. From April 1998 to March 1999, 21 cases were reported, and from April 1999 to March 2000, 43 cases were reported. The infection was most frequent in November. In 1997, 8 cases occurred in November and 1 case occurred in December. In 1998, 13 cases occurred in November and 2 cases occurred in December. In 1999, 1 case occurred in May, 28 cases in November, and 6 cases in December.

All these patients were examined for serum antibody (1) and for the *O. tsutsugamushi* genome whenever the blood from patients in the disease's the acute phase was available (2). The serum antibody was detected using the immunofluorescent technique employing antigens of Gilliam, Karp, Kato, Kawasaki, and Kuroki types (1). Sera showing more than a 4-fold increase of IgM or IgG titers in the disease's convalescent phase in comparison with the acute phase or those showing IgM titers exceeding ×80 in the acute phase were considered positive. The *O. tsutsugamushi* genome was detected using nested PCR using genus *Orientia*-specific primers and type-specific primers (3, 4).

A portion of the data for individual patients is reproduced in Table 1. Sera #98038, #98044, #98045, and #98046 reacted with all of the antigens of Gilliam, Karp, Kato, Kawasaki, and Kuroki, but, with PCR using type-specific primers, the first and the last specimens were positive for only the Kuroki type, while the second and third were positive for only the Kawasaki type. Sera PCR-positive for the Kawasaki type tended to show higher antibody titers for both Gilliam and Kawasaki types (#98043, #98044, and #98045). This is probably because the 56 kDa proteins of the both types shared a common epitope (5). However, some sera (ex., #99033) were positive for only antibody against the Kawasaki type. Use of all the five type antigens, i.e., the Kawasaki and Kuroki type antigens in addition to the three standard antigens, Gilliam, Karp and Kato type antigens, is recommended to increase the sensitivity of antibody detection.

Acute phase sera were generally positive for the genome in PCR. However, some sera, such as #98047, which attained high antibody levels already in the acute phase were negative for the genome.

Table 2 summarizes immunological and PCR data. About 80% of the cases were due to the Kawasaki type, a tendency which is in agreement with the data obtained in Miyazaki (1) and Chiba Prefectures (6). But 3 cases (6.4%) were untypable; this may be due to mutations which made the genome detection impossible using the five sets of primers used.

The work was supported by a grant-in-aid by the Ministry of Health and Welfare of Japan.

*Corresponding author: Fax: +81-45-363-1037

REFERENCES

1. Yamamoto, S., Kawabata, N., Ooura, K., Murata, M. and

Table 1. Representative data of serodiagnosis and typing by PCR

Sample	Days[1]	Antibody Detection										Diag[2]	PCR[3] (Type)
		Gilliam		Karp		Kato		Kawasaki		Kuroki			
		IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG		
#98037	8	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	fn[4]	+(Kw)
#98038	6	40	40	<10	40	<10	20	160	40	160	640	+	+(Kr)
	30	160	160	40	320	80	160	80	160	80	640		
#98043	8	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	+	+(Kw)
	15	40	<10	<10	<10	<10	<10	80	160	<10	<10		
#98044	4	40	<10	<10	<10	<10	<10	160	40	<10	<10	+	+(Kw)
	18	320	320	40	80	20	80	640	640	20	40		
#98045	7	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	+	+(Kw)
	14	160	320	10	160	10	160	320	1280	10	80		
#98046	6	<10	10	<10	20	<10	20	<10	10	20	80	+	+(Kr)
	22	80	320	40	320	40	320	20	20	40	1280		
#98047	5	160	160	160	160	160	160	40	40	160	1280	+	–
#99006	4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	+	+(ut)
	22	<10	<10	<10	<10	<10	<10	80	<10	<10	<10		
#99033	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	+	+(Kw)
	8	<10	<10	<10	<10	<10	<10	40	<10	<10	<10		

[1]Days after onset of illness. [2]Final result of serodiagnosis. [3]Result of PCR (Types: Kw, Kawasaki; Kr, Kuroki; ut, untypable). [4]Final results not obtained.

Table 2. Immunofluorescent Antibody Detection (IF) and Typing by PCR

	Total samples	IF+PCR–	IF+PCR+	IF–PCR+	Typing by PCR				Confirmed cases
					Kp	Kw	Kr	ut	
1997	17	3	6	0	0	5	0	1	9
1998	21	4	10	1	0	8	3	0	15
1999	43	5	26	4	1	24	3	2	35
Total	81	12	42	5	1 (2)	37 (79)	6 (13)	3 (6)*	59

Figures indicate number of samples. IF+PCR–: positive for IF only. IF+PCR+: positive for both IF and PCR. IF–PCR+: positive for PCR only. Typing by PCR: Kp stands for Karp, Kw for Kawasaki, Kr for Kuroki, and ut for untypable. * Percentage of each type.

Minamishima, Y. (1989): Antigenic types of *Rickettsia tsutsugamushi* isolated from patients with Tsutsugamushi fever and their distribution in Miyazaki Prefecture. J. Jpn. Assoc. Infect. Dis., 63, 109-117 (in Japanese).

2. Furuya, Y., Katayama, T. and Yoshida, Y.(1995): Rickettsia. p.1044-1048. *In* Furushou, T. and Imura, H. (eds.). Rinsho DNA Shindanho. Kanehara Press, Tokyo (in Japanese).
3. Furuya, Y., Yoshida, Y., Katayama, T., Yamamoto, S. and Kawamura, A., Jr.(1993): Serotype-specific amplification of *Rickettsia tsutsugamushi* DNA by nested polymerase chain reaction. J. Clin. Microbiol., 31, 1637-1640.
4. Yoshida, Y., Furuya, Y., Katayama, T., Kaiho, I. and Yamamoto, S. (1994): Serotype-specific amplification of *Rickettsia tsutsugamushi* DNA from clinical specimens by nested polymerase chain reaction. J. Jpn. Assoc. Infect. Dis., 68, 601-606 (in Japanese).
5. Furuya, Y., Yamamoto, S., Otu, M., Yoshida, Y., Ohashi, N., Murata, M., Kawabata, N., Tamura, A. and Kawamura, A., Jr.(1991): Use of monoclonal antibodies against *Rickettsia tsutsugamushi* Kawasaki for serodiagnosis by enzyme-linked immunosorbent assay. J. Clin. Microbiol., 29, 340-345.
6. Kaiho, I., Tokieda, M., Yoshida, Y., Furuya, Y., Murata, M., Tanaka, H. and Kawamura, A., Jr. (1993): Epidemiology of Tsutsugamushi disease and typing of isolated *Rickettsia* in Chiba Prefecture. J. Jpn. Assoc. Infect. Dis., 67, 196-201 (in Japanese).