RICKETTSIA SPECIES AMONG TICKS IN AN AREA OF JAPAN ENDEMIC FOR JAPANESE SPOTTED FEVER

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Abstract. Rickettsia diseases, such as Japanese spotted fever, are serious infectious diseases. It is important to periodically survey tick species known for carrying pathogens for the presence of Rickettsia in endemic areas. We collected ticks from the field at 4 locations in the Ise-Shima areas of Mie Prefecture, Japan using a standard flagging method. The DNA samples were obtained from each tick using conventional polymerase chain reaction using primers for both the DNA sequences of the ticks and rickettsia. A total of 347 ticks were collected from the 4 study locations. The predominant species of tick found at 3 of 4 study sites was *Haemaphysalis longicornis* and at the first study site. There was a heterogeneous mix of tick species without a predominant type. Various *Rickettsia* species were found in 80% of the *H. longicornis* ticks but *Rickettisia japonica*, the etiology of Japanese spotted fever, was not detected in any of the studied ticks. Our findings suggest the presence of *R.japonica* is low in the studied areas among the studied ticks.

Keyword: Japanese spotted fever, *Rickettsia japonica*, *Rickettsia tamurae,* endemic area, *Haemophysalis longicornis*

INTRODUCTION

The Japanese spotted fever (JSF) is a serious infectious disease caused by infection with *Rickettsia japonica* (Kondo *et al*, 2010). More than 30 cases of JSF were reported per year in the Ise-Shima areas of Mie Prefecture, Japan during an eight consecutive year period (https://www.pref.mie.lg/jp/Hokan/hp/). JSF develops after the bite of a tick carrying *R. japonica*. The ticks: *Haemaphysalis longicornis* (H.l) (Uchida *et al*, 1995, Ishikura *et al*, 2003, Tabara *et al*, 2011). *Dermacentor taiwanesis* (Fournier *et al*, 2002) and *Haemaphysalis flava* (H.fl) (Fournier *et al*, 2002) have been reported to carry *R. japonica.* Ticks have different species-specific behaviors and life cycles (https://www.cdc.gov/ticks/life_cycle_and_hosts.html). A step in the prevention of JSF is identification of the ticks that carry *R.japonica*. We surveyed ticks known to carry *R.japonica* in an area endemic for JSF in Mie Prefecture, Japan to determine the species of tick associated with JSF and the prevalence of *R.japonica* in the surveyed ticks.

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MATERIALS AND METHODS

Field surveillance

The field surveillance for the studied ticks were conducted in 4 areas of Ise-Shima from November 2014 to February

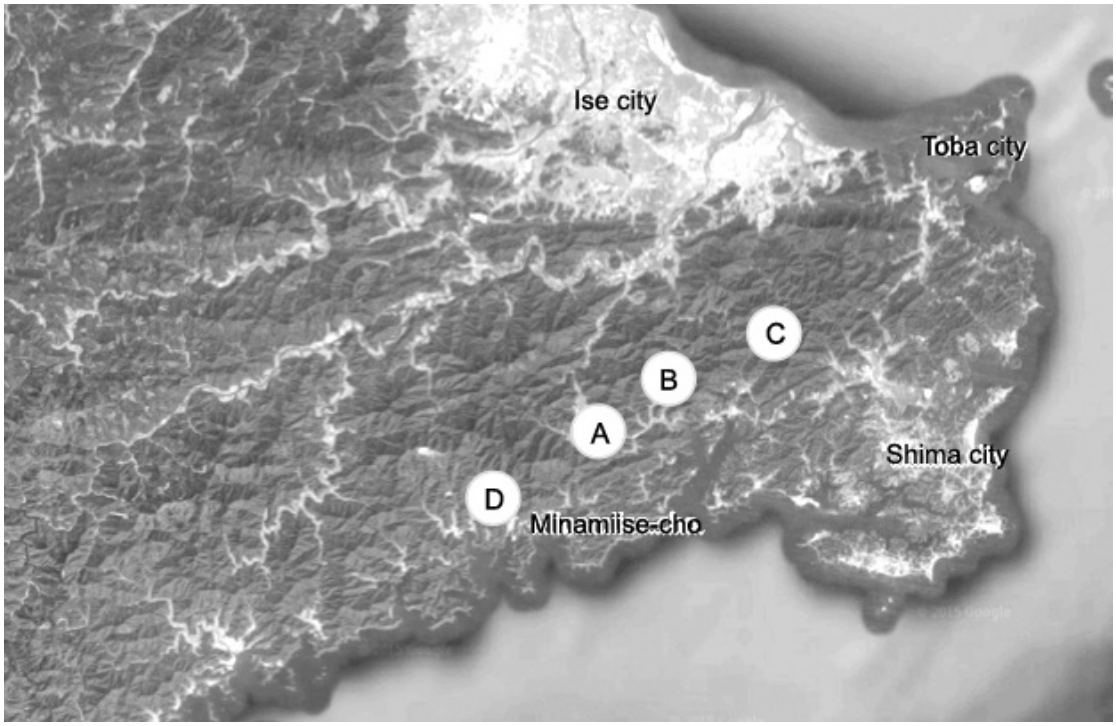

Fig 1–Study areas.

2015 (Fig 1). Area A was a grass field covered with the fallen leaves along the road; Area B was a field of short grass in an orange orchard; Area C was an observation deck for the Fall with many visitors; Area D was a field along an animal trail in a forest. Deer spoor was found in Area D; many cases of JSF had been reported among the residents of nearby villages. The ticks were captured using a standard flagging method. Sampling flag consisted of a 1 m^2 piece of white cotton flannel attached to a 1.5 m wooden dowel (Rulison *et al*, 2013). The collected ticks were placed in 10% ethanol and stored at -80°C until examined.

Detection of *Rickettsia* in surveyed ticks

DNA was extracted from each tick collected using a QIAamp DNA mini kit (QIAGEN, Germantown, MD) according to manufacturer's instructions. The tick specific DNA sequence was amplified by a conventional polymerase chain reaction (PCR) using tick species-specific identification primers (Ushijima *et al*, 2003). The outer membrane of any possible rickettsia protein A (OmpA) was amplified using a specific primer set (Forward: 5′-CAACAAGGTCTTAAAGCCGC-3′ and Reverse: 5′-AGCATTCACTCCCCCTAAAG-3′) we designed. The PCR product sequences were analyzed at Eurofins Genomics (Tokyo, Japan) to detect tick and rickettsia DNA.

RESULTS

A total of 347 ticks were collected (146 larvae, 193 nymphs and 8 adults). A total of 7 species were identified. The distribution and types of ticks found are shown in Table 1. *Haemaphysalis longicornis* was the most common species of tick

Table 1
The numbers and species of ticks captured in the study areas.

Area	Total number	Tick species	Tick growth stages		
			Larva	Nymph	Adult
A	100	H.m	4	1	0
		H.l	64	31	0
		Total	68	32	0
B	67	H.c	0	1	0
		H.l	5	59	0
		H.fl	0	2	0
		Total	5	62	0
C	97	A.t	0	0	1
		H.l	48	41	0
		H.m	2	3	0
		H.c	0	2	0
		Total	50	46	1
D	83	H.fo	0	1	0
		H.l	0	1	0
		H.m	6	34	1
		H.fl	15	2	1
		A.t	1	13	5
		H.j	1	2	0
		Total	23	53	7

H.l, *Haemaphysalis longicornis*; H.m, *Haemaphysalis megaspinosa*; H.fl, *Haemaphysalis flava*; H.c, *Haemaphysalis cornigera*; A.t, *Amblyomma testudinarium*; H.j, *Haemaphysalis japonica*; H.fo, *Haemaphysalis formosensis*.

found in areas A, B and C and the most common species of tick found in Area D was *Haemaphysalis megaspinosa* (H.m). The rickettsia DNA found in the collected ticks is summarized in Table 2. *Rickettsia lon* (R. lon) was the most frequently isolated species of *Rickettsia* in our study and most frequently found in H.l in all 4 study areas. *Rickettsia tamurae* has been reported to be carried primarily by *Amblyomma testudinarium* (A.t) (Fournier *et al*, 2006), but it was detected in H.l in our study as well. No *R. japonica* was detected in any of the collected ticks in our study.

DISCUSSION

Various pathogenic species of *Rickettsia* have been reported in Japan including *R. japonica* (Kondo *et al*, 2010), *R. tamurae* (Imaoka *et al*, 2011) and *R. heilongjiangensis* (Ando *et al*, 2010). Delay in diagnosis and treatment of JSF caused by *R. japonica* can result in life-threating liver disease and disseminated intravascular coagulation (DIC) (Kondo *et al*, 2010). It is important to know the distribution of rickettsia-carrying ticks in JSF endemic areas. *R. japonica*, the cause of JSF, was not detected in our study. *R.tamurae* is a human pathogen causing mild general symptoms (Imaoka *et al*, 2011). *R. tamurae* was detected in a small percentage of ticks in our study.

H.l. has been reported to be a competent host for *R. japonica* (Uchida *et al*, 1995;

Table 2
Prevalence of *Rickettsia* species by tick type at study location.

Study Area	No. (%) of studied ticks positive for *Rickettsia*	Number of ticks	Tick speies	Rickettsia	Tick types positive for *Rickettsia* species		
					Larva	Nymph	Adult
A	83/95 (87.4)	95	H.l	R.L	55	28	0
	1/5 (20)	5	H.m	R.L	1	0	0
B	59/64 (92)	64	H.l	R.L	5	54	0
	0/2 (0)	2	H.fl	R.sp	0	0	0
	0/1 (0)	1	H.c	R.sp	0	0	0
C	64/89 (71.9)	89	H.l	R.L	27	35	0
				R.T	2	0	0
	1/5 (20)	5	H.m	R.L	0	1	0
	0/2 (0)	2	H.c	R.sp	0	0	0
	0/1 (0)	1	A.t	R.sp	0	0	0
D	1/1 (100)	1	H.l	R.L	0	1	0
	7/40 (17.5)	40	H.m	R.L	3	4	0
	0/18 (0)	18	H.fl	R.sp	0	0	0
	6/19 (31.2)	19	A.t	R.L	1	3	0
				RT	0	0	2
	2/3 (66.7)	3	H.j	R.L	1	1	0
	0/1 (0)	1	H.fo	R.sp	0	0	0

H.l, *Haemaphysalis longicornis*; H.m, *Haemaphysalis megaspinosa*; H.fl, *Haemaphysalis flava*; H.c, *Haemaphysalis cornigera*; A.t, *Amblyomma testudinarium*; H.j, *Haemaphysalis japonica*; H.fo, *Haemaphysalis formosensis*; R.L, *Rickettsia lon* type; R.T, *Ricekttsia tamurae*; R.sp, *Rickettsia* species.

Ishikura *et al*, 2003; Tabara *et al*, 2011). H.l was the most common tick in study areas A, B and C. Rickettsia DNA was found in 80% of H.l ticks collected in our study, suggesting it as an effective carrier of *Rickettsia* species, mostly of the R. lon type.

A variety of tick species were detected in area D, an area known as endemic for Rickettsia infections. *R. japonica* was not detected in any area, including area D, which is endemic for JSF, suggesting the sample size may have been too small or the study site inappropriate. Further studies with larger sample sizes in other areas known to be endemic for JSF are needed to determine of the presence of *R.japonica* carrying ticks and their species.

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