TABLE 1. List of *B. henselae* isolates incorporated in this study and corresponding genotypes

Isolate	Origin	No. of genotypes[a]									
		S1	S2	S3	S4	S5	S6	S7	S8	S9	MST
Amber	USA[b]	5	1	1	1	2	2	2	1	1	5
Aron	USA	7	2	5	4	1	2	1	1	3	7
BisQuick	USA	5	2	6	5	2	2	2	1	1	35
Budda	USA	5	1	1	1	2	2	2	1	1	5
Buster Brown	USA	5	1	1	2	2	2	2	1	1	25
Cleo	USA	5	1	1	1	2	2	2	1	1	5
Kody	USA	4	5	5	5	1	2	1	1	3	27
Earl Grey	USA	5	1	1	2	2	2	2	1	1	25
Erick	USA	5	1	1	1	2	2	2	1	1	5
Faleen	USA	5	1	1	1	2	2	2	1	1	5
Gigi	USA	4	5	5	4	1	2	1	1	3	15
Jackie	USA	5	2	1	1	2	2	2	1	1	16
Junior	USA	5	1	1	1	2	2	2	1	1	5
Kelly	USA	8	2	5	4	1	2	1	1	3	8
Kodie	USA	5	1	1	1	2	2	2	1	1	5
Lathious	USA	5	2	6	5	2	2	2	1	1	35
Levi	USA	5	1	1	1	2	2	2	1	1	5
Mew Mew	USA	7	2	5	4	1	2	1	1	3	7
Mitzi	USA	5	1	1	1	2	2	2	1	1	5
Mokka	USA	5	1	1	1	2	2	2	1	1	5
Molly	USA	5	1	1	2	2	2	2	1	1	25
Norman	USA	5	1	1	1	2	2	2	1	1	5
Patches	USA	7	2	5	4	1	2	1	1	3	7
Pyewacket	USA	5	1	1	1	2	2	2	1	1	5
Rafiki	USA	5	1	1	1	2	2	2	1	1	5
Rocket	USA	7	2	5	4	1	2	1	1	3	7
Rum Tum	USA	5	1	1	1	2	2	2	1	1	5
Sabrina	USA	5	2	6	5	2	2	2	1	1	35
Sadie	USA	4	5	5	4	1	2	1	1	3	15
Saki	USA	5	1	1	1	2	2	2	1	1	5
Sam	USA	9	2	5	4	1	2	1	1	3	9
Samantha	USA	5	1	1	1	2	2	2	1	1	5
Sassy	USA	1	1	1	1	2	2	2	1	1	1
Shannon	USA	5	1	1	5	2	2	2	1	1	26
Simba	USA	3	1	1	1	2	2	2	1	1	3
Sinbad	USA	5	1	1	1	4	2	2	1	1	29
Spaz	USA	5	1	1	1	2	2	2	1	1	5
Sunday	USA	5	2	1	5	2	2	2	1	1	28
Sweetie	USA	5	1	1	2	2	2	2	1	1	25
Tabatha	USA	5	1	1	1	2	2	2	1	1	5
Tasha	USA	3	1	6	3	5	4	4	3	2	18
Timothy	USA	5	1	1	1	2	2	2	1	1	5
Toby	USA	5	1	1	1	2	2	2	1	1	5
Tori	USA	4	5	5	5	1	2	1	1	3	27
Zipper	USA	5	2	6	5	2	2	2	1	1	35
Zoe	USA	5	1	1	1	2	3	2	1	1	30
Newmans	USA	4	2	5	4	1	2	2	1	3	33
White	USA	5	2	6	5	2	2	2	1	1	35
Lavery	USA	5	7	6	1	2	2	1	2	1	17
Rae	USA	5	7	6	1	2	2	2	2	1	34
Fairminer	USA	5	2	6	5	2	2	2	1	1	35
Shaw-Lamon	USA	5	2	6	5	2	2	1	1	1	24
Moyle	USA	5	7	6	1	2	2	1	2	1	17
Linnan	USA	5	2	6	5	2	2	1	1	1	24
Silcock	USA	5	2	6	5	2	2	2	1	1	35
Hunt	USA	5	2	6	5	2	2	2	1	1	35
Eichtais	USA	5	2	6	5	2	2	2	1	1	35
Taylor	USA	5	2	6	5	2	2	2	1	1	35
Ramm	USA	5	2	6	5	2	2	2	1	1	35
USA1	USA	5	1	1	1	2	2	2	1	1	5
USA4	USA	4	1	1	1	2	2	2	1	1	4
USA6	USA	4	5	5	4	1	2	1	1	3	15
USA7	USA	3	1	6	3	5	4	4	3	2	18
USA8	USA	5	1	1	1	2	2	2	1	1	5
USA11	USA	4	1	5	4	1	2	1	1	3	19

Continued on following page

TABLE 1—*Continued*

Isolate	Origin	No. of genotypes[a]									
		S1	S2	S3	S4	S5	S6	S7	S8	S9	MST
USA12	USA	3	3	6	3	5	4	4	1	2	11
USA15	USA	4	2	5	4	1	2	1	1	3	14
USA16	USA	3	1	6	3	5	4	4	3	2	18
USA17	USA	5	1	1	1	2	2	2	1	1	5
UR.BH.M.NHC.32	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC.33	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC.34-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC.35	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC.50-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC.52-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC.54-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC.55-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC 56-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC.57-M	France	3	6	2	3	5	4	4	3	2	13
UR.BH.M.NHC.58-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC 59-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC.67	France	5	1	1	1	2	2	4	2	1	31
UR.BH.M.NHC.72-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC 77-M	France	5	1	1	1	2	2	2	1	1	5
UR.BH.M.NHC.78-M	France	5	1	1	1	2	2	2	1	1	5
UR.BH.M.NHC.79-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC 80-H	France	3	5	4	5	2	2	2	1	3	22
UR.BH.M.NHC 82-M	France	5	1	1	1	2	2	2	1	1	5
UR.BH.M.NHC.84-M	France	2	4	1	1	3	1	3	3	2	2
UR.BHM.M.NHC.128	France	3	6	2	3	5	4	4	3	2	13
UR.BHM.M.NHC.129	France	3	6	2	3	5	4	4	3	2	13
UR.BHM.M.NHC.130	France	3	6	2	3	5	4	4	3	2	13
UR.BH.M.NHC 154	France	2	4	1	1	3	1	3	3	2	2
UR.BH.M.NHC 155	France	2	4	1	1	3	1	3	3	2	2
UR.BH.M.NHC 156	France	2	4	1	1	3	1	3	3	2	2
UR.BH.M.NHC 159	France	2	4	1	1	3	1	3	3	2	2
UR.BH.M.NHC 161	France	2	4	1	1	3	1	3	3	2	2
FR96/BK7	Germany	3	5	4	5	2	2	2	1	3	22
FR96/BK26II	Germany	5	1	1	1	2	2	2	1	1	5
FR96/BK36	Germany	3	5	4	5	2	2	2	1	3	22
FR96/BK36II	Germany	5	1	1	1	2	2	2	1	1	5
FR96/BK75	Germany	5	1	1	1	2	2	2	1	1	5
FR96/BK77	Germany	2	4	1	1	3	1	3	3	2	2
FR96/BK78	Germany	2	4	1	1	3	1	3	3	2	2
FR96/BK79	Germany	2	4	1	1	3	1	3	3	2	2
ZF-1	France	3	5	3	5	2	2	2	1	3	12
FIZZ	Switzerland	5	1	1	1	2	2	2	1	1	5
J1	Japan	5	2	6	5	2	2	2	1	1	35
J4	Japan	5	2	5	4	1	2	1	1	3	23
J5	Japan	8	2	5	4	1	2	2	1	3	32
J6	Japan	5	2	6	5	2	2	2	1	1	35
J7	Japan	5	2	6	5	2	2	2	1	1	35
J8	Japan	5	2	6	5	2	2	2	1	1	35
P1	Philippines	5	2	6	5	2	2	2	2	1	38
P2	Philippines	5	2	6	5	2	2	2	2	1	38
P4	Philippines	5	2	6	5	2	2	2	2	1	38
P5	Philippines	5	2	6	5	2	2	1	2	1	37
P6	Philippines	4	2	5	4	1	2	1	2	3	36
P7	Philippines	3	2	5	5	2	2	2	1	1	10
P8	Philippines	3	2	6	5	2	2	2	1	1	21
T1	Thailand	5	2	6	5	2	2	2	1	3	39
T3	Thailand	8	2	5	4	1	2	2	1	3	32
T5	Thailand	6	2	5	4	1	2	2	1	3	6
T6	Thailand	4	2	5	4	1	2	2	1	3	33
T7	Thailand	5	2	6	5	2	2	2	2	1	38
T8	Thailand	5	1	6	5	2	2	2	2	1	20

[a] The description of intergenic spacers S1 to S9 and the primers used for their amplification and sequencing are given in Table 2.
[b] USA, United States.

TABLE 2. The 20 most variable intergenic spacers conserved by both *B. henselae* and *B. quintana* and primers used for amplification and sequencing

Spacer name[a]	Spacer position on the genome[b]	Spacer size (bp)[b]	PCR product size (bp)[b]	Forward primer	Reverse primer
tRNA-Ala/GCA-tRNA-Ile/AUC (S1)[c]	1412349–1412683	335	414	TTGCAAAGCAGGTGCTCTCC	TAAGCGTGAGGTCGGAGGTT
BH2865724-*dut* (S2)	1685859–1686289	431	602	GGTTTTTGCCACGGGTATTT	GGAAGTTCTAAACCTTGTCCATGG
dnaJ related protein-*cobS* (S3)	1828960–1829320	361	490	CAATGGAGGCAACCGTTCTT	GTGATATCGGGTACATTTTCAACTG
pssA-oxidoreductase (S4)	609654–610228	575	709	GATTTTTCTTCCGTGTAGCTTTGT	TGTGCGTAAAAATCGATTCATG
carB-cold shock protein (S5)	1292681–1293066	386	509	AGAAGCTATCGAAGCACTCACAAA	TGAATGAACCCGAAACCTTTAGT
alr-gcvP (S6)	1431110–1431442	333	540	TCAAAGAGGTGATTGGGTAGAGC	CTGTTTCACGTATTGATAATGTTGC
fisK-oxidoreductase (S7)	1799482–1799984	503	594	GCGAACCTTGAGAACTCTGCA	GGGTTTTACACCTTCATTGAGATCA
BH2864883-BH2864884 (S8)	1594026–1594377	352	524	TAACCACATCATCCCCTCCTCT	GAAATAATCATGAAACGCATAAGC
acpP2-malate oxidoreductase (S9)	853898–854063	166	296	CAACTTCACTGATTTCTGCGATAA	CGAGGAGTGGTTAATATGACAGCT
BH16140-BH16150 (S10)	1864960–1865467	508	508	CTCATTACAGAGCAAAACGGATATC	TTATCAAGGTTTGCTTCTACAGCG
dapE-hemN (S11)	76032–76228	197	395	ATGCATATGGTGGATGAGTGTGT	GATTTACAACAACAAGGGCTGGT
phoH-BH02260 (S12)	302238–302400	163	327	CTTATTTTCTCTTTAACGCGCTTT	TCACCTTGGCTTTTACCTGTTGT
Glutathione *S*-transferase–*dapB* (S13)	1383473–1383792	320	441	CTTCTTTTCGCCCTCTTTTAACA	TCGCGTCCCATTCTTCCAT
rpmF-ispA (S14)	1751167–1751490	324	394	GATGGAGAGGTTTTTCGTTTAGG	TGGGCGTGTTTTGCAAGAA
asd-BH12900 (S 15)	1441922–1442299	378	636	TACGCGATGCACCAGGCT	CCGTGTTGTGACCTATCCTGCT
recO-panC (S16)	596596–596744	149	438	TTGTGCAAAGAACTGTTCGTCC	ACCAAACCAATCGAAAATCCTAA
BH16010-*rpsP* (S17)	1846327–1846669	343	461	AGACTGGGAAATTAAAGGCCG	CGTATAGCAGCAGCAAAGCAAG
pgk-gap (S18)	1729282–1729787	506	590	GAACACGTTTTCCTGTGACATCA	GTGATACGGCTGTGGCTTTTG
uvrC-BH05560 (S19)	653261–653650	390	532	AGCTTTTCTTGCTCATTTTCGG	AGCTCAGTCCCTTTCTTATCGC
trwL4-trwL5 (S20)	1805508–1805660	153	280	AGATACATTCGTACGGTGGGGA	CCTGTTGTTATTTTTTGATTGGAG

[a] Intergenic spacer names consist of the name of the 5′-flanking gene combined (-) with the name of the 3′-flanking gene. Flanking open reading frames encoding putative proteins of unknown function are named after their open reading frame number within the *B. henselae* genome (GenBank accession number BX897699).
[b] The positions of the spacers on the genome, the spacer size, and the PCR product size were deduced from *B. henselae* (BX897699).
[c] Spacers S1 to S9 were numbered in descending order of variability.

B. henselae (39 MST types among 126 *B. henselae* isolates; $P < 0.01$).

Phylogenetic classification of MST types. Phylogenetic trees obtained from concatenated spacer sequences using the neighbor-joining (Fig. 3) and maximum parsimony methods showed similar phylogenetic classifications. The 126 tested isolates were grouped into four clusters. Asian isolates were grouped into cluster 1. European isolates were grouped into clusters 2 to 4. In contrast, American isolates did not form a coherent cluster but were spread among the four clusters.

DISCUSSION

In this study, we demonstrated that MST is a highly efficient method for genotyping *B. henselae* at the strain level, with 39 genotypes identified among 126 studied isolates using a combination of nine intergenic spacer sequences. Prior to our study, the most discriminatory genotyping method for *B. henselae*, i.e., MLST using nine genes, had identified seven genotypes among cat and human isolates of *B. henselae* (21). Therefore, MST was more discriminatory than MLST for typing *B. henselae*.

We found *B. henselae* to be significantly more genotypically variable than *B. quintana*, a human pathogen previously identified to be mostly clonal (13) ($P < 0.01$). Such a higher genetic diversity of *B. henselae* is as yet unexplained, despite the studies conducted on the relationship between cat and human isolates. In Germany and The Netherlands, a majority of human isolates were of 16S rRNA gene type I whereas cat isolates mostly belonged to type II (3, 4, 10, 28, 30). In contrast, in Switzerland, France, and the United States, investigators have demonstrated that most of the human isolates of *B. henselae* belonged to 16S rRNA gene type II (5, 8, 17). Iredell et al., using MLST identifying seven genotypes, found that human infection is caused by a limited number of genotypes (21). Therefore, the relationship between human and cat isolates of *B. henselae* remains a puzzling problem. We believe that MST may also be a suitable tool for investigating the dynamics of *B. henselae* populations in humans.

Among the 126 isolates analyzed in this study, we found a significantly higher genotypic heterogeneity among Asian isolates than among European ($P < 0.01$) and American ($P = 0.03$) isolates. This may be explained by the fact that most European isolates originate from only two neighboring coun-

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1   CAATCTTTTTAGAAG----------                                                    (106)
2   CAATCTTTTTAGAAGCAATCTTTTTAGAAG----------                                     (5)
3   CAATCTTTTTAGAAGCAATCTTTTTAGAAGCAATCTTTTTAGAAG----------                      (11)
4   CAATCTTTTTAGAAGCAATCTTTTTAGAAGCAATCTTTTTAGAAGCAATCTTTTTAGAAG----------      (3)
5   CAATCTTTTTAGAAGCAATCTTTTTAGAAGCAATCTTTTTAGAAGCAATCTTTTTAGAAGCAATCTTTTTAGAAG (1)
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FIG. 1. Description of the 15-bp repeated sequences within the tRNA-Ala/GCA-tRNA-Ile/AUC spacer. The first column contains the copy number of repeats. Numbers in parentheses indicate the numbers of strains that have the corresponding repeat numbers.

TABLE 3. Polymorphism characteristics of the nine variable intergenic spacers

Spacer name	No. of nucleotide variations	No. of genotypes	Spacer polymorphism, with reference to Houston-1 strain[a]
tRNA-Ala/GCA-tRNA-Ile/AUC (S1)	5	9	G9A, C49T, 203inserT, C256T, 294VNTR
BH2865724-*dut* (S2)	14.	7	T19C, G31A, C92T, C103T, C113T, C142T, A156G, A162G, C169T, G237T, A289G, C310T, T332dele, T339C
dnaJ-related protein-*cobS* (S3)	8	6	A3G, G12A, A25G, G46A, C84T, T203C, T255C, T264dele
pssA-oxidoreductase (S4)	9	5	A49G, 51inserA, 93inser,[b] G159T, A274G, A306G, T322C, A362G, T484C
carB-cold shock protein (S5)	5	5	51inser,[e] C83A, G145C, T157C, T240C
alr-gcvP (S6)	8	4	C4T, G10A, C60A, G242A, T256G or C, 296dele,[d] A305G, A306G
ftsK-oxidoreductase (S7)	8	4	C324A, G326A, 362inser,[c] G370A, A390C, C432T, A436G, C480T
BH2864883-BH2864884 (S8)	8	3	G19C, A60G, A61G, 69inserT, A88G, C102A, C249A, C282T
acpP2-malate oxidoreductase (S9)	4	3	C28T, A40C, G96A, C114T
Total (9 spacers)	69	39	

[a] The numbers show each variable nucleotide position in reference to the Houston-1 strain. The locus before the number is that within Houston-1, and the locus after the number is a possible variable nucleotide within other strains. inser, insertion; dele, deletion; VNTR, variable number of tandem repeats.
[b] Insertion of CCAGAGTGCTATTCATTAAATAAGTTTGCTTTTAAAAAATATTTCTTG.
[c] Insertion of TTCACCTGTTTCATA.
[d] Deletion of TTTTTG.
[e] Insertion of GTAGGGCA.

tries, France and Germany, and American isolates were mostly obtained from only two states, California and Florida, whereas Asian isolates originate from three countries. However, the phylogenic analysis built by concatenating the nine spacers (Fig. 3) revealed that Asian isolates, despite their apparent genotypic heterogeneity, were phylogenetically homogeneous and were grouped into a single cluster, without any overlap with European isolates. This may suggest that Asian isolates have a more recent common origin. American isolates appeared to be phylogenetically more heterogeneous than other isolates. None of the 39 MST types identified was represented in European, American, and Asian isolates together. Thus, we did not identify any pandemic isolate. However, our data may be updated by future studies incorporating isolates from other geographic origins.

To limit the number of spacers to be sequenced, we propose specific guidelines that facilitate their selection (Fig. 2). In addition, to facilitate usage of MST for genotyping of *B. henselae*, we created an MST-dedicated, free-access online database, i.e., MST-Rick, to which any investigators

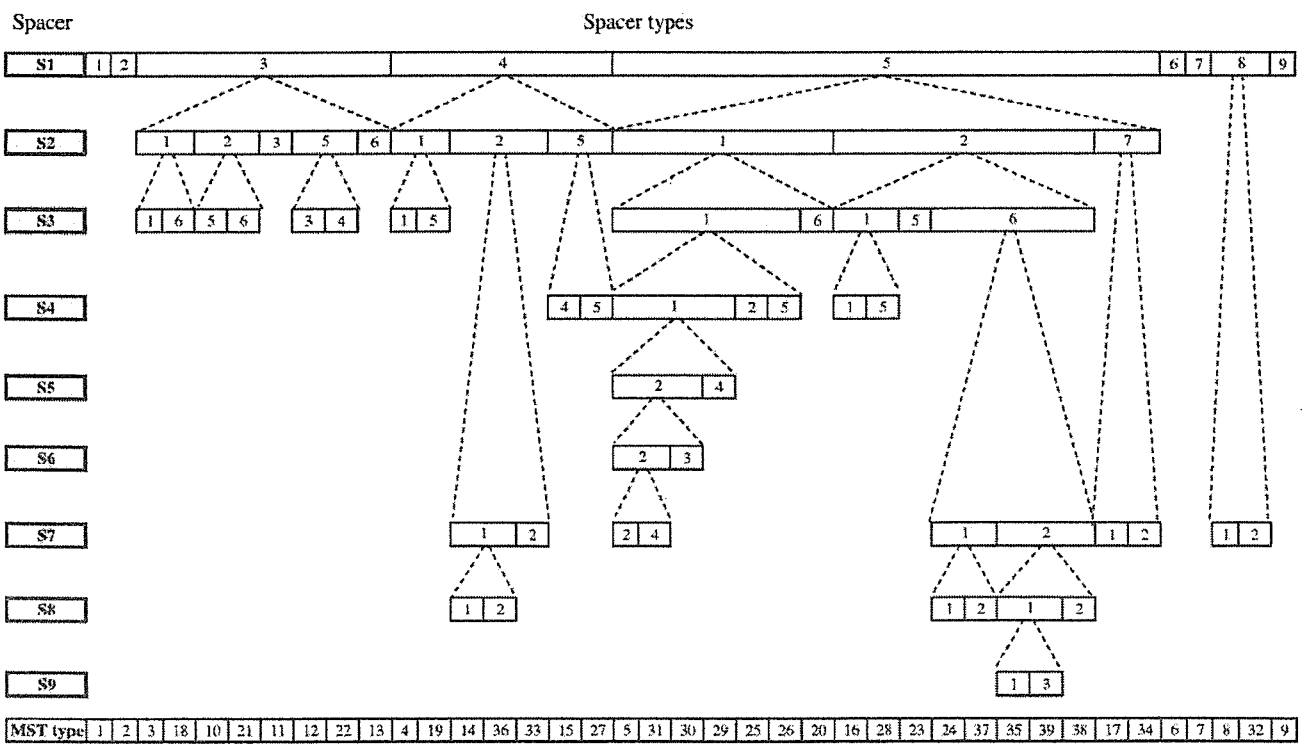

FIG. 2. Guidelines for selection of spacers for MST genotyping of *B. henselae* isolates.

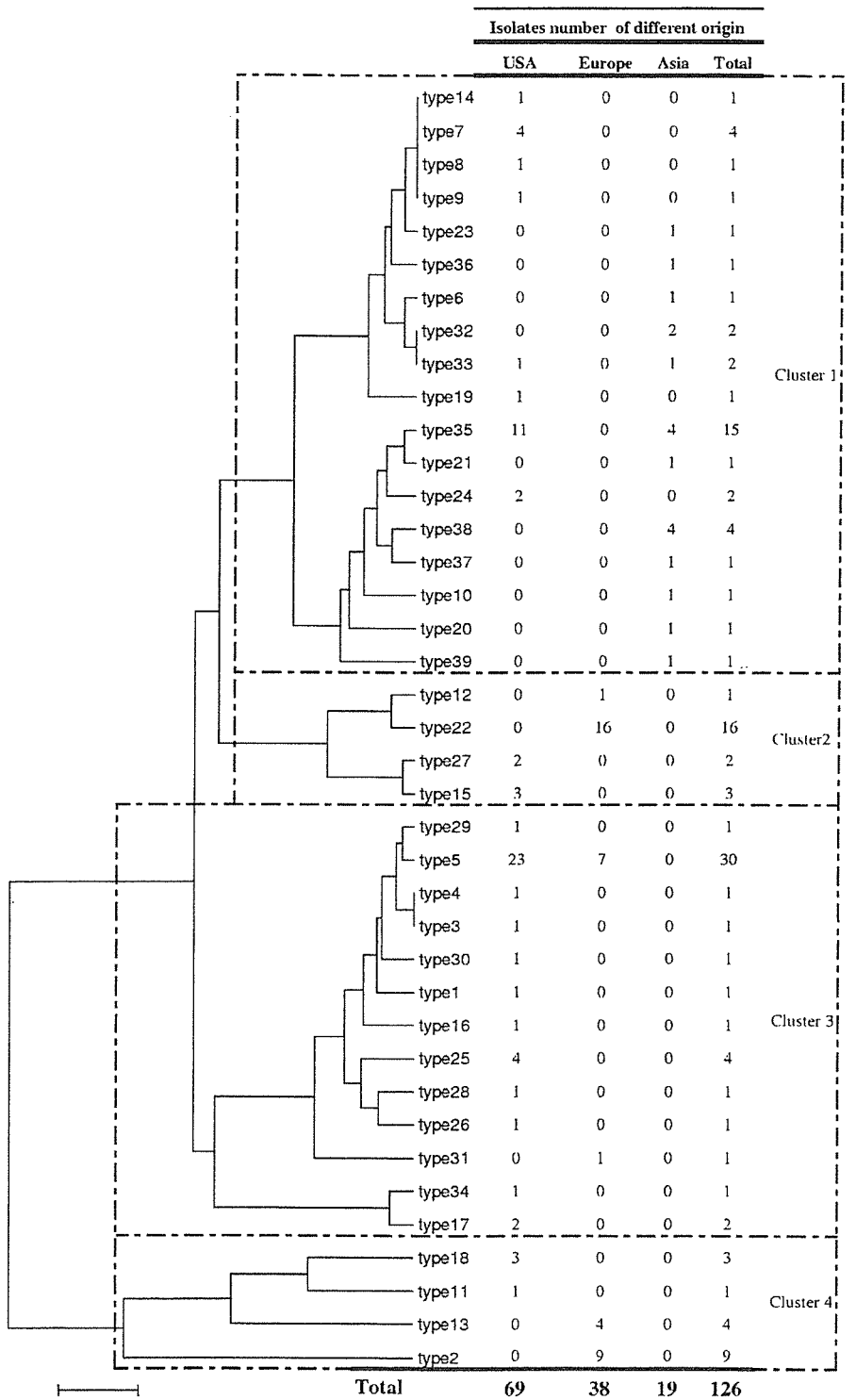

FIG. 3. Dendrogram showing the phylogenetic organization of the 39 MST genotypes, constructed using the neighbor-joining method. Sequences from the nine spacers were concatenated. The scale bar represents a 1% nucleotide sequence variation.

may compare their own spacer sequences (http://ifr48.timone.univ-mrs.fr/MST_BHenselae/mst). Although our study is preliminary and includes a limited number of strains, we hope that our method and database will be used and implemented by other investigators, which would allow frequent updating of the data.

In conclusion, MST using nine variable intergenic spacers identified 39 genotypes among 126 *B. henselae* cat isolates. As

such, MST is the most discriminatory genotyping method for *B. henselae* isolates to date and may be used to investigate the relationships between human and cat isolates of *B. henselae*. Recently, we successfully used MST for genotyping *B. henselae* isolates within lymph node biopsy samples from patients with cat scratch disease (unpublished data). As *B. henselae* is extremely difficult to grow from human specimens, MST might thus serve as both a detection and a genotyping tool.

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All authors have read and approved the final version of the manuscript and do not have any conflict of interest related to this research.

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Short communication

Seroprevalence of *Toxoplasma gondii* antibodies in stray cats and dogs in the Bangkok metropolitan area, Thailand

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Abstract

Cats and dogs are the most popular pet animals worldwide. Cats are the natural reservoir of *Toxoplasma gondii* and excrete the resistant oocyst to environments. On the other hands, dogs play a role in the mechanical transmission of the parasite. Stray cats and dogs in the Bangkok metropolitan area are becoming a public concern because there is a considerable increase in their number annually. These facts indicate the risk of mechanically spreading zoonoses including toxoplasmosis to humans since human acquire the infection from infected mammals, either directly or indirectly. In the present study, the presence of *T. gondii* antibodies was examined in 592 cats and 427 dogs from October 2001 to September 2002 by using a latex agglutination test. *T. gondii* antibodies were detected in 65 (11.0%) of the 592 cats and 40 (9.4%) of the 427 dogs. The antibody titers in the positive animals ranged from 1:64 to 1:2048. Seroprevalence was significantly higher in female cats than in male cats. The present study suggested that *T. gondii* was widespread in the stray animals in the Bangkok metropolitan area; therefore, it is essential to control the number of stray cats and dogs in order to reduce the transmission of toxoplasmosis to animals and humans.

Keywords: Bangkok; Seroprevalence; Thailand; *Toxoplasma gondii*; Stray cats; Stray dogs

1. Introduction

Toxoplasmosis is a zoonosis that affects both animals and humans worldwide. This disease is of economic importance with regard to animal production, and it has become a public health concern since it leads to abortions and neonatal complications in humans. Toxoplasmic encephalitis has been reported as a cause of death in immune compromised individuals with AIDS (Luft et al., 1984). In Thailand, 21.3% HIV-seropositive and 13.1% HIV-seronegative pregnant women have been reported to be positive for *Toxoplasma gondii* antibodies (Chintana et al., 1998). Among the HIV-seropositive individuals with *T. gondii* antibodies, 43.2% exhibited clinical symptoms and signs involving the eyes and the central nervous system (Sukthana et al., 2001).

Cats play an important role in the spread of toxoplasmosis because they are the only animals that excrete resistant oocysts into the environment (Silva et al., 2001). Although the disease is also transmitted transplacentally or by ingesting the meat of *T. gondii*-infected animals, there is evidence that *T. gondii* infection is not maintained in the environment in the absence of cats (Munday, 1972; Wallace et al., 1972; Dubey et al., 1997). Dogs also play a role in the

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mechanical transmission of oocysts to humans (Lindsay et al., 1997), although unlike cats, they do not display clinical signs and the transformation of the stages of the organism does not occur in dogs. The oocysts can be mechanically shed or transported by dogs, thereby contaminating the environment.

In Bangkok, large numbers of stray cats and dogs are found roaming the streets, fresh open markets, public places, and Buddhist monasteries (Jittapalapong et al., 2003). These stray cats and dogs act as sources of many zoonotic diseases such as rabies, cat-scratch disease, ehrlichiosis, and toxoplasmosis.

The latex agglutination test is now widely available as a useful tool for the serological diagnosis of toxoplasmosis. Serological surveys are good indicators of the occurrence of *T. gondii* infection in cats because serologically positive cats probably shed oocysts (Dubey and Thulliez, 1989). In the present study, the seroprevalence of *T. gondii* in stray cats and dogs in the Bangkok metropolitan area was investigated by using a latex agglutination test.

2. Materials and methods

2.1. Animals

From October 2001 to September 2002, 592 stray cats and 427 stray dogs were captured in Buddhist monasteries by monastery caretakers and our staffs after receiving the permission of the Buddhist monks. Blood samples of animals were collected from 41 and 38 districts, respectively, of the Bangkok metropolitan area comprising of a total of 50 districts. Before sample collection, the general condition of cats and dogs were examined thoroughly, and the sex of the individual was noted. Cats were restrained by administering an intramuscular injection of ketamine (10 mg/kg) and xylazine (1–2 mg/kg). Blood samples were collected from the jugular or saphenous veins of cat and dogs, respectively. The blood samples were immediately sent to the Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, and were centrifuged at 500 × *g* for 15 min. The separated sera were stored at −20 °C until analysis.

2.2. Serological assay

The presence of *T. gondii* antibodies was analyzed by the latex agglutination test (LAT) kit (Toxocheck-MT; Eiken Chemical Company, Tanabe, Tokyo, Japan). This test was evaluated as a screening serologic test for toxoplasmosis in animals (Tsubota et al., 1977a,b).

The procedure described in a previous report (Maruyama et al., 2003) was followed accurately. Briefly, 25 μl of latex agglutination buffer was added to each well of a U-shaped 96 well cluster plate. Then 25 μl of 1:8 diluted sera was mixed with the buffer in the first well. Serial two-fold dilutions were performed in all wells and the final 25 μl was discarded. Then 25 μl of *T. gondii*-antigen-coated latex beads were added to each well. The plate was shaken gently and then incubated at room temperature overnight. The cut-off titer for this test was 1:64 according to the manufacturer's instructions in the kit and the end point was decided in each positive sample.

The results obtained were analyzed by the chi-square test, and the level of significance was set at $p < 0.05$.

3. Results

T. gondii antibodies were detected in 65 (11.0%) of the 592 cat blood samples. The proportion of females (13.7%) testing positive for *T. gondii* antibodies was significantly higher than that of males (7.4%) ($p < 0.05$). The antibody titers in the positive cats varied from 1:64 in five cats to 1:2048 in four cats (Table 1).

In dogs, 40 (9.4%) of the 427 blood samples were seropositive; no significant difference was observed between the sexes. The antibody titers of the positive samples ranged from 1:64 in fourteen dogs to 1:2048 in one dog (Table 2).

The seroprevalence of *T. gondii* according to districts is shown in Table 3. The districts showed variations in

Table 1
Prevalence of *T. gondii* antibody and the titers in stray cats in Bangkok metropolitan area

Sex	No. examined	No. (%) positive	No. (%) of samples showing the antibody titers at					
			1:64	1:128	1:256	1:512	1:1024	1:2048
Male	256	19 (7.4)	0	4 (1.6)	4 (1.6)	6 (2.3)	3 (1.2)	2 (0.8)
Female	336	46 (13.7)[a]	5(1.5)	11 (3.3)	11 (3.3)	13 (3.9)	4(1.2)	2 (0.6)
Total	592	65 (11.0)	5(0.8)	15 (2.5)	15 (2.5)	19 (3.2)	7 (1.2)	4 (0.7)

[a] Statistically significant from the rate of male.

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Table 2
Prevalence of *T. gondii* antibody and the titers in stray dogs in Bangkok metropolitan area

Sex	No. examined	No. (%) positive	No. (%) of samples showing the antibody titers at					
			1:64	1:128	1:256	1:512	1:1024	1:2048
Male	174	17 (9.8)	8 (4.6)	7 (4.0)	2 (1.2)	0	0	0
Female	253	23 (9.1)	6 (2.4)	10 (4.0)	5 (2.0)	1(0.4)	0	1 (0.4)
Total	427	40 (9.4)	14 (3.3)	17 (4.0)	7(1.6)	1 (0.2)	1 (0.2)	1 (0.2)

Table 3
Prevalence of *T. gondii* antibody in stray cats and dogs in four Bangkok metropolitan areas

Area	No. of district	District for cat sample		District for dog sample	
		No. examined	No. (%) positive	No. examined	No. (%) positive
East	4	4	3 (75.0)	4	1 (25.0)
West	15	12	7 (58.3)	12	9 (75.0)
South	12	9	5 (55.6)	6	2 (33.3)
North	19	16	12 (75.0)	16	6 (37.5)
Total	50	41	27 (65.9)	38	18 (47.4)

the rate of *T. gondii*-positive cat blood samples; 58.3% districts in the western area and 75% in eastern and northern areas of Bangkok showed positive samples. On the other hand, 25% districts in the eastern area and 75% in the western area had *T. gondii*-positive dog blood samples. In total, 65.9% (27/41) and 47.4% (18/38) of the districts examined had *T. gondii*-positive cat and dog blood samples, respectively; however, this data did not show any statistical significance.

4. Discussion

The seroprevalence of *T. gondii* in cats varied depending on their type (stray or domestic), age, method of testing, and geographic location (Dubey et al., 2002). Maruyama et al. (2003) showed that 5.4% of the pet cats in Japan were positive for *T. gondii*. In the present study, the prevalence of our seropositive stray cats (11.0%) was lower than that of pet cats (23.1%) reported by Nishikawa et al. (1989) and hospitalized cats (57.5%) by Sriwaranard et al. (1981) in Thailand. This variation is probably related to differences in the timing of the studies, the environmental conditions responsible for the dissemination of *T. gondii* infection (Dubey and Beattie, 1988), and the distribution of samples. As compared to previous studies, the present study examined samples collected from various districts in Bangkok and geographically covered more than 80% of Bangkok. The present data on cats showed that *T. gondii* infection was prevalent in approximately 66% of the Bangkok metropolitan area. The seroprevalence of *T. gondii* in cats and dogs was found to vary depending on the area, number of monasteries, density of the animal in each district, and the economic status of the population.

Most cats in Thailand are raised either outdoors or both outdoors and indoors. Since infected stray cats shed oocysts around public places (Jittapalapong et al., 2003), healthy animals and humans may get infected due to the contaminated environment. This study also reinforces the role of stray cats as one of the potential sources of toxoplasmosis transmission to humans in Bangkok.

The prevalence of *T. gondii* is known to be higher in stray dogs as compared other dogs (Riemann et al., 1978; Fan et al., 1998; Ali et al., 2003). In the present study, the prevalence of *T. gondii* infection in stray dogs was found to be 9.4%. This rate was relatively lower than those of other Asian countries; for example, the proportion to the prevalence of *T. gondii* in dogs was 5.5–6.0 times higher in Iran (Ghorbani and Hafizi, 1983), 4.2 times higher in Taiwan (Fan et al., 1998), 4.7 times higher in Japan (Ohsima et al., 1981), and 6.4 times higher in Trinidad and Tobago (Ali et al., 2003), respectively. However, the seroprevalence observed in this study differed from that in a previous report in which the positivity was 6.6% based on the analysis of 150 stray dogs from Bangkok (Nishikawa et al., 1989). The prevalence of infected dogs mostly depended on the population of stray cats and dogs that act as mechanical vectors for toxoplasmosis (Lindsay et al., 1997). Therefore, information on the infectious status of *T. gondii* in dogs is important for assessing the risk to public health because dogs are one of the most popular

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pets and come in close contact with humans. It has been reported that the risk of *T. gondii* exposure in children due to contact with juvenile or young dogs is greater than in the case of contact with cats (Frenkel et al., 1995). Since many stray dogs are found in public areas in Bangkok, they may contaminate the environment and thus expose humans, particularly children, to *T. gondii* infection.

In this study, the sex of dogs was not significantly associated with seroprevalence, as has been reported by other researchers (Dubey, 1985; Ali et al., 2003). On the other hand, the rate of seropositive female cats was significantly higher than that of seropositive male cats. In Japan, no significant differences were observed in the seroprevalence of *T. gondii* in both the sexes of cats (Maruyama et al., 2003). The gender-based differences in seroprevalence between stray dogs and cats in Thailand are presently unclear. Although stray dogs are infected by eating infected rodents (Dubey, 1985) or through oocysts contaminating the environment, further studies are required to determine sources of *T. gondii* infection in dogs.

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Short communication

Characterization of Th1 activation by *Bartonella henselae* stimulation in BALB/c mice: Inhibitory activities of interleukin-10 for the production of interferon-γ in spleen cells

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Abstract

This study was conducted to analyze cytokine production mechanisms in mice after *Bartonella henselae* stimulation. BALB/c mice were inoculated intraperitoneally with 3×10^6 colony forming units of *B. henselae* (Houston-1 strain) twice at 10-day interval. Spleen cells were harvested from the mice and stimulated with the organisms. Following the stimulation, interferon-gamma (IFN-γ) and interleukin-4 (IL-4), IL-10, IL-12 and tumor necrosis factor-alpha (TNF-α) were measured in the culture supernatants of the spleen cells by ELISA. The spleen cells specifically secreted IFN-γ, but not IL-4, indicating that T helper 1 (Th1) cells were activated following *B. henselae* stimulation. In addition, IL-10 and TNF-α productions were also detected in the culture supernatants of spleen cells. Neutralization of IL-10 in the culture supernatants significantly enhanced the production of IFN-γ from the spleen cells stimulated with *B. henselae*. These results indicate that *B. henselae* predominantly stimulated Th1 cells and resulted in secreting IFN-γ, however the production was partially inhibited by IL-10, which was produced simultaneously.

Keywords: *Bartonella henselae*; IL-10; IFN-γ; Mice; TNF-α

1. Introduction

Although high levels of bacteremia with *Bartonella henselae* were found in cats for several months to a few years, most infected cats remain asymptomatic (Kordick et al., 1999). Immune responses against *B. henselae* infection in cats have mainly been studied from the standpoints of humoral immunity (Freeland et al., 1999). Analysis of the immune responses, especially cell-mediated immunity (CMI) is indispensable to understand the mechanisms by which the organisms are eliminated from infected animals, since

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B. henselae is an intracellular pathogen (Kordick and Breitschwerdt, 1995). However, little is known about cytokine responses against *B. henselae* in cats as well as in humans (Kabeya et al., 2006; Papadopoulos et al., 2001). The cat naturally *B. henselae*-infected were shown to promote IL4, but not IFN-γ mRNA expression when the emergence of relapsing bacteremia, suggesting that the selective induction of Th2 immune responses may contribute to establishing the persistent infection of *B. henselae* in naturally infected cats (Kabeya et al., 2006).

It has been reported that despite the fact that mice are not the natural reservoir of *B. henselae*, we used a mouse model to analyze the cellular immune response against *B. henselae*, because it has been well known the immune system of such laboratory animals (Arvand et al., 2001; Kabeya et al., 2003; Karem et al., 1999). Previous investigations have been performed to examine the immune responses against *B. henselae* stimulation in BALB/c or C57BL/6 mice (Arvand et al., 2001; Karem et al., 1999). *B. henselae* elicited cell-mediated immune responses mainly $CD4^+$ helper T cells 1 (Th1) in immunocompetent mice. Karem et al. (1999) also reported that *B. henselae*-primed BALB/c mice induced delayed-type hypersensitivity and the secretion of interferon-gamma (IFN-γ) mediated by $CD4^+$ Th1. However, the mechanism by which $CD4^+$ Th1 response in *B. henselae*-primed mice is induced remains unclear. The aim of this study was to better understand how a Th1 response is specifically induced in experimentally *B. henselae*-inoculated mice through analysis of cytokine expression profiles.

2. Materials and methods

2.1. Bacterial strains

B. henselae strain Houston-1 (ATCC49882) was used in this study. The strain was cultured on heart infusion agar (HIA) plates (DIFCO, USA) containing 5% defibrinated rabbit blood at 35 °C in an atmosphere of 5% CO_2 for 1 week. The bacterial cells were re-suspended in PBS and the concentration was adjusted to OD of 1.0 at 600 nm. Ten-fold serial dilutions were made with PBS and 100 μl of each diluted suspension were plated on two HIA plates containing 5% defibrinated rabbit blood to determine the colony-forming unit (CFU) of the inoculums.

2.2. Experimental animals

Female BALB/c mice were purchased from Nihon CLEA Corp. and used for the experiment at the age of 5 weeks. Three animals were caged together and kept under specific-pathogen-free conditions throughout the study. Animal care was carried out in accordance with the guidelines for the care and use of laboratory animals by College of Bioresource Sciences, Nihon University.

2.3. Inoculation of mice with B. henselae

Three of BALB/c mice were used for each experimental group. The mice were inoculated intraperitoneally twice at 10-day interval with 1 ml of the bacterial suspension containing 3.0×10^6 CFU of *B. henselae* Houston-1 suspended in PBS.

2.4. Induction of cytokine production from mice spleen cells

Spleen was harvested from three mice per group and suspended in complete RPMI medium consist of RPMI1640 (Invitrogen, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum, 200 U/ml penicillin, and 200 μg/ml streptomycin at a concentration of 2×10^6 cells/well of 24-well microplate. The cells were stimulated with *B. henselae* Houston-1 (2×10^6 to 2×10^8 CFU/well) with or without anti-mouse interleukin-10 (IL-10) rat monoclonal antibody (ENDOGEN, MA, USA) or IgG_1 rat isotype control (R and D systems Inc., MN, USA) at a concentration of 40 μg/ml for 24–120 h at 37 °C. Following cultivation, the concentrations of secreted cytokines (IFN-γ, IL-4, IL-10, IL-12 and tumor necrosis factor α; TNF-α) in culture-supernatants were measured by using commercial ELISA kits (Quantikine, R and D systems Inc.).

2.5. Statistical analysis

Differences between the amounts of cytokine in culture conditions (stimulation with versus without stimulant, or stimulation with live versus inactivated organisms) were determined by Student's *t*-test. *P*-values of <0.05 were regarded as significant.

3. Results

3.1. Analysis of cytokine production from spleen cells of B. henselae primed mice following in vitro stimulation

Cytokine production in the culture-supernatant of spleen cells of *B. henselae* Houston-1 primed BALB/c mice was assessed following stimulations with or without antigens at a ratio of cells to bacteria was 1:10 for 24–120 h (Fig. 1). A marked production of IFN-γ (1002.2 pg/ml) was observed following the antigen stimulation for 48 h and reached a plateau over 3000 pg/ml 72 h after the stimulation, while the production was kept at low levels (9.6–10.0 pg/ml) without *B. henselae* stimulation. By contrast, IL-4 productions showed low levels from 12.3 to 31.8 pg/ml in the culture with stimulation and from 11.1 to 13.7 pg/ml in the culture without stimulation throughout the experimental period.

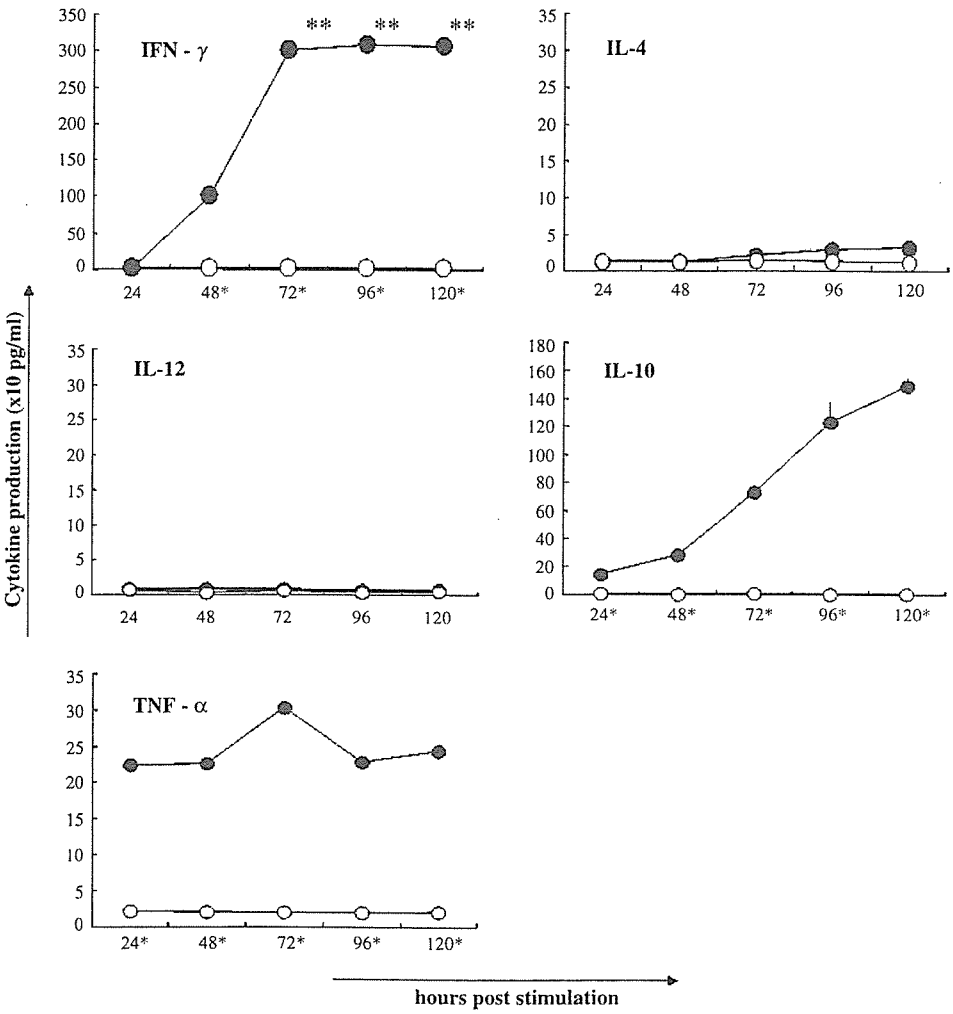

Fig. 1. Cytokine secretion in culture-supernatants of spleen cells from *B. henselae* primed mice. BALB/c mice were intraperitoneally infected with *B. henselae* Houston-1 twice at 10-day intervals. Spleen cells from three mice per group were harvested 5 days after the last inoculation and were cultured with (closed circle) or without (open circle) the organism. Ratio of cells to bacteria was 1:10 from 24 to 120 h. Following cultivation, culture-supernatants were collected and the concentrations of cytokines, such as IFN-γ, IL-4, IL-12, IL-10 and TNF-α were quantitated, using ELISA. (*) $P < 0.01$; (**) production was over 3000 pg/ml.

In order to analyze the mechanisms of specific production of IFN-γ from the spleen cells of *B. henselae* primed mice, we assessed the productions of IL-12 and IL-10 which were representative cytokines of inducing and suppressing Th1 responses, respectively (Fig. 1). The spleen cells did not produce IL-12 both with and without *in vitro* antigen stimulations (5.5–8.3 pg/ml). However, production of IL-10 and TNF-α was specifically increased in the culture supernatants of antigen stimulated spleen cells. The amounts of TNF-α production were small (223.4–302.9 pg/ml), however, the cells secreted TNF-α as early as 24 h after stimulation. Production of IL-10 gradually increased from 141.6 to 1487.0 pg/ml following stimulation, although they remained at low levels from 2.7 to 7.3 pg/ml without stimulation.

3.2. Cytokine production from the primed mice spleen cells by stimulating with live or heat-inactivated B. henselae Houston-1

To examine factors involved in the induction of IFN-γ, IL-10 and TNF-α from *B. henselae* primed mice spleen cells, heat-inactivated organisms were used for *in vitro* stimulation and assessed the cytokine production (Fig. 2). The spleen cells were stimulated

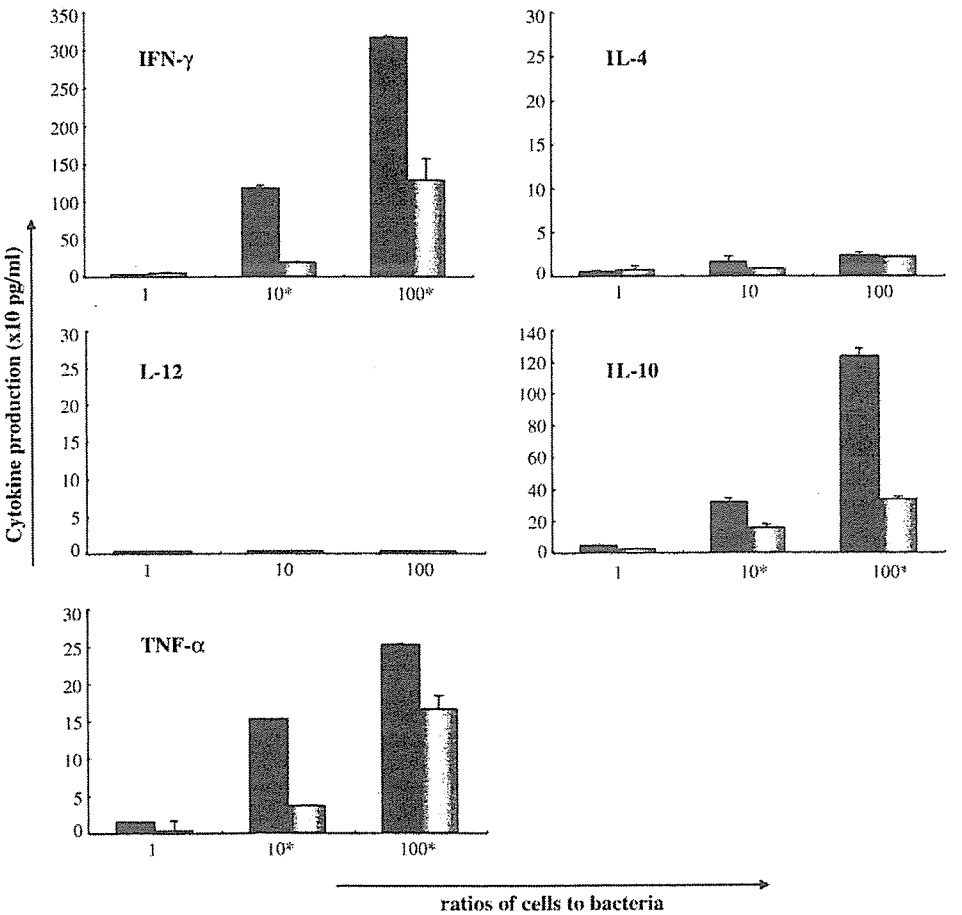

Fig. 2. Cytokine secretion by *in vitro* stimulation with live or heat-inactivated *B. henselae* in culture-supernatants of spleen cells from *B. henselae* primed mice. BALB/c mice were intraperitoneally infected with *B. henselae* Houston-1 twice at 10-day intervals. Spleen cells from three mice per group were harvested 5 days after the last inoculation and were cultured with live (closed bar) or heat-inactivated (gray bar) *B. henselae* Houston-1. Ratios of cells to bacteria were from 1:1 to 1:100 for 120 h. Following cultivation, culture-supernatants were collected and cytokines, including IFN-γ, IL-4, IL-12, IL-10 and TNF-α were quantitated, using ELISA. (*) $P < 0.05$.

with live or heat-inactivated *B. henselae* Houston-1 at different concentrations (ratios of cells to bacteria from 1:1 to 1:100) for 120 h and the amount of the secreted cytokine was calculated by ELISA. The cells produced IFN-γ, IL-10 and TNF-α by stimulating with both live and heat-inactivated organisms in an antigen dose-dependent manner. However, significantly higher levels of production were observed in the cells stimulated with live organisms than the cells stimulated with heat-inactivated organisms ($P < 0.05$). At a ratio of spleen cells to bacteria equal to 1:100, the cells produced IFN-γ, IL-10 and TNF-α at concentrations of 3165.6, 1234.8 and 252.5 pg/ml, respectively when stimulated with live organisms. In comparison, stimulations with heat-inactivated organisms induced production of lower amounts of cytokines (1267.7, 334.3 and 165.2 pg/ml, respectively). Little IL-4 and IL-12 were detected in any cases of stimulation (IL-4: 5.4–21.8 pg/ml, IL-12: 2.4–2.9 pg/ml).

3.3. The effects of IL-10 on the IFN-γ production from B. henselae Houston-1—primed mice spleen cells

To clarify the effects of IL-10 on the IFN-γ production from *B. henselae*-stimulated mice spleen cells, neutralizing antibodies to mouse IL-10 were added to the cultures and the amount of IFN-γ was assessed (Fig. 3). Addition of the neutralizing antibody dramatically enhanced the IFN-γ production (66345.8 pg/ml) compared with cells without the neutralizing antibodies (34227.4 pg/ml), and with cells in which the IgG1 rat isotype control antibody was added (28588.4 pg/ml).

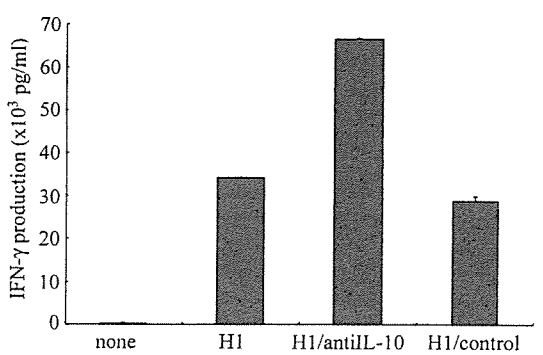

Fig. 3. Effects of neutralization of IL-10 and antigen-specificities on the production of IFN-γ. BALB/c mice were intraperitoneally infected with *B. henselae* Houston-1 twice at 10-day intervals. Spleen cells from three mice per group were harvested 5 days after the last inoculation and were cultured with or without *B. henselae* Houston-1 (H1) at ratio of cells to bacteria was 1:10. When starting the cultivation with *strain* Houston-1, the other wells were cultured in the presence of anti mice IL-10 neutralization (H1/anti IL-10) or isotype control antibodies (H1/control). Following cultivation, culture-supernatants were collected and IFN-γ was quantitated, using ELISA.

4. Discussion

In this study, we demonstrated a specific IFN-γ production from *B. henselae* (Houston-1) -primed mice spleen cells following *in vitro* stimulation with the bacteria. Karem et al. (1999) showed that spleen cells from *B. henselae* primed BALB/c mice secreted large amounts of IFN-γ after stimulation with the same organisms. Spleen cells from unprimed BALB/c mice also produced IFN-γ after *in vitro* stimulation with *B. henselae* although the amount was smaller than those of the primed mice. These results indicate that the production was induced by antigen-specific lymphocytes as well as nonspecific cell-mediated immune responses, including activated macrophages.

Resto-Ruiz et al. (2003) also reported the *B. henselae*-specific IFN-γ secretion from spleen cells of primed A/J mice. They found that *in vitro* stimulation with *B. henselae* also resulted in the significant production of IL-12 from spleen cells of the mice. IL-12 is known to induce Th1-related immune responses, including inductions of IFN-γ by NK and T cells, and resulted in stimulating cytotoxic T lymphocytes and NK cells (Trinchieri, 1998). In contrast, we did not detect any significant production of IL-12 from the spleen cells. This might be caused by the differences of the mice strains used in these experiments. The other possible reason to explain the difference is the ratio of cell to bacteria for the stimulation of spleen cells. We used the ratio of cell to bacteria at 1:1 to 1:100 for *in vitro* stimulation, while Resto-Ruiz et al. applied the ratio of 1:500 (Resto-Ruiz et al., 2003).

Patients with cat-scratch disease also showed no induction of IL-12 in their serum (Papadopoulos et al., 2001). However, the levels of circulating IL-2, IL-6 and IL-10 were shown to be significantly higher in patients with CSD than in healthy individuals

(Papadopoulos et al., 2001). In addition, the production of some inflammatory cytokines such as TNF and IL-1β, and IL-6 was markedly higher in the patients infected with *B. quintana* than those of uninfected individuals. It has also been reported that patients with *B. quintana*-bacteremia showed specific increase of IL-10 production by mononuclear cells (Capo et al., 2003). Because IL-10 is known to suppress several inflammatory mediators, overproduction of IL-10 in bacteremic patients with *B. quintana* may result in establishing a persistent infection as well as other infectious diseases (McGuirk and Mills, 2002). In the present study, we also observed IL-10 secretion from spleen cells of *B. henselae*-primed BALB/c mice and the secretion increased gradually following stimulation with the organisms. Furthermore, secretion of TNF-α was observed just 24 h after *in vitro* stimulation of spleen cells with the organisms and reached a plateau at the same time. Thus, *Bartonella* species, including *B. henselae* stimulate mononuclear cells and/or lymphocytes and the cells secrete IL-10 as well as some inflammatory cytokines, such as TNF-α and IFN-γ. In a mouse model, it has been shown that nitric oxide derived from macrophages activated by IFN-γ play a significant role in eliminating *B. henselae* from infected cells (Musso et al., 2001). We showed that IL-10 induced by stimulation with *B. henselae* partially suppressed the secretion of IFN-γ following the stimulation with the organisms. These results suggest that the organisms may escape the host immune responses, such as the activation of macrophages by inhibiting the induction of IFN-γ by inducing IL-10, simultaneously. However, it has been shown that persistent infection of *B. henselae* does not last for long periods in mice, suggesting that these modulations, such as inhibition of the production of IFN-γ may not affect the infectivity of *B. henselae* in mice. Supporting this hypothesis, *Bartonella* species shows strict host specificities in mice (Kosoy et al., 2000).

Arvand et al. (2001) reported that heat-killed *B. henselae* stimulated spleen cells of the primed C57BL/6 mice and induced proliferation of $CD4^+$ T cells and IFN-γ production. In the present experiment, large amounts of cytokines, including IFN-γ, IL-10 and TNF-α were detected when the spleen cells of BALB/c mice were stimulated with live *B. henselae*, whereas heat-killed organisms induced lower amount of cytokines. These results clearly suggest that some heat-stable components of *B. henselae* as well as secreted factors from live bacteria and/or invasion into cells may be involved in the induction of the cytokines in mice. Recent studies have revealed that *B. henselae* uses type IV secretion systems for intercellular delivery of effector molecules that modify host cellular functions in favor of the pathogen (Schmid et al., 2004). It has also been proven that the type IV secretion systems of *B. henselae* mediates invasion, a NF-κB-dependent pro-inflammatory activation (Schmid et al., 2004). Thus, type IV secretion systems may be involved in the induction of cytokines in mice.

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研究報告

飼育犬および飼い主における下痢菌伝播に関する調査

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要　旨

近年，犬猫などの動物の飼育形態が変化し，飼い主と飼育動物の接触度が密になっており，これに伴って飼い主と飼育動物との間での病原体伝播頻度が増大することが危惧されている．一般の犬飼育者と飼育されている犬との間に起こる病原体伝播のモデルとして，下痢菌の伝播を取り上げて検討した．下痢症状を示した飼育犬とその飼い主21組において，飼い主の同意を得て犬および飼い主の便細菌検査を行った．21名の飼い主および21頭の飼育犬の便培養で赤痢菌，カンピロバクター，サルモネラ菌は陰性であった．したがって，通常の飼育状態であれば，下痢症状を示す飼育犬から健康な飼い主への有害な細菌の糞口感染が起こる可能性は極めて低いと推定された．

目　　的

近年，犬や猫の飼育形態が変化し，以前のように犬は番犬，猫はネズミ取りのために飼うのではなく，愛玩動物として吾が子のように寝食をともにする人々が増加している．これに伴い，飼い主と飼育動物との間で病原体の伝播頻度が増大することが危惧される[1)]．そこで，一般の犬飼育者と飼育されている犬との間に起こる病原体伝播のモデルとして，下痢菌の伝播を取り上げて検討した．

材料と方法

東京都内2カ所，千葉県2カ所，静岡県1カ所の開業獣医師の協力を得て，下痢などを主訴にして獣医科医院を受診した飼育犬およびその飼い主21組において，検査の意義を口頭で説明した後飼い主の了承を得て，便中赤痢菌，サルモネラ菌，カンピロバクターの検出を試みた．糞便検体の採取は栄研化学株式会社製シードスワブを用いて，飼育犬については肛門からの直接採取または排出された便からの間接採取で，飼い主については間接採取で実施した．

背景調査として，飼い主に関しては，年齢，性別，住居環境，基礎疾患の有無，下痢の有無，常用薬，抗菌薬服用の有無を，飼育犬に関しては犬種，年齢，性別，体重，臨床診断，現病歴，抗菌薬投与の有無，既往歴，餌はドッグフードか飼い主と同じか残飯かなど食餌状況，発病直前の摂取食物，飼育状況などを質問した．

病原菌の培養検査はシードスワブを民間臨床検査センターに送付し，検査センターでは，血液寒天培地のほかに，赤痢菌とサルモネラ属菌の選択培地としてSSSB培地を用いて，またカンピロバクター用選択培地としてCCDA培地を用いて実施した．

なお，検査に当たっては飼い主に検査目的を説明した後に同意を得てから実施し，結果は飼い主の個人情報を入れずに集計した．

1）東京都立駒込病院小児科　2）杉山獣医科　3）おゆみの動物病院　4）東京獣医科病院　5）佐藤獣医科　6）イトウペットクリニック

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結　　果

1．飼い主の背景

飼い主21名の年齢分布は20歳代が2名，30歳代が7名，40歳代が7名，50歳代が3名，60歳代が2名であった．

性別では男性10名，女性9名，記載なし2名であった．

飼い主の住居環境は，一戸建て住宅に住む飼い主が19名，集合住宅に住む飼い主が1名，記載なしが1名であった．

健康状態調査では，基礎疾患のない飼い主が18名，何らかの基礎疾患がある飼い主が3名で，疾患の内訳は高血圧，胃全摘，自律神経失調が各1名であった．何らかの薬剤を常用している飼い主が6名おり，他の15名に常用薬はなかった．検査時に抗菌薬を服用していた飼い主はいなかった．また，下痢症状を訴えた飼い主が3名いた．

2．飼育犬の背景

犬種では，雑種犬が5頭，ミニチュアダックスが2頭の他，柴犬，コリーなどが1頭ずつであった．

飼育犬の年齢は，1歳が3頭，2歳が3頭，3歳が3頭，4歳が1頭，5歳が4頭，6～7歳が1頭，8～10歳が2頭，11～14歳が3頭，年齢不明1頭であった．

獣医科医院受診理由は，下痢が14例，血便が1例，下痢と血便が3例，下痢・嘔吐が1例，記載なしが2例であった．

飼育環境は，室内のみの飼育が10例，室内および戸外が8例，戸外のみが2例，記載なしが1例であった．

飼育環境では，単一飼育例が16例，多頭飼育が4例であり，記載なしが1例であった．当該犬の他に飼育している頭数は1～2頭であった．犬以外の動物を飼育している例は，猫2例，ウサギ，フェレットが各1例，記載なしが1例あり，残る16例は犬以外の動物を飼育していなかった．

飼育犬の餌は17例が市販のドッグフードを与え，4例は市販のドッグフードの他に飼い主と同じ食物を与えており，残飯のみを与えていた飼い主はいなかった．

3．糞便の細菌検査結果

糞便から赤痢菌，サルモネラ菌，カンピロバクターが検出された飼い主はいなかった．また，飼育犬からも上記3菌種は検出されなかった．

検出された菌種は，飼育犬でも飼い主でも*Escherichia coli*(*E. coli*)が最も多かった．*E. coli*以外の飼育犬と飼い主で共通に検出された菌種は*Pseudomonas aruginosa*(*P. aruginosa*)と*Klebsiella pneumoniae*(*K. pneumoniae*)が1件ずつであった(表1)．また，下痢を訴えた飼い主2名からは*E.coli*のみが，1名からは*E. coli*と*K. pneumoniae*が検出された．

考　　察

今回の調査では，下痢などの消化器症状を呈して獣医科医院を受診した飼育犬の便からも，その飼い主の便からも赤痢菌，サルモネラ菌，カンピロバクターは検出されなかった．この結果から，飼育犬の下痢では上記細菌が原因になることは多くないものと推定された．また，上記細菌による飼育犬の下痢がなかったことは，飼育犬のほとんどが市販のドッグフードを与えられており，以前のように残飯を与えられる飼育犬がないことによると思われる．飼育犬と飼い主に共通して検出された細菌としては，大腸菌以外には*P. aruginosa*と*K. pneumoniae*が1件ずつあった．これらの菌が飼育犬から飼い主に感染した可能性は否定できないが，*P. aruginosa*は雑菌として飼育犬と飼い主に定着していた可能性も[2]，また飼育犬と飼い主が共通の汚染された環境から感染した可能性もある．また，*K. pneumoniae*は約5％の健康人の呼吸器や腸管にみられるので[3]，たまたま飼育犬と飼い主から同時に検出されたことも考えられる．したがって，過度の接触を避け，衛生的に飼育していれば，下痢症状を示す飼育犬から健康な飼い主への有害な細菌の糞口感染が起こる可能性は極めて低いと推定された．

文　　献

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表1　下痢症状を呈した飼い犬とその飼い主の糞便から分離された菌

飼い犬	主　訴	分離菌1	分離菌2	分離菌3	分離菌4	飼い主	分離菌1	分離菌2	分離菌3
1	記載なし	*C. freundii*	*K. pneumoniae*	*E. cloacae*		1	*E. coil*		
2	下　痢	*E. coli*				2	*C. freundii*	*E. cloacae*	*K. pneumoniae*
3	下　痢	*E. coli*	*E. cloacae*	*K. pneumoniae*		3	*K. oxytoca*	*E. coil*	
4	下　痢	*E. coli*				4 *	*E. coil*		
5	下　痢	*E. coli*				5	*E. coil*	*K. pneumoniae*	
6	下　痢	*E. coli*	*E. aerogenes*	*Enterococcus* spp.		6	*E. coil*		
7	下　痢	*E. coli*				7	*E. cloacae*	*K. pneumoniae*	*P. aeruginosa*
8	下　痢	*C. freundii*	*S. marcescens*			8	*K. oxytoca*	*C. freundii*	
9	記載なし	*E. coli*				9	*E. coil*		
10	下　痢	*E. coli*	*K. pneumoniae*			10	*E. coil*	*P. aeruginosa*	
11	下　痢	*P. mirabilis*	*C. freundii*			11	*K. pneumoniae*	*E. cloacae*	*P. aeruginosa*
12	下　痢	*C. freundii*	*K. pneumoniae*	*P. aeruginosa*		12	*E. coil*	*Bacillus* spp.	
13	下　痢	*E. coli*	*E. cloacae*	*S. marcescens*		13	*K. pneumoniae*	*C. freundii*	
14	下　痢	*E. coli*	*P. aeruginosa*			14	*E. coil*	*K. pneumoniae*	
15	血　便	*E. coli*				15	*E. coil*	*Bacillus* spp.	
16	下　痢	*E. coli*	*P. aeruginosa*			16	*E. coil*	*P. aeruginosa*	
17	下　痢	*E. coli*	*K. pneumoniae*			17	*C. freundii*	*K. pneumoniae*	
18	嘔吐，下痢	*E. coli*				18	*E. coil*		
19	下痢，血便	*E. coli*	*E. aerogenes*	*P. mirabilis*	*P. aeruginosa*	19*	*E. coil*	*K. pneumoniae*	
20	下痢，血便	*E. coli*				20*	*E. coil*		
21	下痢，血便	*E. coli*				21	*E. coil*	*C. freundii*	

*：下痢あり