Parasitology **Full Paper** **Revised**

20 July 2005

Detection of Rickettsial DNA in Ixodid Ticks Recovered from Dogs and Cats in Japan

Hiroko HIRAOKA[1)], Yojiro SHIMADA[2)], Yoshimi SAKATA[3)], Malaika WATANABE[1)], Kazuhito ITAMOTO[1)], Masaru OKUDA[1)] and Hisashi INOKUMA* [4)]

[1)] Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, [2)] Nippon Zenyaku Kogyo Co., Ltd, Koriyama, Fukushima 963-0196, [3)] Merial Japan Ltd., Tokyo 100-0014 and [4)] Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan

Correspondence:

* To whom all correspondence and reprint requests should be addressed.

Hisashi INOKUMA

TEL/FAX (+81)-155-49-5370

e-mail: inokuma@obihiro.ac.jp

Running Head: RICKETTSIA IN TICKS FROM DOGS AND CATS

ABSTRACT. DNA from ticks recovered from 1137 dogs and 133 cats from all over Japan were examined for Rickettsia infection by citrate synthase gene (gltA)-based PCR and partial nucleotide sequencing. A total of 91 dog tick samples and 18 cat tick samples showed a single band of the appropriate size in the nested PCR. Sequence analysis was successfully performed on 102 samples. DNA of Rickettsia japonica or closely related Rickettsia spp. strains were detected from 38 ticks in 16 prefectures mainly in western Japan. The other 33, detected from 13 prefectures including Hokkaido and Okinawa, were found to be Rickettsia helvetica or closely related strains. A total of 29 DNA that showed highest homology with Rickettsia akari or closely related strains were detected in 19 prefectures, widespread throughout Japan. Rickettsia canada-like DNA was detected from Haemaphysalis sp. removed from a dog in Fukuoka, and 'Candidatus Rickettsia tarasevichiae'-like DNA was from Ixodes sp. removed from a dog in Hokkaido.

KEY WORDS: canine, feline, epidemiology, Rickettsia, tick.

要約

全国の犬と猫から回収されたマダニからのリケッチアDNAの検出 ----------

平岡 博子[1)], 島田 洋二郎[2)], 坂田 義美[3)], 渡辺 麻麗香[1)], 板本 和仁[1)], 奥田 優[1)], 猪熊 壽[4)] ([1)]山口大学農学部、[2)]日本全薬工業(株)、[3)]メリアル・ジャパン(株)、[4)]帯広畜産大学畜産学部)----------

クエン酸合成酵素遺伝子配列に基づいて設計されたリケッチア属特異的 nested PCR を用いて全国の犬1137 頭と猫 133 頭から回収されたマダニからリケッチア DNA を検出した。陽性検体については PCR 産物の塩基配列を決定した。犬由来マダニ91 検体, 猫由来マダニ18 検体がPCR陽性を示し, うち 102 検体の遺伝子解析が成功した。西日本の 13 府県から検出された 38 検体が Rickettsia japonica またはその近縁種と, 北海道と沖縄を含む 13 道府県から検出された 33 検体が Rickettsia helvetica またはその近縁種と, また北海道から鹿児島の 19 都道府県から検出された 29 検体が Rickettsia akari またはその近縁種とそれぞれ高い相同性を示した。さらに福岡県の犬由来チマダニ属マダニからは Rickettsia canada 近縁種、また北海道の犬由来マダニ属マダニからは‘Candidatus Rickettsia tarasevichiae’近縁種のそれぞれ DNA 断片が検出された。

Humans and animals are often exposed to a large number of tick species, depending on the distribution of these arthropod vectors in the environment. Recently, interest in ticks of domestic animals has been increasing, because of emerging and re-emerging tick-borne diseases and their zoonotic nature, including rickettsial, bacterial and protozoal pathogens. Rickettsiae belong to the order Rickettsiales and are obligate intracellular, gram-negative bacteria. Several species cause disease in humans and other animals and have a worldwide distribution. This genus was subdivided into three groups, the typhus group (TG), the ancestral group and the spotted fever group (SFG) on the basis of phenotypic criteria [2].

In Japan, Rickettsia japonica, classified into the SFG Rickettsia, was discovered to be the causative agent of Japanese spotted fever (JSF) [7]. The JSF patients have been mainly identified in the western part of Japan since 1984, when the first patient was reported in Tokushima prefecture. Recently, many Rickettsia spp., including Rickettsia helvetica and Rickettsia felis, have been detected from ticks in Japan [2, 6]. However, this wide variety of Rickettsia species in Japan has been examined only in limited localities and the overall distribution of Rickettsia in Japan has not yet been determined. In this study, the detection and analysis of Rickettsia species from ticks recovered from domestic dogs and cats all over Japan was attempted using molecular methods including PCR and sequence analysis of the citrate synthase gene (gltA).

MATERIALS AND METHODS

Ticks and extraction of DNA: A total of 4122 ticks were recovered from 1137 domestic dogs and a total of 287 ticks were recovered from 133 domestic cats from 47 prefectures in Japan and stored in 70% ethanol for morphological identification [12, 13]. After identification, one tick per dog or cat was selected for screening analysis. All the ticks selected were semi- or fully-engorged adult females or nymphs. Total DNA was extracted with a method described previously [4].

Amplification and sequencing of rickettsial gltA gene: A nested PCR was used to detect

rickettsial gltA gene fragments from the ticks. The first PCR amplification was performed in a 25-μl reaction mixture containing 5 μl of each DNA template with a genus specific primer set, RpCS.877p and RpCS.1273r [11]. A 1:100 dilution of the first PCR product with DW, was used as a template for the second PCR, using an inner primer set; RpCS.896f (GGC TAA TGA AGC GGT AAT AA), PpCS.1258n (ATT GCA AAA AGT ACA GTG AAC), that was designed based on the common sequence of the gltA of the genus Rickettsia. The first and second PCR were carried out under the following conditions: 35 cycles of denaturation (94 C, 60 sec), annealing (54 C, 60 sec) and extension (72 C, 90 sec). The second PCR product was electrophoresed at 100V in 2% agarose gel (Wako Chemicals Ind) for 30 min, stained with ethidium bromide, and verified by UV illumination. The 363bp PCR product with a positive reaction was purified using the QIA PCR purification kit (QIAGEN) for direct sequence analysis with a Perkin-Elmer ABI Prism 377 automated DNA sequencer at the DNA Core Facility of the Center for Gene Research, Yamaguchi University.

Sequence analysis: The sequence data of the PCR products was analyzed using the BLAST 2.0 program (National Center for Biotechnology Information) for homology search. The determined sequences were then analyzed for phylogenetic relationships with other sequences registered in GenBank. Multiple alignment analysis, the determination of pair-wise percent identities of the sequences, distance matrix calculations and the construction of phylogenetic trees were all performed with the ClustalW program version 1.8 in the DNA data bank of Japan. Tree figures were generated using the Tree View program version 1.6.6. The GenBank accession numbers of the gltA gene sequences of other species used to analyze the data are as follows: R. prowazekii, M17149; R. japonica, U59724; R. akari, U41752; R. felis, U33922; R. slovaca, U59725; R. conorii, U59730; R. cadada, U59713; R. honei, AF022817; R. helvetica, U59723; R. australis, U59718; R. montana, U74756; R. massiliae, U59719; 'Candidatus Rickettsia tarasevichiae', AF503167; Rickettsia sp. AT1, AB114796; Rickettsia sp. In56, AB114819; Rickettsia sp. IO1, AB114797; Rickettsia sp. IP2, AB114801; Rickettsia sp. Hf151, AB114815;

Rickettsia sp. IM1, AF394900; Rickettsia sp. FLA1, AF394898; Rickettsia sp. DT1, AF394897.

RESULTS

DNA was successfully extracted from a total of 1137 dog ticks and 133 cat ticks. A total of 91 dog tick samples and 18 cat tick samples showed a single band of the appropriate size in the nested PCR. The dogs and cats infested with these particular ticks did not show any clinical signs of rickettsial infection such as fever, rush and arthritis. The peripheral blood of these dogs and cats was not available for analysis.

Approximately 322bp of the rickettsial gltA sequences excluding the primer region were determined. A total of 102 PCR products were successfully analyzed for the partial nucleotide sequence (GenBank accession numbers; AB204416 to AB204517). The determined sequences were divided into 4 groups based on their homologies, obtained through BLAST and phylogenetic analyses (Fig1). A total of 38 ticks were classified into the same group as R. japonica and related species, including strains FLA1, DT1 and Hf151 which have been recently detected from ticks in Japan [5, 6] (Group1). These rickettsial DNA fragments showed highest homology of 99.1 to 100% with R. japonica or one of the related strains FLA1, DT1 and Hf151. The other 33 ticks that showed 99.3 to 100% nucleotide sequence homology with R. helvetica, or closely related strains IM1 and IP2, and IO1 detected from *Ixodes* ticks in Japan, were clustered into Group 2. A total of 29 ticks were clustered into Group 3. This cluster contains 1 tick that showed 100% homology with Rickettsia sp. AT1, closely related to R. akari detected in Japan [6], and 28 ticks that showed 99.1 to 100% homology with Rickettsia sp. In56 [5]. One of the remaining 2 ticks (Group4) showed highest homology of 99.1% with R. canada, and another showed 99.8% homology with 'C. R. tarasevichiae' [14].

The distribution of the rickettsial genes detected in this study is summarized in Table 1 and Fig.2. The rickettsial DNA in Group1 was detected from 15 prefectures mainly in western Japan. Group2 rickettsial DNA was detected from 13 prefectures from Hokkaido to Okinawa prefectures.

Group3 rickettsial DNA was detected from 19 prefectures from Hokkaido to Kagoshima. In Group4, R. canada-like rickettsial DNA was detected in Fukuoka Prefecture, and 'C. R. tarasevichiae'-like DNA was from Hokkaido.

The information on tick stages and host animals for the positive tick samples are summarized in Table 1. Rickettsial DNAs in Group1 were detected from 22 H.longicornis (7 females, 3 males and 12 nymphs), 8 H. flava (4 females, 2 males and 2 nymphs), 1 female of H. campanulata, 5 Haemaphysalis sp. (2 females, 2 nymphs and 1 larva) and1 female of Ixodes sp. Most of them were recovered from dogs and only two H. longicornis were from cats. Group2 rickettsial DNAs were from 10 H. longicornis (6 females and 4 nymphs), 2 females of H. flava, 2 females of H. campanulata, 9 Haemaphysalis sp. (4 females and 5 nymphs), 2 females of I. ovatus, 1 female of I. persulcatus, 5 females of Ixodes sp. and 2 Rhipicephalus sanguineus (1 female and 1 nymph). Only one I. persulcatus tick was recovered from a cat and the rest of them were recovered from dogs. DNAs in Group3 were detected from 14 females of I. nipponensis (7 from dogs and 7 from cats), 4 females of I. ovatus (1 from a dog and 3 from cats), 4 H. flava (3 females and 1 nymph, 3 from dogs and 1 from a cat), 3 females of H. longicornis (2 from dogs and 1 from a cat), 2 Ixodes sp. (1 female and 1 larva from 2 dogs) and 2 Haemaphysalis sp. (1 female from a dog and another from a cat). In Group4, R. canada-like rickettsial DNA was detected from a female Haemaphysalis sp. recovered from a dog, and 'C. R. tarasevichiae'-like DNA was from a female Ixodes sp. recovered from a dog. Most of the unidentified ticks were partly- or fully-engorged larvae or nymphs.

DISCUSSION

In the present study, ticks recovered from dogs and cats were used for the epidemiological study of Rickettsia in Japan. One tick was selected from each dog and cat for screening purposes. All the selected ticks were fully- or semi-engorged adults or nymphs, meaning they contained peripheral blood of the canine or feline host. Thus, a positive PCR result would lead to two

possibilities of infection, either the tick or the animal host. From the nested PCR results, 109 samples were positive and these ticks were widely distributed throughout Japan. Successfully determined 102 rickettsial gltA sequences were divided into 4 groups based on their sequence analyses.

In Group1, rickettsial DNAs closely related to R. japonica or Rickettsia spp. strains DT1 FLA1, and Hf151, were detected from 38 ticks, mainly in western Japan. DT1 and FLA1, isolated from Dermacentor taiwanensis and H. flava, respectively, were found to be closely related to R. japonica [6]. Rickettsia sp. Hf151 detected from H. flava is thought to be a new species of the SFG rickettsiae and is particular to Japan [5]. It is not clear whether the Rickettsia spp. strains DT1, FLA1 and Hf151 are pathogenic to humans and/or animals. As R. japonica is a well known pathogen of Japanese spotted fever in Japan [7], it is important to distinguish R. japonica from other related strains. Unfortunately the 322bp of the rickettsial gltA analyzed in this study was too short to determine the strains accurately. More sequence analyses using other genes should be carried out for this purpose. However, in the present study, rickettsial DNAs of R. japonica or closely related strains were detected from several prefectures, including Tokyo, Ishikawa, Kyoto, Nara, Okayama and Tottori, where the pathogens or JSF patients have never been detected before [5, 6, 8]. D. taiwanensis and H. flava are confirmed vectors of R. japonica [6] and H. longicornis is thought to be a potential vector of the pathogen [15]. In this study, 57.9% of Group1 rickettsial DNA was detected from H. longicornis, the predominant tick species of dogs and cats. These results suggest that H. longicornis may be a vector of R. japonica and closely related strains.

In Group2, R. helvetica and closely related strains IM1, IP2 and IO1, are included [2, 6]. In the present study, 33 detected DNA were found to be R. helvetica or closely related strains. The DNA was detected from Hokkaido, northern Japan, to Okinawa, southern most islands. Previously R. helvetica was known to exist only in European countries [1]. Recently, several Rickettsia spp. closely related to R. helvetica were detected from Ixodes ticks in Hokkaido, Akita, Fukushima, Kumamoto and Kagoshima prefectures [2, 6]. R. helvetica and its close strains were found to be

more widespread in Japan than previously reported. It is possible that this group of Rickettsia might be more widely distributed than expected. Furthermore, most of the R. helvetica and related DNA were detected from Haemaphysalis ticks in this study, although suspected vectors of R. helvetica and related species are Ixodes spp [2]. Whether or not Haemaphysalis is a vector of R. helvetica or closely related strains in Japan remains to be seen, because it is not clear whether the rickettsial DNA came from the tick or the host. DNA fragments of R. helvetica-like strains were also detected from 2 Rh. sanguineus ticks recovered from 2 dogs in Okinawa, in this study. This is the first record where DNA of R. helvetica or a close strain was detected from Rh. sanguineus and also the first such finding in the sub-tropics. Rh. sanguineus is a tick that prefers canine hosts and is known to be a vector of R. conorii, an etiological agent of Mediterranean spotted fever [9]. However, R. conorii was not detected in this study.

R. akari is a pathogen of rickettsial pox and has been detected in the U.S.A., Ukraine, Korea and Slovenia [10]. Although R. akari has never been detected in Japan, Rickettsia spp. strains AT1 and In56 that are closely related to R. akari have recently been detected in Japan [5, 6]. The pathogenicity of these strains, however, remains to be elucidated. In the present study, 1 rickettisal DNA which showed 100% nucleotide sequence identity to strain AT1 and 28 rickettsial DNA which showed high nucleotide sequence identity to strain In56, was identified and categorized into Group 3. Rickettsial DNA in Group 3 was widely distributed from northern to southern Japan. More than half of the R. akari and related DNA were detected from Ixodes nipponensis and Ixodes ovatus ticks, in this study. These ticks may be possible vectors of this agent. Furthermore, cats were more related to this Rickettsia than other Rickettsia species, although direct evidence is required to demonstrate the relationship between cats, Ixodes spp. and this group of Rickettsia.

R. canada is widespread in northern America and its vector is H. leptorispalustris. The pathogenesis of R. canada is not clear, but there is a report that R. canada causes a Rocky Mountain spotted fever-like disease and acute cerebrovasculitis in humans [3]. R. canada or a related species has never been detected or isolated in Japan, thus this is the first record of this Rickettsia species in

Japan. In this study, a DNA fragment similar to R. canada was detected from a tick recovered from a dog in Fukuoka prefecture, but the origin and history of overseas travel of the dog were both unknown. Another R. canada-like DNA was detected from an Ixodes sp. tick recovered from a dog in Hokkaido. The gltA sequence showed 99.8% similarity with 'C. Rickettsia tarasevichiae' which was recently detected from I. persulcatus in Russia [14]. In Russia, 9.27 to 20.5% of I. persulcatus have 'C. R. tarasevichiae', but its pathogenicity is still unclear [16]. 'C. Rickettsia tarasevichiae' has never been detected or isolated outside of Russia, including Japan. The dog from which the tick was recovered showed no symptoms except for heavy tick infestation. Although the origin and history of overseas travel of this dog were unknown, it is possible that 'C. Rickettsia tarasevichiae' is present in northern Japan where the tick vector I. persulcatus can be found [12].

Four groups of Rickettsiae, including possible new pathogens, were detected in this study. Although more epidemiological studies are required to clarify the distribution, vectors, and carriers of the *Rickettsia*, dogs and cats may be possible carriers of the agents. It is also important for both medical and veterinary workers to be aware of the variety of rickettsial pathogens present in Japan.

ACKNOWLEDGMENTS. The authors would like to acknowledge the technical expertise of the DNA Core facility of the Center for Gene Research, Yamaguchi University, supported by a Grant-in-aid from the Ministry of Education, Science, Sports and Culture of Japan. They would also like to thank Nippon Zenyaku Kogyo Co., Ltd. for collecting and sending the tick samples. This work was supported in part by Merial Japan Ltd., National Institute of Animal Health, and a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

REFFERENCES

1. Fournier, P. E., Dumler, J. S., Greub, G., Zhang, J., Wu, Y. and Raoult, D. 2003. Gene sequence-based criteria for identification of new Rickettsia isolates and description of Rickettsia heilongjiangensis sp. nov. J. Clin. Microbiol. **41**: 5456-5465
2. Fournier, P. E., Fujita, H., Takada, N. and Raoult, D. 2002. Genetic identification of Rickettsiae isolated from ticks in Japan. J. Clin. Microbiol. **40**: 2176-2181.

3. Hechemy, K. E., Fox, J. A., Groschel, D. H. M., Hayden F. G. and Wenzel, R. P. 1991. Immunoblot studies to analyse antibody to the Rickettsia typhi group antigen in sera from patients with acute febrile cerebrovasculitis. J. Clin. Microbiol. **29**: 2559-2565.

4. Inokuma, H., Yoshizaki, Y., Shimada, Y., Sakata, Y., Okuda, M. and Onishi, T. 2003. Epidemiological survey of Babesia species in Japan performed with specimens from ticks collected from dogs and detection of new Babesia DNA closely related to Babesia odocoilei and Babesia divergens DNA. J. Clin. Microbiol. **41**: 3494-3498.

5. Ishikura, M., Ando, S., Shinagawa, Y., Matsuura, K., Hasegawa, S., Nakayama, T., Fujita, H. and Watanabe, M. 2003. Phylogenetic analysis of spotted fever group rickettsiae based on gltA, 17-kDa, and rOmpA genes amplified by PCR from ticks in Japan. Microbiol. Immunol. **47**: 823-832.

6. Ishikura, M., Fujita, H., Ando, S., Matsuura, K. and Watanabe, M. 2002. Phylogenetic analysis of spotted fever group rickettsiae isolated from ticks in Japan. Microbiol. Immunol. **46**: 241-247.

7. Mahara, F., Koga, K., Sawaga, S., Taniguchi, T., Shigemi, F., Suto, T., Tsuboi, Y., Ooya, A., Koyama, H.,Uchiyama, T. and Uchida, T. 1985. The first report of the rickettsial infection of spotted fever group in Japan: three clinical cases. Jpn. J. Assoc. Infect. Dis. **59**: 1165-1172.

8. Noda, S., Yamamoto, S. and Honda, T. 2004. Detection of Japanese spotted fever rickettsiae DNA from ixodid ticks and seasonal occurrence of ixodid tick population on the Osumi Peninsula, Kagoshima Prefecture. Med. Entomol. Zool. **55**: 21-28.

9. Perter, O., Raoult, D. and Gilot, B. 1990. Isolation by a sensitive centrifugation cell culture system of 52 strains of spotted fever group rickettsiae from ticks collected in France. J. Clin. Microbiol. **28**: 1597-1599.

10. Roux, V. and Raoult, D. 1997. Global genetic diversity of spotted fever group rickettsiae. pp. 53-62. In: Rikettsial Infection and Immunity.

11. Roux, V., Rydkina, E., Eremeeva, M. and Raoult, D. 1997. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. Int. J. Syst. Bacteriol. **47**: 252-261.

12. Shimada, Y., Beppu, T., Inokuma, H., Okuda, M. and Onishi, T. 2003. Ixodid tick species recovered from domestic dogs in Japan. Med. Vet. Entomol. **17**: 38-45.

13. Shimada, Y., Inokuma, H., Beppu, T., Okuda, M. and Onishi, T. 2003. Survey of ixodid tick species on

domestic cat in Japan. Vet. Parasitol. **111**: 231-239.

14. Shpynov, S., Fournier, P. E., Rudakov, N. and Raoult, D. 2003. "Candidatus Rickettsia tarasevichiae" in Ixodes persulcatus ticks collected in Russia. Ann. N. Y. Acad. Sci. **990**: 162-172.

15. Uchida, T., Yan, Y. and Kitaoka, S. 1995. Detection of Rickettsia japonica in Haemaphysalis longicornis ticks by restriction fragment length polymorphism of PCR product. J. Clin. Microbiol. **33**: 824-828

16. Wenzel, R. P., Hayden, F. G., Groschel, D. H., Salata, R. A., Young, W. S., Greenlee, J. E., Newman, S., Miller, P. J., Hechemy, K. E. and Burgdorfer, W. 1986. Acute febrine cerebrovasculitis: a syndrome of unknown, perhaps rickettsial, cause. Ann. Med. Intern. **104**: 606-615.

Figure legends

Fig.1. Phylogenetic relationship of various Rickettsia spp. registered in GenBank and 102 partial sequences determined in this study based on the nucleotide sequences of citrate synthase gene. The neighbor-joining method was used to construct the phylogenetic tree with the Clustal W program. The scale bar represents 1 % divergence.

Fig.2. The distribution of rickettsial DNA detected from ticks removed from dogs and cats in Japan. Prefectures that showed positive samples are in black in (A) R. japonica and related species, (B) R. helvetica and related species, (C) R. akari and related species, and (D) R. canada and related species. Each number represents a prefecture. 1: Hokkaido, 2: Aomori, 3: Iwate, 4: Akita, 5; Yamagata, 6: Miyagi, 7: Fukushima, 8: Tochigi, 9: Gunma, 10: Ibaraki, 11: Saitama, 12: Chiba, 13: Tokyo, 14: Kanagawa, 15: Yamanashi, 16: Nagano, 17: Shizuoka, 18: Niigata, 19: Toyama, 20: Ishikawa, 21: Fukui, 22: Gifu, 23: Aichi, 24: Shiga, 25: Mie, 26: Kyoto, 27: Osaka, 28: Nara, 29: Wakayama, 30: Hyogo, 31: Tottori, 32: Okayama, 33: Shimane, 34: Hiroshima, 35: Yamaguchi, 36: Kagawa, 37: Tokushima, 38: Kochi, 39: Ehime, 40: Fukuoka, 41: Saga, 42: Nagasaki, 43: Ohita, 44: Kumamoto, 45: Miyazaki, 46: Kagoshima, 47: Okinawa.

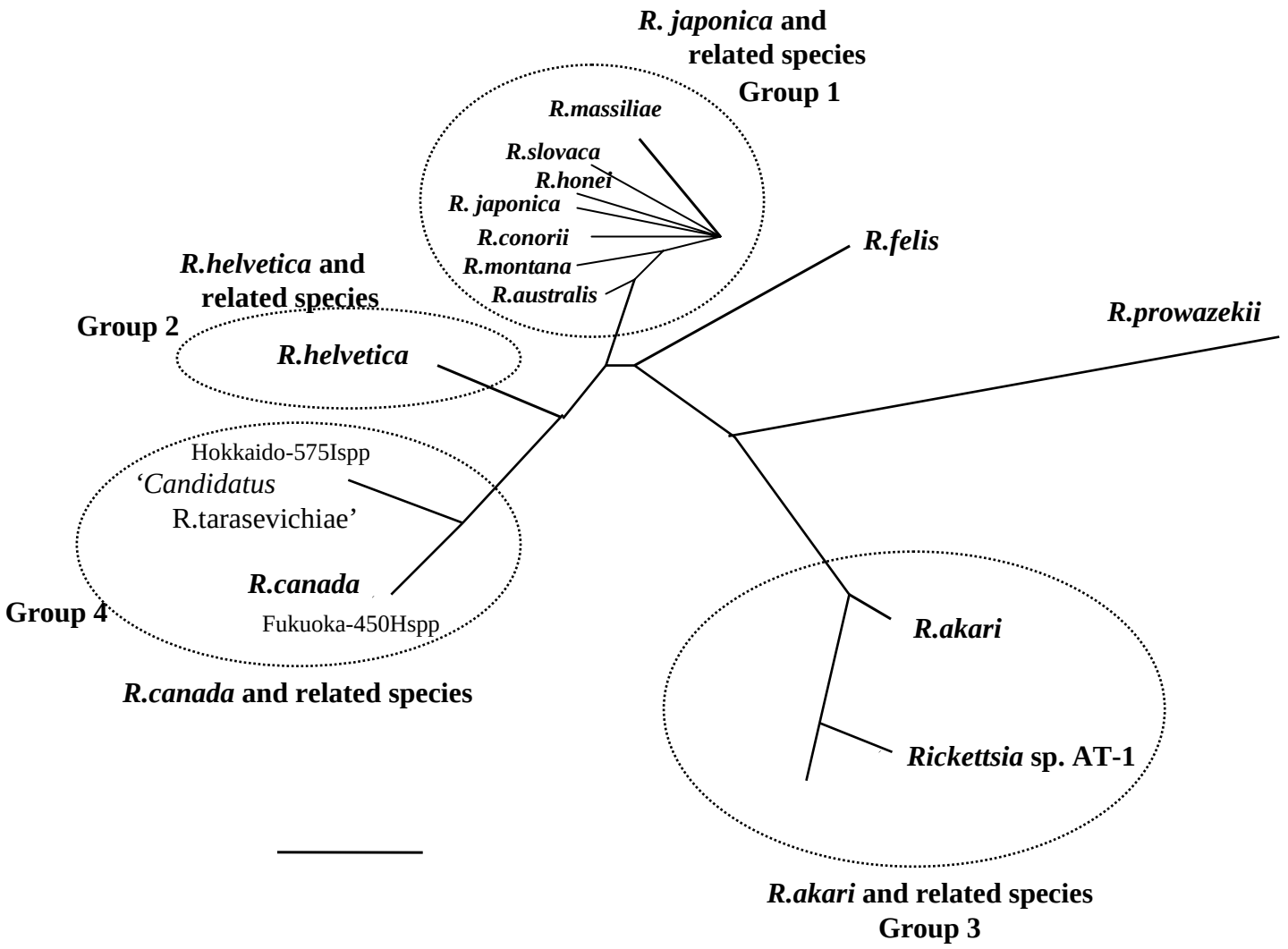
R. japonica and
related species
Group 1
R.massiliae
R.slovaca
R.honei
R. japonica
R.conorii
R.montana
R.australis
R.felis
R.prowazekii
R.helvetica and
related species
Group 2
R.helvetica
Hokkaido-575Ispp
'Candidatus
R.tarasevichiae'
R.canada
Fukuoka-450Hspp
Group 4
R.canada and related species
R.akari
Rickettsia sp. AT-1
R.akari and related species
Group 3

Fig.1

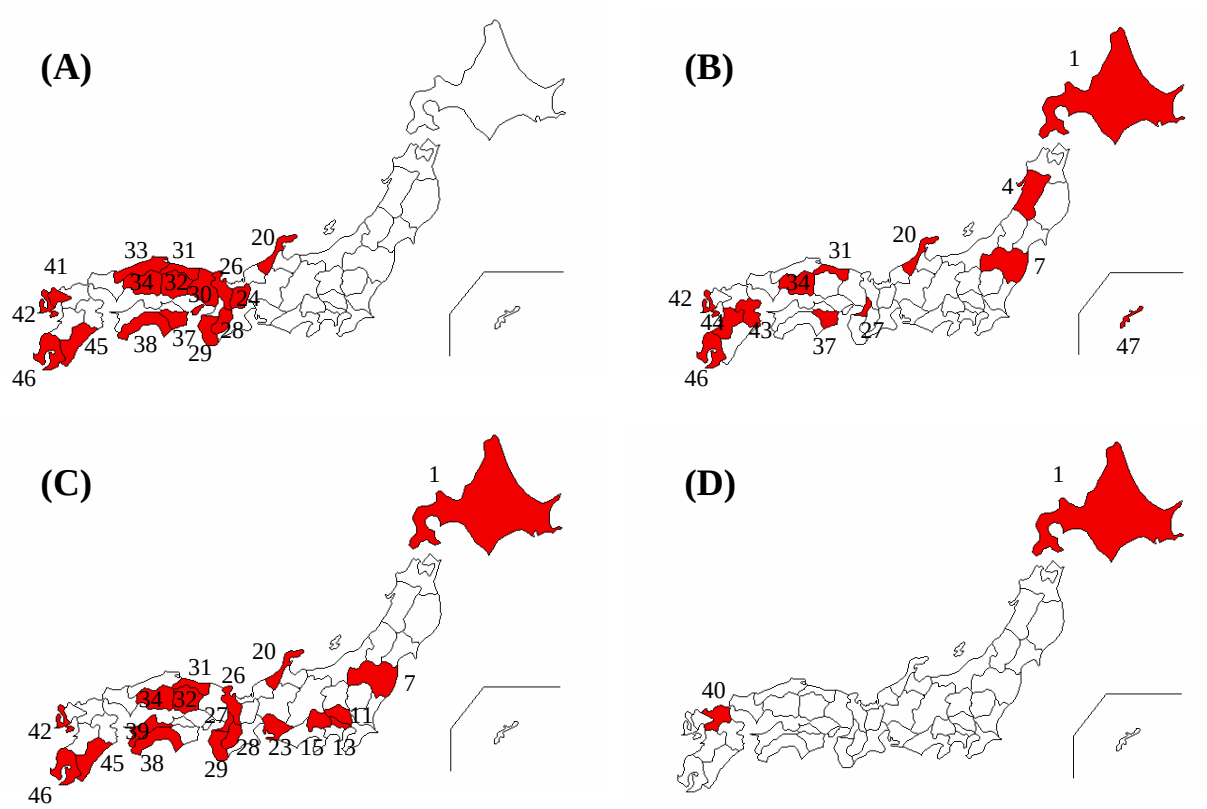
(A)
41
33
31
20
26
34
32
30
24
42
28
45
38
37
29
46
(B)
1
4
20
31
7
34
42
42
43
37
27
47
46
(C)
1
20
31
26
7
34
32
27
11
42
39
28
23
15
13
45
38
29
46
(D)
1
40

Fig.2

Table 1 Prefectures, species, stages and host animals of positive ticks for Rickettsial PCR

Group	Prefecture	Tick No.	Species	Stage	Host	Accession No.
1	Ishikawa	205	I	F	dog	AB204416
1	Kyoto	281	H	L	dog	AB204418
1	Kyoto	838	HL	N	dog	AB204454
1	Kyoto	847	HL	N	dog	AB204455
1	Kyoto	848	HL	N	dog	AB204456
1	Nara	297	HL	N	dog	AB204419
1	Nara	871	HL	F	dog	AB204459
1	Wakayama	307	HF	F	dog	AB204421
1	Wakayama	308	HF	M	dog	AB204422
1	Wakayama	314	HL	N	dog	AB204423
1	Wakayama	318	HF	M	dog	AB204424
1	Wakayama	880	HL	M	dog	AB204461
1	Hyogo	328	HL	N	dog	AB204425
1	Tottori	912	HF	F	dog	AB204463
1	Tottori	921	HF	F	dog	AB204464
1	Tottori	926	H	F	dog	AB204465
1	Tottori	928	HF	F	dog	AB204467
1	Tottori	951	HL	F	dog	AB204468
1	Shimane	361	H	N	dog	AB204427
1	Okayama	343	HL	F	dog	AB204426
1	Hiroshima	377	H	F	dog	AB204428
1	Hiroshima	386	HF	N	dog	AB204429
1	Hiroshima	967	HL	F	dog	AB204469
1	Kochi	1109	I	F	dog	AB204497
1	Tokushima	415	HL	F	dog	AB204430
1	Saga	458	HF	N	dog	AB204433
1	Saga	462	HL	N	dog	AB204434
1	Saga	1038	HL	M	dog	AB204475
1	Saga	1039	HL	N	dog	AB204476
1	Shiga	237	HL	N	dog	AB204417
1	Nagasaki	116	HL	M	cat	AB204513
1	Nagasaki	1041	HL	N	dog	AB204477
1	Nagasaki	1043	HL	F	dog	AB204478
1	Nagasaki	1057	HL	F	dog	AB204483
1	Miyazaki	128	HL	N	cat	AB204515
1	Miyazaki	513	H	N	dog	AB204435
1	Miyazaki	1093	HC	F	dog	AB204496
1	Kagoshima	519	HL	N	dog	AB204436
2	Hokkaido	2	IP	F	cat	AB204503
2	Hokkaido	559	I	F	dog	AB204437
2	Hokkaido	562	I	F	dog	AB204438
2	Hokkaido	567	I	F	dog	AB204439
2	Hokkaido	569	I	F	dog	AB204440
2	Hokkaido	572	I	F	dog	AB204441
2	Akita	617	HL	F	dog	AB204443
2	Fukushima	625	HL	N	dog	AB204444
2	Ishikawa	745	IO	F	dog	AB204449
2	Osaka	860	H	F	dog	AB204457
2	Tottori	927	HF	F	dog	AB204466
2	Hiroshima	1025	IO	F	dog	AB204473
2	Tokushima	1008	HC	F	dog	AB204471
2	Nagasaki	1052	HL	F	dog	AB204479
2	Nagasaki	1054	HF	F	dog	AB204480
2	Nagasaki	1055	HL	N	dog	AB204481
2	Nagasaki	1056	HL	N	dog	AB204482
2	Oita	1058	HL	F	dog	AB204484
2	Oita	1059	H	N	dog	AB204485
2	Oita	1060	H	F	dog	AB204486
2	Oita	1061	H	N	dog	AB204487
2	Oita	1062	H	F	dog	AB204488
2	Oita	1064	H	N	dog	AB204489
2	Kumamoto	1067	H	N	dog	AB204490
2	Kumamoto	1068	H	N	dog	AB204491
2	Kumamoto	1069	HL	F	dog	AB204492
2	Kumamoto	1071	HL	F	dog	AB204493
2	Kumamoto	1073	HC	F	dog	AB204474
2	Kumamoto	1074	HL	F	dog	AB204494
2	Kumamoto	1076	HL	N	dog	AB204495
2	Kagoshima	1118	H	F	dog	AB204498
2	Okinawa	1124	RS	N	dog	AB204500
2	Okinawa	1125	RS	F	dog	AB204501
3	Hokkaido	1	IO	F	cat	AB204502
3	Fukushima	112	IN	F	cat	AB204512
3	Fukushima	634	HF	N	dog	AB204445
3	Saitama	667	IN	F	dog	AB204446
3	Saitama	672	IN	F	dog	AB204447
3	Tokyo	689	HF	F	dog	AB204448
3	Yamanashi	27	IN	F	cat	AB204504
3	Aichi	788	IN	F	dog	AB204451
3	Aichi	795	HL	F	dog	AB204452
3	Aichi	798	I	F	dog	AB204453
3	Ishikawa	47	IN	F	cat	AB204505
3	Ishikawa	751	IN	F	dog	AB204450
3	Kyoto	63	IN	F	cat	AB204506
3	Osaka	865	IN	F	dog	AB204458
3	Nara	303	HL	F	dog	AB204420
3	Wakayama	878	IN	F	dog	AB204460
3	Wakayama	893	I	L	dog	AB204462
3	Tottori	86	H	F	cat	AB204507
3	Tottori	88	IO	F	cat	AB204508
3	Tottori	90	HF	F	cat	AB204509
3	Okayama	97	IN	F	cat	AB204510
3	Hiroshima	100	IO	F	cat	AB204511
3	Hiroshima	983	IO	F	dog	AB204470
3	Ehime	444	H	F	dog	AB204431
3	Kochi	1014	IN	F	dog	AB204472
3	Nagasaki	120	HL	F	cat	AB204514
3	Miyazaki	129	IN	F	cat	AB204516
3	Kagoshima	130	IN	F	cat	AB204517
3	Kagoshima	1107	HF	F	dog	AB204499
4	Hokkaido	575	I	F	dog	AB204442
4	Fukuoka	450	H	F	dog	AB204432

HL: *Haemaphysalis longicornis*, HF: *H. flava*, HC: *H.campanulata*, H: *Haempahysalis* sp., IP: *Ixodes persulcatus*, IO: *I. ovatus*, IN: *I. nipponensis*, I: *Ixodes* sp., RS: *Rhipicephalus sanguineus*, F: Femals, M: Male, N: Nymph, L: Larva

Accession numbers are registered sequences in GenBank.