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Current Status of Tsutsugamushi Disease in Korea

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INTRODUCTION

Tsutsugamushi disease is a *Rickettsia tsutsugamushi*-bearing mite-borne illness characterized by fever, headache, rash, and an eschar at the site of inoculating chigger bite. It is endemic in southern and eastern Asia including the Republic of Korea, Northern Australia, and Western Pacific Islands(WHO, 1974 ; Rapmund, 1984).

During the Korean war in 1951, six cases of tsutsugamushi disease were reported among United Nation's military personnel(Munro-Faure and Missen, 1951 ; 406 MGL3, 1951 ; Fuller and Smadel, 1954). However, tsutsugamushi disease was unfamiliar to Koreans until 1986 when Korean patients were diagnosed as having tsutsugamushi disease(Lee et al., 1986 ; Lyi, 1986 ; Chang and Kang, 1987). Thereafter the reported incidence of tsutsugamushi disease has increased sharply in Korea with poorly understood reasons.

The result of nation-wide seroepidemiological and microbiological surveys from 1986 to 1993 revealed that seropositive the rate to *R. tsutsugamushi* among patients with acute febrile illness during these years varied from about 27.7 % to 51 %(Chang et al., 1989b, 1992, 1994). Tsutsugashi disease is regarded as one of the most prevalent diseases in Korea that occur especially in autumn.

This review will cover epidemological surveillence of the tsutsugamushi disease, and characteristics of *R. tsutsugamushi* and vector mites in Korea.

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Historical review of tsutsugamushi disease in Korea

In 1951 during the Korean War, Munro-Faure and Missen(1951) reported five cases of tsutsugamushi disease among British military personnel and in the same year, 406 Military General Laboratory U.S.A. in Tokyo, Japan reported one seropositive case among US Marine Corps at Masan, in southern part of Korea diagnosed by Weil-Felix reaction(1951).

In 1953, Fuller and Smadel(1954) isolated *R. tsutsugamushi* from a patient in the U.S. military. He sent the blood samples to Walter Reed Army Hospital in the United States for the isolation. At that time, *R. tsutsugamushi* was isolated from wild rodents (*Apodemus agrarius, Microtus fortis, Micromys minutus*) which were captured in the Kanghwa, Chulwon and Yeonchun area for the purpose of demonstration of causative agent of Hemorrhagic fever with renal syndrome(12). Moreover *R. tsutsugamushi* was isolated from the mites of these rodents(Jackson et al., 1957).

In 1964, Chun et al.(1965) tested 83 sera from healthy Korean residents for the antibodies to *R. tsutsugamushi* by Weil-Felix reaction and found eight seropositive cases. These suggested the possibility that tsutsugamushi disease existed since or before Korean War. But, surprisingly there was no report on the tsutsugamushi disease until 1986.

In 1986, Lee et al.(1986) and Lyi(1986) reported 9 and 21 tsutsugamushi disease patients respectively among Korean residents diagnosed by indirect immunofluorescence testing. Chang and Kang(1987) isolated two strains of *R. tsutsugamushi* from Korean patients in 1986.

Reported cases of tsutsugamushi diseases in Korea are summarizd briefly in Table 1.

Table 1. Chronological review of Tsutsugamushi disease in Korea.

reporter	year	area	cases	diagnostic method
Munro-Faure A and Missen MD	1951	near limjin river	2	Weil-Felix
		West battle field	3	Weil-Felix
		Masan	1	Weil-Felix
Fuller HS and Smadel JE	1953		2	Isolation
Jackson EB, et al.	1952	Jipo-Li	3	Detection after aminal inoculation
	1952	Jipo-Li	16/82	Isolation
	-	Songgu-Li	2/10	Isolation
	1953	Kuemhwa	1/1	Isolation
		Yeonchon		
		Mungye-Li		
		Kowang-Li		
		Jipo-Li		Isolation
Chun CH, et al.	1965	near DMZ	8/83	Weil-Felix
Lee JS, et al.	1984-1985		9	IF
Lyi KS	1985	Chinhae	21	IF
Chang WH and	1986	Kyunggi	2	Isolation

Transmission of *R. tsutsugamushi* and vector

The species of the vector mite that have been reported to transmit *R. tsutsugamushi* to human include *Leptotrombidium flecherii, L. akamushi, L. deliense, L. scutellare* and *L. pallidum*(Traub & Wisseman, 1975). However, only *Leptotrombidium pallidum* (Fig. 1) has so far been proven as a *R. tsutsugamushi* bearing mite in Korea, although the existance of *L. scutellare* was reported(Rhee et al., 1991, 1992).

The life cycle of the mite consists of egg-larva-nymph-adult stages(Fig. 2). Ususally these mites are free-living in soil and feed on insect eggs(Dohany et al., 1978), but in the larval stage, feeding on vertebrated blood is required for the metamorphosis into eight-legged nymphs and subsequently into adults. In this stage, the transient parasitism of mites on wild rodents becomes established and transmission of *R. tsutsugamushi* can occur(Traub et al., 1975 ; Takahashi et al., 1990). Similarly, when a human is exposed to a chigger harbouring *R. tsutsugamushi* in a chigger infested area, human infection of *R. tsutsugamushi* is established. This is why tsutsugamsuhi disease is also called chigger-borne typhus(Joklik et al., 1988).

An infected mite can transmit *R. tsutsugamushi* transovarially(from adult to egg)(Takahashi et al., 1988) and transstadially(from larva to nymph to adult). Trombiculid mites function as both vector and reservoir of tsutsugamushi disease.

The chigger of *L. pallida* appears in spring(March) and autumn(October to November) in a natural environment(Dohany et al., 1979). This phenomenon suggests that tsutsugamushi disease can occur between March to May and October to December. In reality, epidemiological survey has revealed that the majority of tsutsugamushi disease occurs between

Fig. 1. *Leptotrobidium pallidum* collected at Pyungtaek.

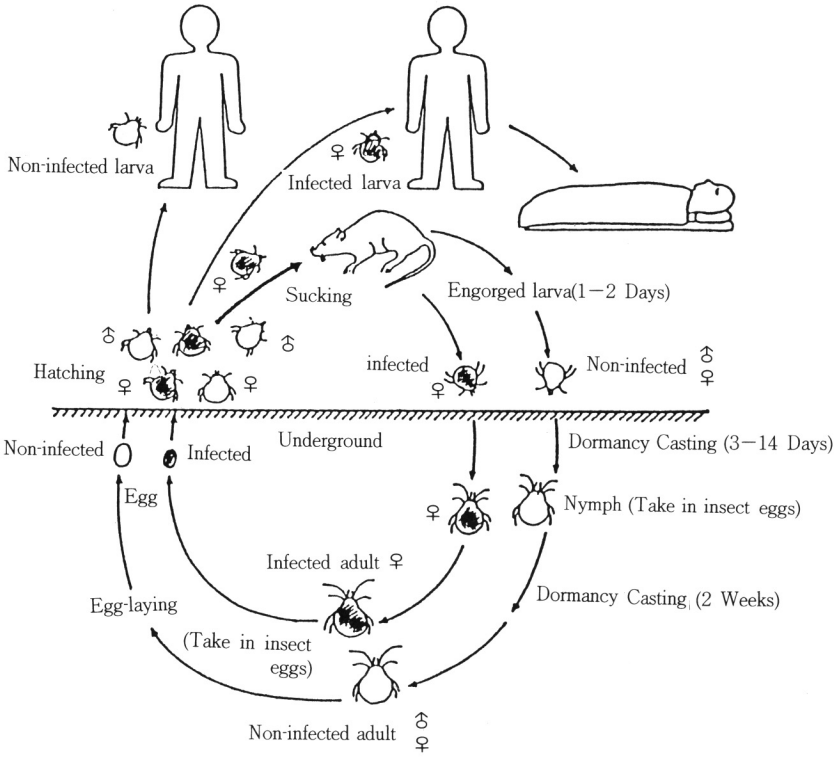

Fig. 2. Life cycle of Leptotrombidium and transmission of *R. tsutsugamushi*.

October to December(Chang et al., 1989b, 1992, 1994).

Phylogenetic status of *Richettsia tsutsugamushi* in genus Rickettsia

Rickettsia tsutsugamushi is an obligatory intracellular microorganism and has a characteristic outer membrane structure which consists of a well developed outer layer and poorly developed middle and inner layers(Silverman & Wisseman, 1978). Habitation of *R. tsutsugamushi* is restricted in the cytoplasm of infected cell(Urakami et al., 1983; Tamura, 1988) whereas spotted fever group(SFG) rickettsia grow in both cytoplasm and nucleus. These morphological and physiological properties could differentiate *R. tsutsugamushi* from other members in the genus Rickettsia.

In a recent molecular phylogenic study using 16S rRNA gene, the average sequence divergence between *R. tsutsugamushi* and member of SFG(spotted fever group) and TG(typhus group) rickettsia has been shown to be approximately 12 %, five times greater than the largest distance separating any other species pair within Rickettsia(Fig. 3)(Sothard and Fuerst, 1994). According to these features, some suggest that *R. tsutsugamushi* is not closely related to other members of Rickettsia and may represent a separate genus. But at present, it is included with the members of genus Rickettia and regarded as a problematic genus from a taxonomical view point.

Distribution of serotypes

R. tsutsugamushi is subdivided into various serotypes by its antigenic nature. Originally Gilliam, Karp and Kato were considered as major antigenic types of *R. tsutsugamushi* and term prototypes were used. The other antigenic types were first recognized in Thailand by Elisberg et al.(1968), and five antigenically different strains of *R. tsutsugamushi*(TA686, TA716, TA763, TH1817 and TA678) were characterized(Shirai et al., 1979; Shirai, 1980; Johnson et al., 1982). Recently, Tamura et al.(1984), and Yamamoto and

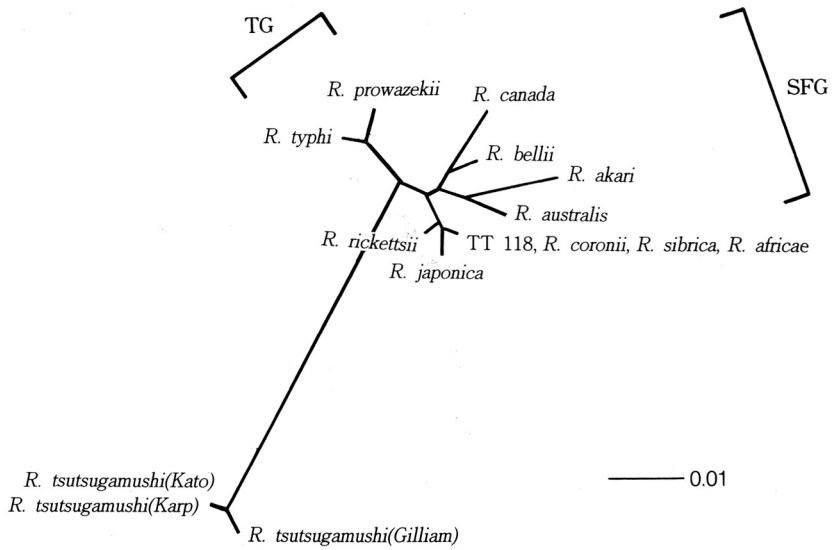

Fig. 3. Phylogenetic tree constructed by Fitch-Margoliasch algrithm based on partial sequences of 16S rRNA genes in genus Rickettsia. These sequences were retrieved from GenBank(National Library of Medicine, USA) and accession number of sequences were U17256(Gilliam), U17258(Kato), U17257(Karp), L36107(*R. coronii*), U11014(*R. bellii*), U11021(*R. rickettsii*), U12459(*R. australis*), M21789(*R. prowazekii*), L36104(*R. canada*), L36098(*R. africae*), L36218(*R. sibrica*), U12458(*R. akari*), L36221(*R. typhi*), L36220(TT-118), and L36213(*R. japonica*). TG indicates typhus group rickettsia, SFG means spotted fever group rickettsia.

Kawatobe(1989) and Ohashi et al.(1990) reported the presence of three other strains of *R. tsutsugamushi*, such as Shimokoshi, Kuroki and Kawasaki strains in Japan.

A total of 137 strains of *R. tsutsugamushi* were isolated from patients and analyzed for antigenic characteristics using strain-specific monoclonal antibodies(Chang et al., 1990; Chang and Kang, 1992). All these isolates were classified into four serotypes; ten were identified as Gilliam, 13 isolates as Karp, 111 isolates as Boryong which has a strain specific epitope that not found in other previously reported strains and serotypes of three isolates were undetermined. Boryong strains were distributed predominantly in the southern part of Korea and other strains such as Gilliam and Karp were found in the mid portion of Korea. The geographical distribution of *R. tsutsugamushi* isolated in Korea is shown in Table 2.

Clinical manifestation

Approximately 1 to 3 weeks after being bitten by infected an chigger, humans abrubtly develop chills, headache and high fever(about 40℃)(Joklik et al., 1988). The spectrum of clinical severity of untreated

Table 2. Geographic distribution of *R. tsutsugamushi* isolated in Korea.

Area	number of isolates				
	Gilliam	Karp	Boryong	unclassified	total
Kyunggi	7	7	3	3	20
Kangwon	1	0	0	0	1
Chungbuk	0	4	4	0	8
Chungnam	1	2	70	0	73
Chonbuk	0	0	13	0	13
Kyungnam	1	0	21	0	22
total	10	13	111	3	137

tsutsugamushi disease seen in different outbreaks ranges from inapparent or mild to severe or fatal (Wyngaarden and Smith, 1985). There are some differences among patients, and additional symptoms such as nausea, vomitting, cough, myalgia, abdominal pain, and sore throat etc, can occur(Chang et al., 1989a ; Lee et al., 1989 ; Choi et al., 1989 ; Chun et al., 1989 ; Kwon et al., 1989 ; Park et al., 1991 ; Peck et al., 1991 ; Park et al., 1993)(Table 3). About one week after the onset of fever, maculopapular rash may appear on the trunk and later become generalized ; it may last for about 5 to 10 days. This skin rash of scrub typhus is classically heralded by a local cutaneous lesion, which evolves from a small indurated or vesicular lesion into an ulcerated lesion present at the time of the onset of symptoms. When this skin lesion is covered by a black scab, it is called eschar. There is lymphadenopathy usually near the eschar.

Typical clinical course of tsutsugamushi disease and change of antibody titers to *R. tsutsugamushi* are presented in Fig. 4.

Seropositive rate among normal residents and wild rodents

It is possible to detect patients who have suffered from tsutsugamushi disease including subclinical infection with the past two years by checking antibody titers against the *R. tsutsugamushi*, because the antibody titers usually decline to less than 1 : 10 within two years after infection. Chang et al.(1989) surveyed the seropositivies among 9,384 healthy residents on July, 1987 and reported a seropositive rate of 4.7%. These results and geographical difference of seropositivities are shown in Table 4.

Seological survey in rodents was also performed by Lee et al.(Lee and Joo, 1989) during 1986-1987. This

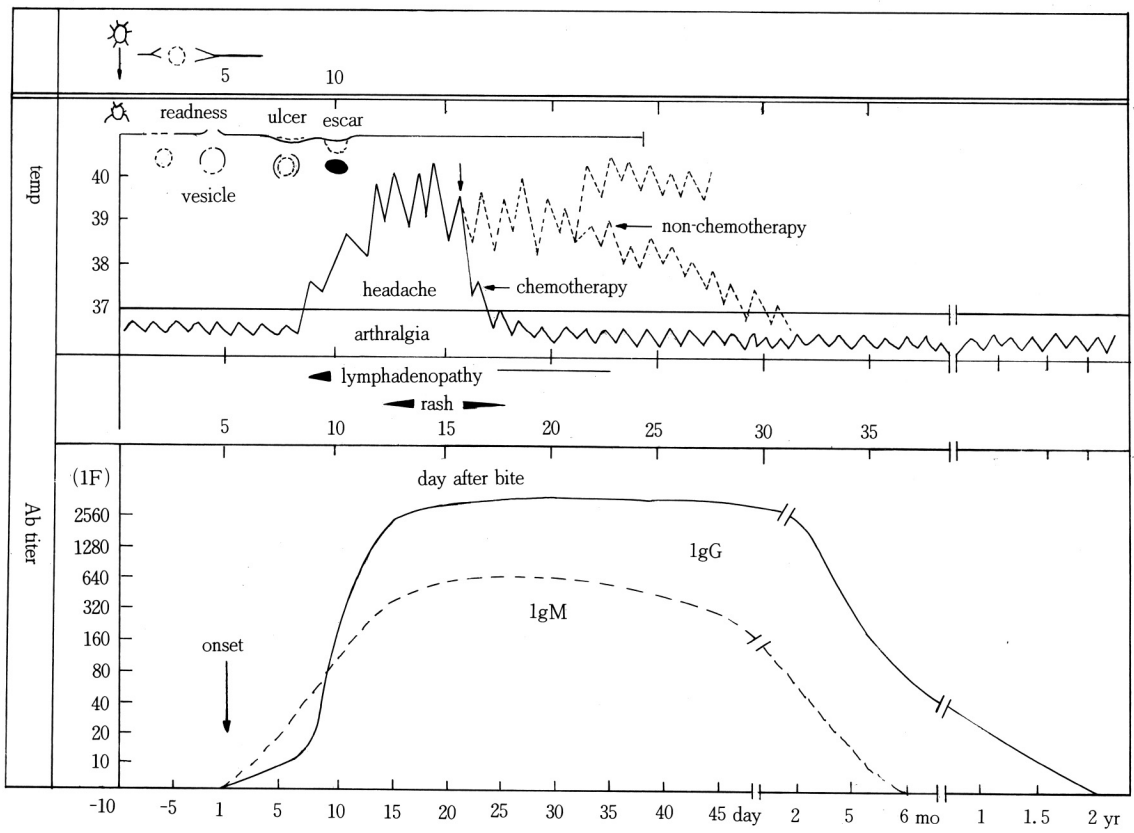

Fig. 4. Clinical course of tsutsugamushi disease and change of antibody titer to *R. tsutsugamushi*.

Table 3. Major clinical manifestation of tsutsugamushi disease.

clinical manifestation	incidence rate (number of positive/number of infection(%))								
	Kyungsang namdo(37)*	Chollabukdo (38)	Chungchung namdo(38)	Chungchung namdo(39)	Seoul(40)	Kyunggido (41)	Kangwondo(42)	Kyunsang bukdo(43)	Kyungsang namdo(44)
fever and chill	18/18(100)	38/38(100)	30/30(100)	75/75(100)	43/44(100)	21/24(88)	24/24(100)	23/26(88)	28/28(100)
myalcia	11/18(61)	35/38(92)	25/28(89)	62/75(83)	31/43(72)	9/24(38)	24/24(100)	17/26(65)	7/28(25)
headache	14/18(78)	33/38(87)	26/30(92)	75/75(100)	36/43(84)	17/24(71)	23/24(96)	14/26(54)	18/28(63)
backache		32/38(84)	13/27(48)		6/43(14)		19/24(79)		
nausea/vometing	6/18(33)	24/38(63)	15/26(57)	27/63(43)	14/43(33)	10/24(71)	17/24(71)	8(26)	11/28(40)
prostration		23/38(61)							
anorexia	4/18(22)	20/20(100)		33/63(52)					
cough/sputum	14/18(78)	18/38(47)	15/29(51)	22/75(29)	21/43(50)	7/24(29)	14/24(58)	2/26(8)	18/28(63)
abdomnal pain		16/38(42)	14/27(51)		10/43(23)	12/24(50)	9/24(38)	7/26(27)	15/28(54)
sore throat	5/18(28)	15/38(40)	17/27(62)		9/40(23)			3/26(12)	5/28(18)
ocular pain		13/38(34)	13/27(48)		3/38(8)				1/28(4)
arthralgia		12/38(32)	15/27(55)		12/35(34)	5/24(21)		4/26(43)	4/28(15)
dyspnea	2/18(11)	8/38(21)	1/27(4)		7/43(16)		16/24(67)	1/26(4)	2/28(7)
epistaxia		4/38(11)							
blood-tinged sputum		3/38(8)	2/26(7)		8/43(19)				
mental change	1/18(6)	1/38(3)				5/24(21)			
malaise		73/75(97)						4/26(15)	
dizziness		29/63(46)							
diarrhea	2/18(11)					6/24(25)		2/26(8)	
skin rash	15/18(83)	36/38(95)	29/30(96)	83/84(99)	16/44(36)	4/24(17)		18/26(69)	24/28(87)
eschar	14/18(78)	33/38(87)	28/30(93)	71/80(89)	23/44(52)	3/24(13)	11/24(46)	18/26(69)	24/28(87)
conjunctival injection	3/18(17)	30/38(79)	14/27(51)	31/78(40)	23/41(58)		15/24(63)	8/26(31)	24/28(87)
facial flushing		24/38(63)							
pharyngeal injection		21/38(55)						3/26(12)	
lymphadenopathy	2/18(11)	21/38(55)	15/28(53)	42/60(70)	10/44(23)	3/24(13)		2/26(8)	24/28(87)
CVA tenderness		15/38(40)	3/25(12)		7/41(17)	7/24(29)		4/26(15)	
bradycardia	6/18(33)	15/38(40)							
tachycadia	4/18(22)	12/38(32)				6/24(25)		2/26(8)	
hypotension	1/18(6)	6/38(16)				5/24(21)		3/26(12)	
hepatomegaly	1/18(6)	5/38(13)	3/26(11)		17/44(39)			2/26(8)	24/28(87)
facial edema		2/38(5)							3/28(11)
genelized edema		1/38(3)	9/27(33)		10/43(29)				
nuchal rigidity		1/38(3)						4/26(15)	
rales		1/38(3)							
splenomegaly			0/26(0)		6/44(14)		2/24(2)		
crackles			0/10(0)		14/43(33)				
convulsion									1/28(4)

* : Numbers in parenthesis indicates the number of reference.

Table 4. Seropositive rates of normal residents.

reporter	area	No. of samples	No. of positives	seropositivities	origin of samples
Chang *et al.*(9)	Kyungnam(Kuchang)	248	11	4.4	residents
	Chonnam(Wanjoo)	145	54	37.2	
	Kangwon(Wonsung)	255	13	5.1	
	Kangwon(Chulwon)	411	15	3.6	
	Cuhngnam(Kongjoo)	112	10	8.9	
	Chungnam(Boryong)	292	25	8.6	
	cheju island	226	22	9.7	

Table 5. Seropositive rates of wild rodents.

reporter	area	No. of samples	No. of positives	seropositivities (%)	origin of samples
Lee *et al.*(45)	house mice	139	7	5.0	rodents
	A. agrarius	275	162	58.9	
	Microtus spp.	9	7	77.7	

survey showed seropositive rates of the house mouse(5%), *Apodemus agrarius*(58.9%) and *Microtus* spp.(77.7 %)(Table 5), suggesting a high prevalence rate of tsutsugamushi disease in Korea.

Seropositive rate to *R. tsutsugamushi* among patient with acute febrile disease

Once tsutsugamushi diseases were confirmed serologically and bacteriologically(Lee et al., 1986; Lyi, 1986; Chang et al., 1987), many of acute febrile illnesses occurred in autumn have been diagnosed as tsutsugamushi disease. At that time, there were difficulties in differentiating tsutsugamushi disease from other acute febrile illnesses such as leptospirosis, murine typhus and hemorrhagic fever with renal syndrome due to similar clinical manifestations and there might be misdiagnosed cases of febrile illness.

Table 6. Seropositive cases to *R. tsutsugamushi* among patients with acute febrile episodes by year, 1986-1993.

province	No.of test	No.of positive	positive rate(%)
1986	1,141	215	18.8
1987	1,773	376	21.2
1988	1,761	544	20.9
1989	2,295	808	35.2
1990	2,921	777	26.6
1991	4,250	1,890	44.5
1992	5,668	2,116	37.3
1993	5,381	1,994	37.1

Cut-off point of seropositive ; 1 : 80

However, at present, tsutsugamushi disease is known as the most prevalent febrile illness in Korea. The nation-wide survey after 1986(Chang et al., 1989b, 1992, 1994) revealed that about 40% of febrill illnesses are diagnosed as tsutsugamushi disease.

1) Regional prevalence

Tsutsugamushi disease occurs in all regions of Korea including Cheju island, tsutsugamushi disease patients totaled 8,720 among 25,190 acute febrile patients between 1986 and 1993(Table 6). Prevalence was the highest in Kyungnam province(21.2%) followed by Chonnam, Chungnam, Kyungbuk, Chonbuk, and Kunggi province in order of decreasing prevalence(Table 7 and Fig. 5).

Table 7. Distribution of seropositive cases to *R. tsutsugamushi* among patients with acute febrile episodes by province in Korea, 1986-1993.

province	No.of test	No.of positive	Occurrence rate(%)
Seoul	2,489	342(13.7)	3.9
Kyunggi	5,087	1,001(19.7)	11.5
Kangwon	1,174	267(22.7)	3.1
Chungbuk	1,012	336(33.2)	3.9
Chungnam	2,488	1,135(45.6)	13.0
Kyungbuk	2,824	1,058(37.5)	12.1
Kyungnam	3,829	1,849(48.3)	21.2
Chonbuk	2,064	923(44.7)	10.6
Chonnam	2,295	1,273(55.5)	14.6
Cheju	182	87(47.8)	1.0
N.I.	1,746	449(25.7)	5.1

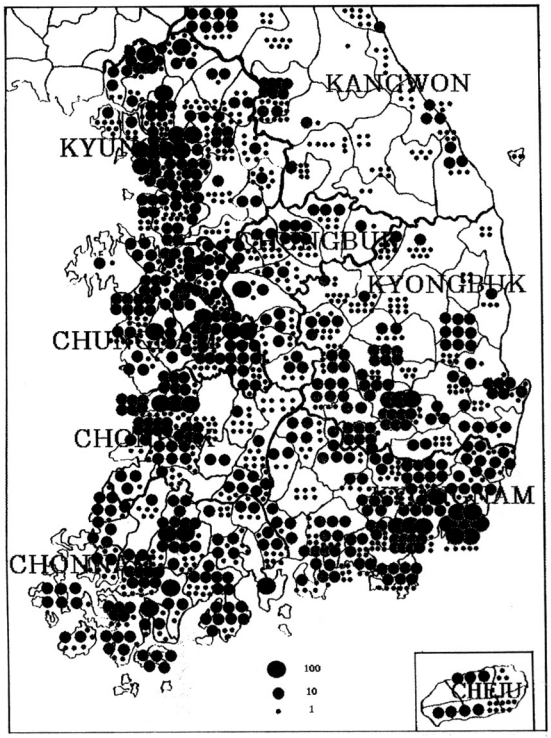

Fig. 5. Distribution of seropositive cases to *R. tsutsugamushi* among patients with acute febrile episodes by country in Korea, 1986-1993.

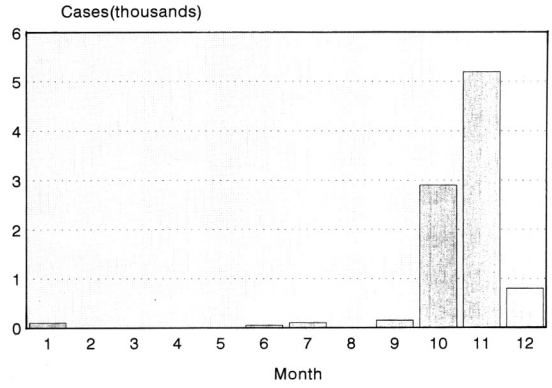

Fig. 6. Monthly distribution of seropositive cases to R. tsutsugamushi in Korea, 1986-1993.

2) Seasonal incidence

The months of peak incidence are October and November(90%) and December(7%) according to the analysis of 8,200 patients with tsutsugamushi disease. Although the incidence is low, there have been several reports in May, June, and July(Chang et al., 1989)(Fig. 6).

Antigens

R. tsutsugamushi is classified into Gram negative bacteria, but in contrast to other gram negative bacteria, *R. tsutsugamushi* possesses neither lipopolysaccharide nor glycoproteins(Amano et al., 1987). Major antigens of *R. tsutsugamushi* are known as protein antigens(Tamura et al., 1985). The electrophoresis analysis of protein profile using whole cell extracts of purified *R. tsutsugamushi* reveals about 30 major proteins. Among them, are 110, 70, 60, 56, 47, and 25 KDa proteins known as the major surface antigenic components(Urakami et al., 1986a, 1986b); 47 KDa and 70 KDa proteins are thought to be related to the group specific antigens due to cross reactivity of antisera against heterologous types and 56 proteins are related to the type specific antigens. The protein of 56 KDa is the most abundant protein in cell surface and shown a strong reactivity with most patients' sera. Moreover, most of type-specific monoclonal antibodies reacted with the 56 KDa protein and comparisons of nucleotide sequences of this protein genes supported the type specificity of the proteins(Tamura et al., 1985; Urakami et al., 1986b; Stover et al., 1990a, 1990b).

1) 56 KDa protein

Since the significance of the 56 KDa protein in serotype differentiation was recognized, molecular biologic approaches for analysis of its biologic function have been performed in several laboratories. A surface protein that was originally reported as 60 KDa(Hanson, 1985) identified as the same protein as the 56 KDa protein. The sequence analysis of 56 KDa protein encoding gene from various serotypes revealed that this protein is composed of 521-534 amino acids and four variable domains(VD) have been identified on the basis of amino acid sequence(Ohashi et al., 1992). These VD regions are thought to be important for serotype differentiation. In comparison of nucleotide and amino acid sequences, serotype Boryong has almost 100% homology to serotype Kuroki, and 89 % homology with serotype Karp. Kuroki strain is known as avirulent to mice whereas Boryong is highly virulent, suggesting that other pathogenic factors must be related to development of tsutsugamushi disease.

Computer analysis of nucleotide sequences revealed the phylogenetic relationships among various serotypes in genus Rickettsia(Fig. 7). In this result, Korean isolates Boryong are very closely relatied to Kuroki. These studies may contribute to the serotype classification from the taxonomical view point.

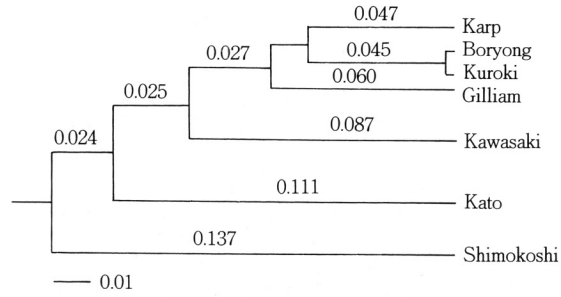

Fig. 7. Phyolgenetic tree of *R. tsutsugamushi* based on the nucleotide sequences of 56 KDa protein This tree was constructed with unweighted pair-group method of analysis in the program pachage Phylip. The numbers indicate evolutionary distances.

Diagnosis

Tsutsugamushi disease is initially suspected by the presence of clinical manifestations such as high fever, skin rash and eschar formation. However, laboratory tests are required to differentiate tsutsugamushi disease from other febrile diseases such as leptospirosis, hemorrhagic fever with renal syndrome, and other rickettsial diseases. The laboratory test being used for this purpose is usually based on the serological response of host to *R. tsutsugamushi*. However, other tests such as PCR method and isolation of *R. tsutsugamushi* based are tried.

1) Serodiagnosis

Serological method is the most commonly used diagnostic method in Korea. Usually diagonsis of tsutsugamushi disease is accomplished by demonstration of rising antibodies titer against *R. tsutsugamushi*. Serum antibodies are measured at 3 to 5 day intervals after the onset of illness and diagnosis of tsutsugamushi disease is made by demonstrating a fourfold or greater increase in antibody titer.

Different serotypes have different surface antigens and do not cross react with each other. Therefore, all different serotypes presenting in endemic area must be emplyed in serologic tests. In Korea, serotypes Boryong as well as Karp, Kato, Gilliam must be used as antigens for serological diagnosis.

(1) Immunofluorescence test

The indirect immunofluorescence(IF) test which has been reconmended as the standard diagnostic method by WHO is a reliable and widely used method(Bozeman and Elisberg, 1963).

(2) Immunoperoxidase test

This method has been developed by Suto(1980) using purified *R. tsutsugamushi* as an antigen. In contrast to IF test, this method does not require special equipment such as fluorescence microscope and dark room and long term storage of tested specimen is possible.

(3) Enzyme linked immunosorbent assay(ELISA)

Dash et al.(1979) developed the ELISA method using purified *R. tsutsugamushi* whole cell as antigen. Currently an ELISA method is available using recombinant 56 KDa protein, which was prepared by recombinant DNA technology(Kim et al., 1993a). The recombinant DNA technolgy has solved the problem of tedious antigen preparation.

ELISA method has several advantages in that the result is more objective and, due to automatization, the procedure has become simpler and more rapid in comparison to other serological methods. This method could be used for a mass-screening tool.

(4) Passive hemagglutination(PHA) test

This method has been developed by Kim et al.(Kim et al., 1993b) using purified 56 KDa recombinant protein which is chemically attached on the surface of sheep RBC. The PHA test has shown a high sensitivity and specificity and does not require any special equipment(microcope or reader). This method is appropriate for a diagnostic method in community based clinics.

2) PCR method

The PCR method is to detect *R. tsutsugamushi* DNA by amplification of specific target DNA sequence. High sensitivity of this method make it possible to detect *R. tsutsugamushi* in early stage of the illness when antibody titers are not high enough yet to be detected.

Currently available PCR methods are usually based on the 56 KDa protein gene which has been highly recommended as a reliable diagnostic protein gene(Kelly et al., 1990 ; Furuya et al., 1991). Furuya et al.(1993) reported a serotype specific PCR method ; serotypes could be determined without isolation of *R. tsutsugamushi*. Kee et al. (1994) reported a group-

specific PCR method based on 120 KDa protein gene.

3) Isolation of the causative agent

A definite diagnosis of tsutsugamushi disease requires detection of the causative agent. One may attempt to isolate the organism especially in the early stage of illness. However, rickettsial isolation may take too long a time to influence patient managememt and it requires experienced personnel. Isolation is of less value in the diagnosis of the illness than other diagnostic methods, but it is highly required for characterizaion of *R. tsutsugamushi* prevalent in the endemic area.

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