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（ 総括・分担 ）研究報告書

狂犬病の診断技術向上に必要な検査系の開発に関する研究

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研究要旨：狂犬病は海外からの侵入が憂慮される動物由来感染症であり、国内に侵入した感染動物を早期に察知するためには実験室内におけるウイルス学的な診断技術の向上と普及が求められる。これまで、「動物由来感染症のサーベイランス手法の開発に関する研究（平成16年から平成18年）」で安全で簡便な抗原診断・遺伝子診断系の開発を行ってきたが、狂犬病は希少感染症であり確立した診断系による検査が頻繁に行われるわけではない。したがって、狂犬病の検査系を普及するためには頻回にしか行われない検査の技術伝達や成績判定を安全に正しくかつ容易にできることが望まれる。そこで、今回は狂犬病の抗原診断に使用される抗体の力価・感度および反応性を簡単に検証できる方法として、組換え技術により抗原診断（DFA法、直接蛍光抗体法）の標的である狂犬病ウイルスN蛋白を培養細胞（BHK細胞）に発現させて、生ウイルスを使用しないで安全かつ安価に再生産できる検査抗体の反応性を検証できる抗原スライドを作成した。

A. 研究目的

これまで、「動物由来感染症のサーベイランス手法の開発に関する研究（平成16年から平成18年）」において、安全で簡便な抗原診断・遺伝子診断系の開発を行ってきたが、狂犬病は希少感染症であり確立した診断系による検査が頻繁に行われるわけではない。したがって、狂犬病の検査系を普及するためには頻回にしか行われない検査の技術伝達や成績判定を安全に正しくかつ容易にできることが望まれる。

そこで、今回は、狂犬病の抗原診断（DFA法、直接蛍光抗体法）に使用される抗体の力価・感度および反応性を容易に検証するために、組換え技術を利用して抗原診断の標的蛋白（狂犬病ウイルスのN蛋白（RV-N））を培養細胞に発現させて、安全かつ安価に再生産できる生ウイルスを使用しないで抗体の反応性等を検証可能な抗原スライドの作出を目的とした。

B. 研究方法

RV-N発現プラスミドの作成

狂犬病ウイルス CVS11 株の N 蛋白をコードする遺伝子を pcDNA3.1 V5/His にクローニングして、RV-N/pcDNA3.1 プラスミドを作成した。

RV-N の発現

N 蛋白の発現は、RV-N/pcDNA3.1 プラスミドを BHK (baby hamster kidney) 細胞と MNA (mouse neuroblastoma)にトランスフェクトして行った。

トランスフェクション

1）0.8μg/100μl の RV-N/pcDNA3.1 プラスミドと 2μl/100μl の Lipofectamine 2000 (Invitrogen)を無血清 E-MEM 培地で調整して、等量混合の後に室温で 20 分以上静置。
2）E-MEM 培地（含 10%FBS）を用いて、BHK 細胞を 1.5×10^6 個/ml、MNA 細胞を 1.0×10^6 個/ml に調製して 1）のプラスミド液とそれぞれの細胞液を 1:2 として 4-6 時間緩やかに転倒混和。
3）抗原発現の陰性対照はプラスミド液を加えない無血清 E-MEM 培地とした。
4）転倒混和した 2）液を、8 穴ガラススライドの各ウェルに 100μl ずつアプライして、37℃、CO_2 incubator で培養。
5）24 時間後、E-MEM 培地（含 10%FBS）を交換してさらに 24 時間培養。
6）培養後の 8 穴ガラススライドは、PBS 洗浄後に 100%アセトンで 30 分固定して乾燥の後、使用時まで-80℃または室温で保存。

発現させた RV-N 抗原の保存性と安定性

RV-N を発現した細胞スライドは、-80℃で 1、2、7 ヶ月、室温で 1、2、3、4 週間保存して DFA 法によって培養細胞に発現させた抗原の保存性と安定性を検討した。

DFA 法

抗体には FITC Anti-Rabies Monoclonal Globulin (Fujirebio)を使用した。抗体液は PBS で 50 倍に希釈してエバンスブルーを終濃度 0.002%となるように加え、0.45μm フィルターを通してから使用した。抗体は、8 穴ガラススライド各ウェルに 100μl ずつ重層して室温で 30 分反応させた。反応後は PBS で洗浄して蛍光顕微鏡で観察を行った。

C. 研究結果

RV-N の発現と抗原性

RV-N/pcDNA3.1 プラスミドをトランスフェクトしてスライド上で培養した BHK 細胞、MNA 細胞いずれの細胞も RV-N の発現が DFA により確認できたが、MNA 細胞における RV-N の発現効率と蛍光強度は BHK スライドより低かった。RV-N を発現した BHK スライドの蛍光像を図 1 に示した。一方、陰性対照としたプラスミド非導入 BHK 細胞では蛍光が認められず、蛍光は RV-N 特異的であると考えられた。蛍光は細胞質内に均質に

広がっており、ウイルス感染細胞で一般的に見られる封入体様の構造は認められなかった。

RV-N を発現した細胞の安定性

RV-N/pcDNA3.1 プラスミドのトランスフェクションからアセトン固定に至る行程において、RV-N を発現させた MNA 細胞は BHK 細胞と比較してアセトン固定の際に細胞が培養したスライド表面から容易にはがれ落ちることが明かとなった。

発現させた RV-N 抗原の保存性と安定性

RV-N を発現した細胞スライドは、-80℃の保存では少なくとも 7 ヶ月間、室温保存で少なくとも 1 ヶ月の間、RV-N に対する抗体の反応性（蛍光強度、蛍光陽性細胞数）に変化が認められなかった。

D. 考察

狂犬病は希少感染症であり確立した診断系による検査が頻繁に行われるわけではない。したがって、狂犬病の検査系を普及するためには頻回にしか行われない検査の技術伝達や成績判定を安全に正しくかつ容易にできることが望まれる。

今回、組換え技術を利用して狂犬病ウイルスの N 蛋白（RV-N）を培養細胞に発現させたスライドを作成して、DFA（直接蛍光抗体）法等の抗原検出系で使用する検査抗体の反応性等の検証を可能にした。また、RV-N 発現細胞スライドは安全で安価に再生産が可能である。

今後は、作出した RV-N 発現細胞スライドを使用することになる自治体の関係機関等の協力を得て、抗原の安定性と使用法等の課題点について検討を加える予定である。

E. 結論

組換え技術を利用して狂犬病ウイルスの N 蛋白（RV-N）を培養細胞に発現させたスライドを作成して、狂犬病の抗原診断（DFA 法、直接蛍光抗体法）に使用される抗体の力価・感度および反応性を容易に検証可能とした。

本研究の成果は、これまでに開発した狂犬病の抗原検出系を自治体等の関係機関において正しく習得・検証するための教材としても活用可能であり、国内に侵入した感染動物を早期に察知するために必要とされる狂犬病の実験室内診断技術の向上とその普及に大きな波及効果があると考えられた。

F. 健康危険情報

なし

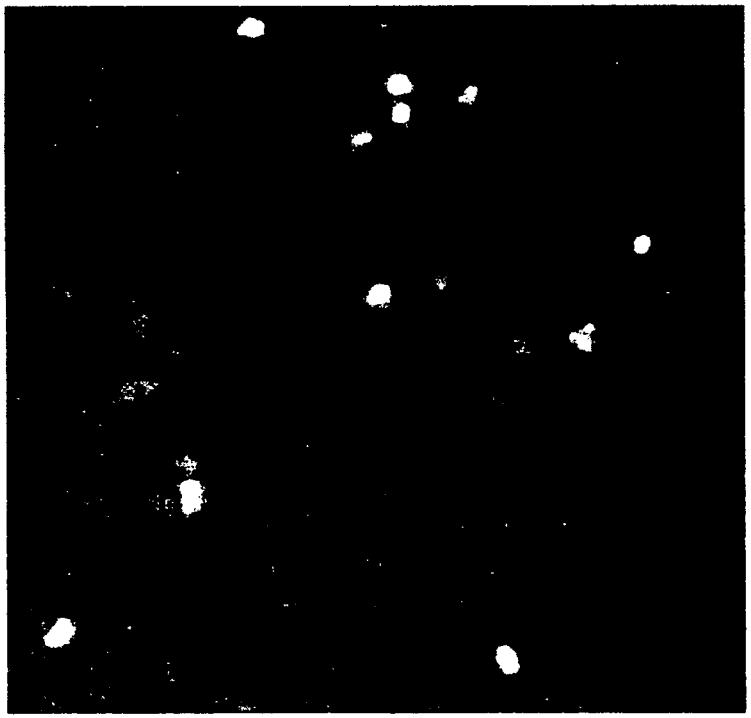
G. 研究発表

なし

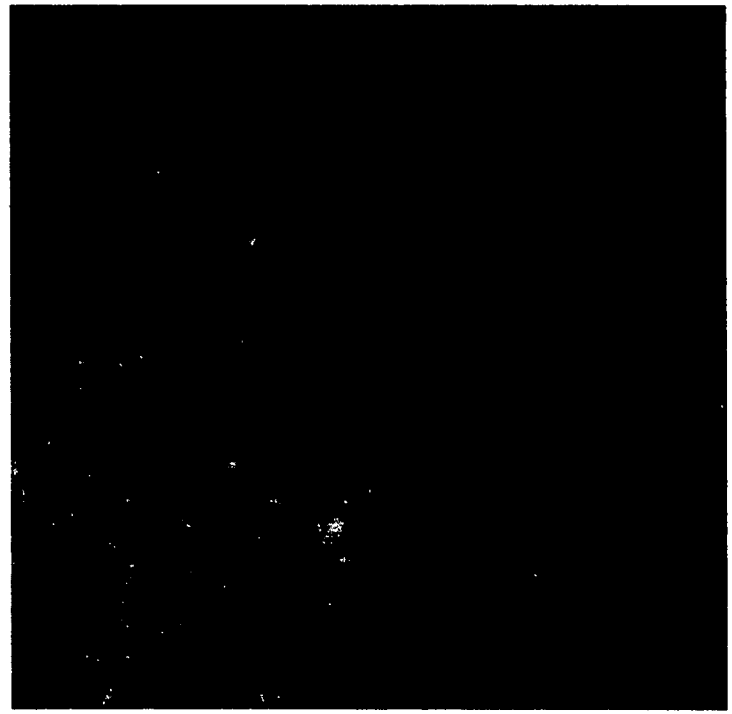
H. 知的財産権の出願・登録状況（予定を含む）

なし

図1　RV-N発現BHK細胞スライドを用いたDFA



RV-N発現BHK細胞



陰性対照BHK細胞

Ⅲ. 研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表レイアウト（参考）

雑誌

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ORIGINAL ARTICLE

Detection of *Streptobacillus* spp. in feral rats by specific polymerase chain reaction

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List of Abbreviations: 16S rRNA, 16S ribosomal RNA; PCR, polymerase chain reaction; *R. norvegicus*, *Rattus norvegicus*; *R. rattus*, *Rattus rattus*; *S. moniliformis*, *Streptobacillus moniliformis*; SPF, specific pathogen free.

Key words
16S rRNA gene, polymerase chain reaction, rat-bite fever, *Streptobacillus* spp.

ABSTRACT

***Streptobacillus moniliformis* is an etiological agent of rat-bite fever and Haverhill fever in human infection. As the currently available methods for identifying the causative bacteria are not satisfactory, we attempted to establish them by PCR using newly designed primers for the 16S rRNA gene of *S. moniliformis*. We then determined the prevalence of *Streptobacillus* spp. in two species of feral rats that inhabit an urban region in Japan, because information on the prevalence of the bacteria in feral rats is obscure. The use of PCR with newly designed primers showed that an extremely high proportion of *R. norvegicus* harbored the bacteria (61/66, 92%), whereas the prevalence was only 58% in *R. rattus* (30/52). The nucleotide sequence analysis of the 16S rRNA gene of *Streptobacillus* spp. isolated from oral swabs of feral rats showed at least two different types of bacteria among isolates from *R. norvegicus* and *R. rattus*.**

Streptobacillus moniliformis is a type of bacteria indigenous to the oral cavity of rats and other animal species, such as mice, guinea-pigs, gerbils, ferrets, cats, dogs, koalas, and non-human primates (1) and has been isolated from apparently healthy animals (2). However, *S. moniliformis* infection in humans may result in rat-bite fever or Haverhill fever. Two to 10 days after exposure to the bacteria through a bite or abrasion by rats or through the ingestion of water or food contaminated by rat feces containing the bacteria, acute symptoms such as fever, malaise, muscle pain, articular inflammation, and maculopapular, petechial, or pustular rash develop (3). *S. moniliformis* infection in humans has been reported worldwide, and mortality has been estimated to be 13% when untreated (3). However, the incidence of human infection might be underestimated because rat-bite fever is not only an uncommon disease, but the bacteria are difficult to isolate by conventional culture methods without the use of a special culture medium, such as ATCC medium 488 broth (*Streptobacillus* medium) (1, 2, 4).

To our knowledge, two studies have reported that *S. moniliformis* can be identified by PCR (5, 6). Andre *et al.* (5) used universal primers for the detection of the 16S rRNA gene. This method requires subsequent nucleotide sequencing for identification, and the results may not be conclusive if the specimen contains several species of bacteria. Boot *et al.* (6) reported the use of PCR using specific primers for the 16S rRNA gene to detect *S. moniliformis*, but, in our preliminary study, some non-specific amplification was observed.

In the present study, we attempted to design new primers that would enable us to detect *S. moniliformis* more specifically. We also applied PCR using newly designed primers to study the prevalence of the bacteria in laboratory rats and in two species of feral rats that inhabit an urban region.

Table 1. Prevalence of *Streptobacillus* spp. in feral and laboratory-reared SPF rats

	n	PCR positive	Positive (%)
Feral rats			
Norway rat (*R. norvegicus*)	66	61	92
Black rat (*R. rattus*)	52	30	58
Total	118	91	77
Laboratory rats			
Fisher 344	28	0	0
Wistar	26	0	0
Total	54	0	0

Materials and methods

Rat samples

Feral *R. norvegicus* and *R. rattus* were captured at several urban areas of Tokyo and its vicinity in Japan. Most of the *R. norvegicus* were caught outdoors, whereas *R. rattus* were all captured inside buildings (Table 1). Oral swabs were obtained and kept at 4 °C until cultivation. SPF Fisher 344 and Wister rats (*R. norvegicus*) were obtained from Japan SLC, Hamamatsu, Japan.

Cultures and isolations

Oral swabs were suspended in ATCC medium 488 broth (*Streptobacillus* medium: heart infusion broth containing 0.9% peptone, 0.045% glucose, and 18.2% horse serum) and incubated overnight at 37 °C under an atmosphere of 5% CO_2. For the isolation of *Streptobacillus* spp., aliquots were inoculated in an ATCC medium 488 agar plate containing colisin nalidixic acid and sulfamethoxazole-trimethoprim, and the plate was incubated at 37 °C under anaerobic conditions (7).

Bacterial strains and extraction of genomic DNA

The bacterial strains used in the present study are listed in Table 2. Bacterial species belonging to the family *Fusobacteriaceae* (*Fusobacterium* spp. and *Leptotrichia buccalis*) and commensal species of the oral cavity of humans and animals were included. Three reference strains of *S. moniliformis* (ATCC14647, ATCC49567, and ATCC49940) were purchased from American Type Culture Collection (Manassas, VA, USA). Strains of *Fusobacterium*, *Leptotrichia*, and *Ensifer* were obtained from RIKEN BioResource Center (Wako, Saitama, Japan). *Fusobacterium nucleatum* GTC 04469 was from Gifu University (Gifu, Japan). Bacterial strains readily available in our laboratory and used in the previous study (8) were also included. The reference and new isolates of *Streptobacillus* spp. were cultured on ATCC medium 488 agar plates at 37 °C overnight under an atmosphere of 5% CO_2, and DNA was extracted using SepaGene (Sanko Junyaku, Tokyo, Japan) according to the protocol supplied by the manufacturer. DNA from non-*Streptobacillus* strains was also prepared as described previously (8).

Table 2. Bacterial strains and polymerase chain reaction results

Bacterial strains	Amplification by primers S5/AS2	S/AS
S. moniliformis, ATCC14647	+	+
S. moniliformis, ATCC49567	+	+
S. moniliformis, ATCC49940	+	+
Bacillus anthracis, PAII	–	–
Bacillus cereus, NBRC3466	–	–
Bacillus subtilis, 3	–	–
Brucella abortus, 544	–	–
Brucella canis, QE13	–	–
Brucella melitensis, 16M	–	–
Brucella suis, 1330	–	–
Capnocytophaga canimorsus, ATCC35979	–	–
Capnocytophaga cynodegmi, ATCC49044	–	–
Capnocytophaga sputigena, ATCC33612	–	–
Coxiella burnettii, Nine Mile	–	–
Ensifer meliloti, JCM20682	–	–
Escherichia coli, DH5 alpha	–	–
Francisella tularensis, LVS	–	–
Fusobacterium equinum, JCM11174	–	–
Fusobacterium necrophorum, JCM3718	–	–
Fusobacterium nucleatum, GTC 04469	–	+
Fusobacterium varium, JCM3721	–	+
Haemophilus influenzae, Type B	–	–
Klebsiella pneumoniae, ATCC13883	–	–
Leptotrichia buccalis, JCM12969	–	+
Listeria monocytogenes, ATCC15315	–	–
Mycobacterium tuberculosis, ATCC27294	–	–
Ochrobactrum anthropi, ATCC49187	–	–
Pseudomonas aeruginosa, KH683	–	–
Pasteurella aerogenes, ATCC27883	–	–
Pasteurella canis, ATCC43326	–	–
Pasteurella dagmatis, ATCC43325	–	–
Pasteurella gallinarum, ATCC13361	–	–
Pasteurella multocida, ATCC12947	–	–
Staphylococcus aureus, ATCC29247	–	–
Yersinia enterocolitica, Pa177	–	–
Yersinia pestis, Yreka	–	–
Yersinia pseudotuberculosis, 319	–	–

PCR and sequence analysis

Bacterial cells cultured in ATCC medium 488 broth overnight were collected by centrifugation at 8900 × *g* for

Table 3. Primers used in the present study

Primer name	Sequence	Target length	Location† (Z35305)
S5	5′-CATACTCGGAATAAGATGG-3′	269 bp	965-983
AS2	5′-GCTTAGCTCCTCTTTGTAC-3′		1233-1215
S	5′-GCTTAACACATGCAAATCTAT-3′	296 bp	39-59
AS	5′-AGTAAGGGCCGTATCTCA-3′		334-317
27f	5′-AGAGTTTGATCCTGGCTCAG-3′	1482 bp	1-20
1492r	5′-GGCTACCTTGTTACGACTT-3′		1482-1464

Primers S5 and AS2 were newly designated in this study. S and AS were prepared according to reference 6. 27f and 1492r were prepared according to reference 10.
†Genbank accession number.

3 min and were then resuspended in 200 μL TE (10 mM Tris-HCl, 1 mM EDTA-2Na, pH 8.0). After being heated at 99 °C for 15 min, the clarified supernatant fluid was used as a template for specific amplification of *Streptobacillus* spp. DNA.

S5 and AS2 primers, which target the 16S rRNA gene of *S. moniliformis*, were newly designed (Table 3). PCR with S5 and AS2 primers was performed using puReTaq Ready-to-Go PCR beads (GE Healthcare Bio-Science Corp., Piscataway, NJ, USA) in a 25 μL reaction volume containing 5 pmol (0.5 μl) of each primer, 2.5 μl cultured supernatant fluid, or 2.5 ng (2.5 μl) template bacterial DNA for verification. The PCR program consisted of initial denaturation at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 20 sec, annealing at 57 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. A touchdown PCR procedure, which was used by Boot *et al.* (6), was also examined using both pairs of primers: S/AS and S5/AS2 (Table 3).

Sequencing templates from reference ATCC strains of *S. moniliformis* and isolates were prepared by PCR using the universal primers for the 16S rRNA gene of eubacteria, 27f and 1492r (Table 3) (9). Then, the PCR products were purified using GPX PCR DNA and Gel Band Purification Kit (GE Healthcare Bio-Science Corp.) according to the manufacturer's instructions. The purity of products was also inspected by electrophoresis on agarose gels. Purified PCR products were adjusted to concentrations of 5–10 ng/μL and sequenced using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's directions with the sequencing primers 27f, S, S5, AS, AS2, and 1492r. Sequencing reaction products were purified using Centrisep Spin Columns (Princeton Separations, Adelphia, NJ, USA), dried, and resuspended in 20 μL Hi-Di formamide before capillary electrophoresis on ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The sequencing data sets were exported from the instrument and aligned using the GENETYX-MAC Ver.13.0 software (GENETYX Corp., Tokyo, Japan).

Biochemical tests

ATCC strains of *S. moniliformis* and isolates were tested with API 20E (API Laboratory Products, Hampshire, UK), IDtest NF-18 (Nissui Pharmaceutical Co., Tokyo, Japan), and BACTOLABO oxidase test (Wako Pure Chemical Industries, Osaka, Japan), in accordance with the manufacturer's instructions. Included tests are listed in Table 4.

RESULTS

Detection of *S. moniliformis* gene by PCR

We first compared the specificity of newly designed primers (S5 and AS2) with those (S and AS) reported previously (6). As summarized in Table 2, specific amplification of *S. moniliformis* DNA was achieved with the primers S5 and AS2 (Fig. 1 and Table 2) without non-specific amplification of genes from other bacterial strains. In contrast, the S and AS primers amplified DNA fragments not only from *S. moniliformis* but also from *Fusobacterium* and *Leptotrichia*, which indicated that the primers S5 and AS2 are superior for the specific detection of *S. moniliformis* (Fig. 1, Table 2). Similar results were obtained when the touchdown PCR was performed (6). There was no apparent difference in the sensitivity between the PCR using different sets of primers. DNA samples of 1–5 pg were necessary for both methods.

The clarified supernatant fluid from bacterial culture in ATCC medium 488 broth was then examined by PCR using the S5 and AS2 primers to detect the presence of *S. moniliformis*-specific sequences. PCR results indicated an extremely high proportion of *R. norvegicus* (61/66, 92%); however, the prevalence of *Streptobacillus* spp.

Table 4. Biochemical characteristics of ATCC strains and isolates

	ATCC strains			*R. norvegicus* isolates				*Rattus rattus* isolates		
	ATCC No.			DDBJ Accession no.						
Test	14647	49567	49940	AB330754	AB330755	AB330756	AB330757	AB330758	AB330759	AB330760
Oxidase	–	–	–	–	–	–	–	–	–	–
Indole	–	–	–	–	–	–	–	–	–	–
H_2S production	–	–	–	–	–	–	–	–	–	–
Nitrate reduction	+	+	+	+	+	+	+	+	+	+
Acetoin production	–	–	–	–	–	–	–	–	–	–
Amygdalin fermentation	–	–	–	–	–	–	–	–	–	–
Esculin in hydrolysis	–	–	+	–	–	W	W	–	–	–
Urea hydrolysis	–	–	–	–	–	–	–	–	–	–
Citrate utilization	–	–	–	–	–	–	–	–	–	–
Arginine dihydrolase	–	–	–	–	–	–	–	–	–	–
Beta-galactosidase	–	–	–	–	–	–	–	–	–	–
Gelatinase	–	–	–	–	–	–	–	–	–	–
Lysine decarboxylase	–	–	–	–	–	–	–	–	–	–
Ornithine decarboxylase	–	–	–	–	–	–	–	–	–	–
Tryptophan deaminase	–	–	–	–	–	–	–	–	–	–
Urease	–	–	–	–	–	–	–	–	–	–
Acid produced from:										
Arabinose	W	W	W	W	W	W	W	W	W	W
Fructose	+	+	+	+	+	+	+	+	+	+
Galactose	–	–	–	–	–	–	–	–	–	–
Glucose	W	+	+	W	+	+	+	+	+	+
Inositol	–	–	–	–	–	–	–	–	–	–
Lactose	–	–	–	–	–	–	–	–	–	–
Maltose	+	+	+	+	+	+	+	+	+	+
Mannitol	–	–	–	–	–	–	–	–	–	–
Melibiose	–	–	–	–	–	–	–	–	–	–
Rhamnose	–	–	–	–	–	–	–	–	–	–
Sorbitol	–	–	–	–	–	–	–	–	–	–
Sucrose	–	–	–	–	–	–	–	–	–	–
Xylose	+	+	+	+	+	+	+	+	+	+

W, weak reaction.

among *R. rattus* was much lower (30/52, 58%). All the laboratory SPF rats tested negative (Table 1).

Isolation of *Streptobacillus* spp.

After incubation for 24 hr on an ATCC medium 488 agar plate, several colonies of bacteria resistant to antimicrobial agents were observed. Colonies with a diameter of approximately 0.1 mm were chosen and tested by PCR with the S5 and AS2 primers. After examination of more than 1000 colonies, only four colonies obtained from *R. norvegicus* and three from *R. rattus* tested positive. The colonies that formed on the ATCC medium 488 agar appeared translucent and greyish white. When cultured in the ATCC medium 488 broth, cotton-puff-like colonies, which were typical in the culture of *S. moniliformis* (10–13), showed agglomeration (Fig. 2a).

Microscopic observation showed that the isolates were pleomorphic Gram-negative bacilli with irregular, lateral bulbar swellings (Fig. 2b).

Biochemical character of isolates and ATCC strains

As summarized in Table 4, the biochemical characteristics of three reference ATCC strains of *S. moniliformis* and seven isolates were almost identical. All isolates and ATCC strains had positive reactions for nitrate reduction. Although the esculin hydrolysis test gave variable results, depending on the strain, other tests on all strains gave identical results.

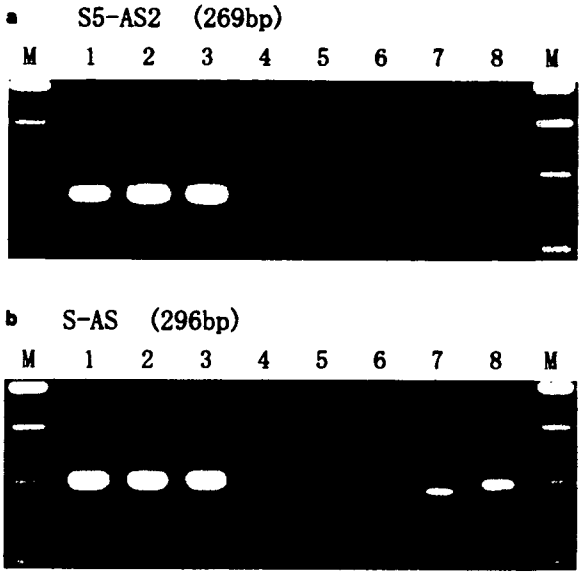

Fig. 1. Specificity of PCR amplifications using (a) S5-AS2 primers and (b) S-AS primers. M, size marker. Lanes 1–3, *S. moniliformis* (ATCC14647, 49567, and 49940). Lanes 4–7, *Fusobacterium* spp. (*F. equinum* JCM11174, *F. necrophorum* JCM3718, *F. nucleatum* GTC 04469, and *F. varium* JCM3721). Lane 8, *Leptotrichia buccalis* JCM12969.

Sequence analysis of the 16S rRNA gene

The nucleotide sequences of the 16S rRNA genes were determined for four isolates from *R. norvegicus* (DDBJ Accession nos. AB330754, AB330755, AB330756, and AB330757), and they were compared with that of the ATCC14647 strain of *S. moniliformis*. A high degree of homology (99.7–99.9%) was observed among them. In contrast, three isolates from *R. rattus* (AB330758, AB330759, and AB330760) were more distantly related, and the homology level was low (97.8–98.3%). Sequence homology between isolates from *R. norvegicus* and *R. rattus* varied from 97.6 to 98.5%. These sequence variations were mostly observed in the region at nucleotide positions 1 to 300 in the 16S rRNA gene (Fig. 3).

DISCUSSION

Feral rats are reservoirs for several zoonotic agents besides *Streptobacillus*, such as *Leptospira, Coxiella, Salmonella, Yesinia, Toxoplasma*, and Hantavirus (14, 15), and are implicated in the transmission of these pathogens to humans in urban environments (16). *S. moniliformis* bacteria are indigenous to the oral cavity of rats and cause rat-bite fever in infected humans (17). *S. moniliformis* infection of

Fig. 2. Typical cotton-puff-like colonies (a) and Gram stain (b) of an isolate from *R. norvegicus*. The bacteria were cultured in ATCC medium 488 broth under an atmosphere of 5% CO_2 (24 hr, 37 °C).

	DDBJ Accession No.	0	37-49	62-69	129-34	159-64	173-5	185-10	253
ATCC14647			TATGTATGAAATG	CATAGACT	AGAAAT	GTAGTA	CTA	GGAGAG	T
ATCC49567			**********	********	******	******	***	******	*
ATCC49940			**********	********	******	******	***	******	*
R. norvegicus isolates	AB330754		**********	********	******	******	***	******	*
	AB330755		**********	********	******	******	***	******	*
	AB330756		**********	********	******	******	***	******	*
	AB330757		**********	********	******	******	***	******	*
R. rattus isolates	AB330758		A****TAATT*AC	GT**AGAG	G****C	A**TA*	A**	TA*TTT	G
	AB330759		*****TAATT***	G***AGAG	******	*A***G	**T	******	G
	AB330760		*****TAATT***	G***AGAG	******	*A***G	**T	******	G

355	430-1	792-4	938	956	981	1112	1122	1153	1234-5	1376	1401	1408	1444
A	GA	GTG	G	A	T	C	G	-	TC	-	C	G	
*	**	***	*	*	*	*	*	-	**	T	*	*	
*	**	***	*	*	*	*	*	-	**	T	*	*	
*	**	***	*	*	*	*	*	T	**	T	*	*	
*	**	***	*	G	*	*	*	-	-*	T	-	-	
*	**	***	*	*	*	*	*	-	-*	T	-	*	
*	**	***	*	*	*	*	*	-	-*	T	*	*	
C	**	***	*	*	C	T	A	T	**	T	*	*	
*	AG	A*-	A	*	C	*	*	T	-A	T	-	-	
*	AG	A*-	A	*	C	*	*	T	-A	T	-	-	

Fig. 3. 16S rRNA sequence comparison among isolates and ATCC strains. The 16S rRNA gene from ATCC strain 14647 was used as the reference sequence. The nucleotides that differ from those of the reference sequence are shown. The asterisk and minus in the columns show concordance and deletion with a reference, respectively.

humans has been reported worldwide (2). More than 200 cases of rat-bite fever have been documented in the USA (4). Feral rats, as well as those kept as pets or in schools, are considered to be the source animals (11). Two fatal cases of rat-bite fever were recently reported in the USA after exposure to *R. norvegicus* (17).

In the present study, we designed new primers that amplify the 16S rRNA gene of *Streptobacillus* spp. more specifically than previously designed primers (6) (Fig. 1, Table 2). The use of PCR with the newly designed primers to detect bacteria showed that *Streptobacillus* spp. was highly prevalent among feral rats in urban regions. Of

66 captured *R. norvegicus*, 61 (92%) tested positive for *Streptobacillus*, whereas a lower proportion (58%, 30/52) of *R. rattus* was shown to carry the bacteria (Table 1).

We also attempted to isolate *Streptobacillus* spp. using ATCC 488 agar plate containing colisin nalidixic acid and sulfamethoxazole-trimethoprim under anaerobic conditions. Isolation of bacteria was hampered because of the presence of numerous bacterial colonies of non-*Streptobacillus* spp. that were resistant to the antimicrobials. Therefore, only seven isolates could be obtained from more than 1000 colonies grown from 91 PCR-positive oral swabs.

Biochemical examinations showed that seven isolates were nearly identical to three reference ATCC strains of *S. moniliformis* (Table 4). However, the results of H_2S production (7), nitrate reduction (7, 18), and acid production from xylose (18, 19) did not agree with previous results. This discrepancy might have resulted from the fastidious nature of these organisms and from the use of different basal media (7).

Sequence analysis of the 16S rRNA gene suggested that at least two different types were present among isolates (Fig. 3). It was also suggested that different species of the genus *Rattus* (*R. norvegicus* and *R. rattus*) might harbor different types of bacterial strains. Because the rats were captured in the urban area of Tokyo and its vicinity, where two species of rats were sympatric, the presence of distinct bacterial strains in different species of rats was probably due to co-evolution of the bacteria with their hosts rather than to differences in the geographical distribution of the bacteria. It might be intriguing to study whether there would be any differences in pathogenicity among bacteria maintained by different species of rats. However, to ascertain whether different types of bacteria are taxonomically *S. moniliformis*, more detailed genotypic (e.g. DNA/DNA homology) as well as phenotypic characterization of the isolates in question is necessary.

Although the absence of *Streptobacillus* spp. is not a prerequisite for SPF rats raised as laboratory experimental animals, none of the SPF rats were found to be infected with *Streptobacillus* spp. when tested using PCR. Because individuals handling laboratory rats might be exposed to the organism more frequently than the general public, rats raised in the conventional environment should be tested for the presence of bacteria.

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IASR

痂皮のPCRにより*Streptobacillus moniliformis*を検出した鼠咬症の一例

(Vol.28 p 226-227:2007年8月号)

鼠咬症(Rat-bite fever)は、鼠などに咬まれ*Streptobacillus moniliformis*や*Spirillum minus*に感染することにより、特徴的な皮疹・発熱・関節痛をきたす稀な全身性感染症である。今回、その特徴的な皮疹より鼠咬症を疑い、痂皮のPCRにより*S. moniliformis*を検出した一例を報告する。

症例:74歳、女性。
初診日:2007年5月7日。
主訴:四肢・顔面の紅斑、関節痛。
家族歴:特記なし。
既往歴:47歳時に子宮筋腫にて子宮、卵巣摘出。72歳時に左腎細胞癌のため左腎摘出。

現病歴:2007年4月27日自宅で鼠(頭胴長15cm)に右手の第2、3指を咬まれる(図1)。5月2日より関節痛、筋痛、全身倦怠感が出現。5月7日に四肢に紅斑が出現し、当院を受診。

初診時現症:体温37.0℃、全身倦怠感。手掌・足底を含む四肢末梢側優位に、大豆大までの軽度浸潤を触れる紅斑が多発(図2)。上肢では紅斑は癒合傾向を示し、顔面は額部を中心にびまん性紅斑。皮疹に掻痒感等の自覚症状はなし。四肢の大小関節痛・腰背部痛・筋把握痛。結膜に充血はなく、口腔内にコプリック斑や、舌に白苔の付着はなし。頸部・鼠径等の表在リンパ節は触知できず。

血液検査所見:WBC 11,500/mm^3(好中球84.7%、好酸球0.2%、好塩基球0.1%、単球2.1%、リンパ球12.9%)、Hb 12.5g/dl、Plt 15.4万/mm^3、CRP4.22mg/dl。肝、腎機能、電解質に明らかな異常値は認めず。2007年5月7日麻疹IgM(EIA)0.13、麻疹IgG(EIA)11.7、5月16日麻疹IgG(EIA)13.8。

血液培養:陰性(5月8日)。
病理組織学的所見:初診時に上肢の紅斑部より皮膚生検を実施。真皮の血管周囲に軽度のリンパ球浸潤。真皮に接する皮下脂肪織では一部の血管周囲にリンパ球、好中球の高度な集簇。

治療および経過:5月8日夜に悪寒・戦慄とともに39℃台の発熱が出現。刺し口ははっきりしなかったが、山中での作業をしていたことから日本紅斑熱やつつが虫病を疑い、ミノサイクリン(MINO)200mg/日を開始。5月12日より解熱し、四肢・顔面の紅斑は消退し、手掌・足底に点状紫斑が残存。関節痛は腰背部のみが残存。鼠に咬まれた既往より鼠咬症の可能性も考え、国立感染症研究所にて、リケッチアの検査とともに*S. moniliformis*の16S-rRNA遺伝子特異的PCRを実施。その結果、鼠咬部痂皮(図1)より*S. moniliformis*遺伝子を検出。臨床経過も含め、鼠咬症と診断し、MINO 200mgを14日間投与。以降は、発熱なかったが腰背部痛のみが持続。6月9日になり、再度38℃台の発熱。血液培養陰性だが、鼠咬症の再燃と考え、6月11日よりMINO 200mg/日の投与を開始。しかし熱型が改善しないため、13日よりピペラシリン(PIPC)4g/日へ変更。変更後、熱型・腰背部痛は徐々に改善し、16日には解熱。PIPCを10日間継続し、軽快退院。外来にて経過観察しているが現在のところ再燃なし。

考察:鼠咬症は*S. moniliformis*や*S. minus*による人獣共通感染症である。*S. moniliformis*は好気性あるいは通性嫌気性のグラム陰性桿菌で、一部の齧歯目の口腔内常在菌として存在し、咬傷や引っかき傷より感染する。2～10日の潜伏期を経て、高熱、多発関節痛、筋痛、皮疹と全身に症状が出現する。文献的には、関節痛は肘、膝、腰背部など大関節が中心であり、皮疹は手掌、足底を中心とした紅斑であり、膿瘍を伴うものもあるとされる。

本症例では、発症まで6日間、発熱は間欠的で、悪寒、戦慄を伴い、急性期のインフルエンザを思わせるほどの重篤感を伴った。皮疹は、手掌、足底を含む四肢の末梢優位に、大豆大の紅斑が出現した(図2)。紅斑は癒合傾向を示

し、消退後は紫斑となった。他疾患と比べ、特異的な皮疹のため、一度経験すれば、皮疹と詳細な病歴摂取により診断可能と実感した。多発関節痛は、入院当初は動けないほどの痛みであった。

一般に、*S. moniliformis* の分離培養は、血液・関節液から可能であるが、特殊な培地を必要として困難なことが多い。近年では、PCRで患者の体液より*S. moniliformis* 遺伝子の検出により診断されることもある。本症例では、血液からは検出されなかったものの、鼠咬部痂皮のPCRで*S. moniliformis* 遺伝子を検出し、確定診断にいたった。

治療は、ペニシリン系の抗菌薬が第一選択であり、テトラサイクリンも有効とされる。自然治癒する場合もあるが、心内膜炎、心筋炎、脳炎、深部膿瘍などを合併した場合高い死亡率を有する。また、治療が完全でないと再発する場合があるとされる。自験例では、当初、リケッチア感染症も疑っていたためMINOを投与し、いったん軽快するも、再燃した。ペニシリンに変更後は、熱型も著明に改善し、残存していた腰背部痛も軽快した。海外の文献では1カ月投与を行っている症例もあり、抗菌薬の種類、使用量、使用期間に関しては、臨床経過をみながらの注意深い判断が必要だろう。

近年、本邦では鼠咬症の症例報告はほとんどない。理由として、衛生環境の改善や内服抗菌薬の薬効向上が挙げられる。しかし、一般に知られていない疾患であるため、中毒疹とされている例もあると思われる。重篤化する危険性のある疾患のため、初期診断が大切であり、鑑別診断に上げるべき疾患である。

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Short Communication

Simultaneous Detection of the Genus *Brucella* by Combinatorial PCR

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SUMMARY: We have developed a combinatorial polymerase chain reaction (PCR) procedure to identify four major species of the genus *Brucella* simultaneously. Four pairs of primers targeting the genes encoding a cell surface protein (*BCSP31*) and outer membrane proteins (*omp2b*, *omp2a* and *omp31*) were prepared. PCR using these primers gave rise to specific patterns of amplification for each *Brucella* spp. examined in this study. *B. abortus* could be identified when fragments of *BCSP31* and *omp2b/2a* were amplified by *B. abortus*-specific primers. *B. melitensis* could be identified by the amplification of fragments of *BCSP31*, *omp2b/2a* and *omp31* using pair of primers B4/B5, JRF/JPR-ab and *omp31*. Identification of *B. canis* could be achieved when the amplicons of *omp2b/2a* were detected by *B. canis*-specific primers, as could the identification of *BCSP31* and *omp31*. If specific amplifications occurred using all pairs of primers, the strain was identified as *B. suis*. Combinatorial PCR reported here thus appeared to be an ideal method of identifying *Brucella* spp., the causative pathogen of human brucellosis.

Brucellosis, a zoonosis caused by bacteria belonging to the genus *Brucella*, is endemic in various parts of the world, especially in countries of the Mediterranean region, Asia, Africa and South America (1-3). Among the species of the genus *Brucella*, the four major causative agents of human brucellosis are *B. melitensis*, *B. abortus*, *B. suis* and *B. canis*, although their natural hosts are usually confined to goats and sheep, cattle, pigs and dogs, respectively (1-3). Moreover, some species of the genus *Brucella* are considered to be potential agents for bioterrorism (4).

Microbiological, serological and molecular techniques are commonly used for the diagnosis of brucellosis (1,2,5). Microbiological tests such as the isolation of bacteria from host tissues or blood cultures followed by bacteriological characterization remain important, although they are tedious and time-consuming (2,5). The most widely used serological tests, i.e., tube agglutination tests using inactivated *B. abortus* or *B. canis* as antigens, show some degree of cross-reaction with other bacterial strains (1,2). Moreover, it is difficult to serologically distinguish the species within the genus *Brucella* using the tube agglutination test (1,2).

Among molecular techniques, polymerase chain reaction (PCR) is one of the most useful tools for the diagnosis of brucellosis. It has been reported that identification of the genus *Brucella*, but not of the species within the genus, can be performed by PCR using primers targeting highly conserved regions such as the *BCSP31* (6) or 16S-rRNA (7). As regards the differentiation of species and/or biovars of *Brucella* within the genus, several laboratories have reported PCR procedures using highly specific primers and/or stringent assay conditions. For example, it was reported that *B. abortus* could be distinguished from *B. melitensis* by species-specific PCR targeting IS711 using primers designed based on the nucleotide sequences of *B. abortus* (8,9). *B. suis* could also be discriminated from *B. abortus* using primer pairs designed according to *B. suis*-specific sequences (10). Furthermore, identification of *B. canis* could be accomplished by using specific primers designed to amplify *virB2* (11).

In Japan, the prevalence of brucellosis is quite low, but cases of *B. melitensis* infection have recently been reported (12,13). It remains possible that some people in Japan currently suffer from brucellosis, since canine brucellosis caused by *B. canis* still exists in this country. Therefore, a reliable diagnostic system capable of distinguishing between the four species of the genus *Brucella*, including *B. canis*, remains necessary. In the present study, we attempted to develop a PCR approach that could be used to identify the four major species of the genus *Brucella* simultaneously using newly designed primers.

Here, we used 11 strains belonging to the genus *Brucella* and 23 strains of non-*Brucella* bacteria (Table 1). *Brucella* strains were cultured on sheep blood agar plates and the DNA was isolated using SepaGene (Sanko Junyaku, Tokyo, Japan) according to the protocol supplied by the manufacturer. DNA from non-*Brucella* strains was also prepared.

Isolated DNA was amplified using puReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Science Corp., Piscataway, N.J., USA) by PCR consisting of initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 1 min, annealing at 65°C for 1 min and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min.

The primers designed and used for the simultaneous identification of four major *Brucella* spp. are listed in Table 2. The results are shown in Fig. 1 and summarized in Table 1. A pair of primers, B4/B5, was previously reported to amplify a 224-bp DNA fragment from a gene encoding a 31-kDa cell surface protein (*BCSP31*) that is well conserved in all *Brucella* spp. (M20404) (6). We have confirmed that this pair of primers is specific for the genus *Brucella*, since no PCR product was detected when DNA from bacteria other than *Brucella* spp. was used as templates (Table 1). The gene encoding *Brucella* major outer membrane protein 2 (*omp2*) has two related regions, *omp2b* and *omp2a*, and these two regions are 85% homologous and oriented in opposite directions (U26438) (14). Leal-Klevezas et al. reported that a 193-bp fragment could be amplified with a pair of primers, JPF/JPR, from *B. abortus*, *B. melitensis* and *B. suis*, but not from

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Table 1. Bacterial strains used in this study and the results of PCR

Species	Strain	*BCSP31*	*omp2b*		*omp2a*		*omp31*
		B4/B5	JPF/JPR-ab	JPF/JPR-ca	JPF/JPR-ab	JPF/JPR-ca	1S/1AS
Brucella abortus	544[1]	+	+	−	+	−	−
Brucella abortus	Takanashi[1]	+	+	−	+	−	−
Brucella abortus	125[2]	+	+	−	+	−	−
Brucella melitensis	16M[1]	+	+	−	+	−	+
Brucella melitensis	HagiwaraB1[1]	+	+	−	+	−	+
Brucella melitensis	TWCC40430[3]	+	+	−	+	−	+
Brucella melitensis	H17-298[4]	+	+	−	+	−	+
Brucella suis	1330[1]	+	+	−	−	+	+
Brucella suis	S-13[1]	+	+	−	−	+	+
Brucella canis	QE13[1]	+	−	+	−	+	+
Brucella canis	Shizuoka03[5]	+	−	+	−	+	+
Yersinia pestis	Yreka	−	−	−	−	−	−
Yersinia pestis	A1122	−	−	−	−	−	−
Yersinia enterocolitica	Pa2369 (O3)	−	−	−	−	−	−
Yersinia enterocolitica	Pa9571 (O5)	−	−	−	−	−	−
Yersinia enterocolitica	Pa12986 (O8)	−	−	−	−	−	−
Yersinia enterocolitica	Pa177 (O9)	−	−	−	−	−	−
Yersinia pseudotuberculosis	319	−	−	−	−	−	−
Bacillus anthracis	PAII	−	−	−	−	−	−
Bacillus cereus	NBRC3466	−	−	−	−	−	−
Bacillus subtilis	3	−	−	−	−	−	−
Francisella tularensis	LVS	−	−	−	−	−	−
Coxiella burnetii	Nine Mile	−	−	−	−	−	−
Eschericia coli	DH5 alpha	−	−	−	−	−	−
Haemofilus influenzae	Type B	−	−	−	−	−	−
Klebsiella pneumoniae	ATCC13883	−	−	−	−	−	−
Listeria monocytogenes	ATCC15315	−	−	−	−	−	−
Mycobacterium tuberculosis	ATCC27294	−	−	−	−	−	−
Pasteurella aerogenes	ATCC27883	−	−	−	−	−	−
Pasteurella multocida	ATCC12947	−	−	−	−	−	−
Staphylococcus aureus	ATCC29247	−	−	−	−	−	−
Streptobacillus moniliformis	ATCC14647	−	−	−	−	−	−
Ochrobactrum anthropi	ATCC49187	−	−	−	−	−	−
Ochrobactrum anthropi	ATCC49687	−	−	−	−	−	−

[1]: Bacterial strains were supplied from National Institute of Animal Health, Tsukuba, Ibaraki, Japan.
[2]: Heat-inactivated bacteria, which was commercially available, was obtained from National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan as an antigen for a tube agglutination test.
[3]: A new isolate from blood of an imported brucellosis patient was supplied from Tokyo Women's Medical University, Tokyo, Japan.
[4]: A new isolate from blood of an imported brucellosis patient was supplied from Tokyo Metropolitan Institute of Public Health, Tokyo, Japan.
[5]: A new isolate from a piece of liver of an aborted puppy was isolated in our laboratory.

Table 2. Primers designated in this study

Target gene	Primer name	Sequence	Target length	GenBank accession	Location
BCSP31	B4 (S)[1]	5′-Tgg CTC ggT TgC CAA TAT CAA	224 bp	M20404	789-809
	B5 (AS)[1]	5′-CgC gCT TgC CTT TCA ggT CTg		M20404	1012-992
omp2	JPF (S)[2]	5′-gCg CTC Agg CTg CCg ACg CAA		U26438	2110-2130
	JPR-ab (AS)	5′-CAT TgC ggT Cgg TAC Cgg Ag	186 bp	U26438	2295-2276
	JPR-ca (AS)	5′-CCT TTA CgA TCC gAg CCg gTA	187 bp	U26439	2296-2276
omp31	1S (S)	5′-gTT CgC TCg ACg TAA CAg CTg	249 bp	AF366073	218-238
	1AS (AS)	5′-gAC CgC Cgg TAC CAT AAA CCA		AF366073	446-466

Primers 1) and 2) were prepared according to reference 6 and 8, respectively. Others were newly designated in this study.

B. canis (15). In this study, we designed two novel antisense primers, JPR-ab and JPR-ca, which are specific for *B. abortus* (U26438) and *B. canis* (U26439), respectively. In *B. abortus* and *B. melitensis*, it was observed that 186-bp fragments from both *omp2b* and *omp2a* regions were amplified by PCR with the pair of primers, JPF/JPR-ab. In contrast, since the nucleotide sequences of target regions of *B. canis* differ from those of *B. abortus* and *B. melitensis*, the *B. canis* fragments *omp2b* and *omp2a* were amplified only when the JPR-ca primer was used together with the JPF primer. On the other hand, since the sequences of amplicons of *omp2b* and *omp2a* of *B. suis* (U26443) are identical to those of *B. abortus* and

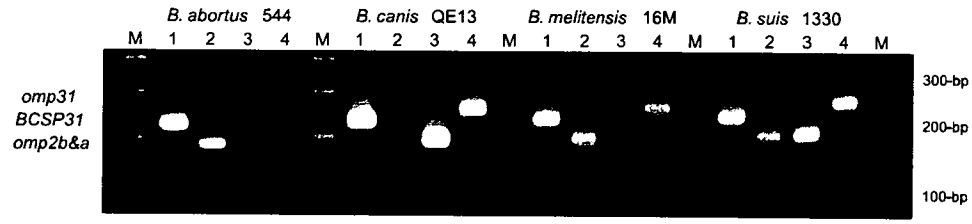

Fig.1. Detection patterns of four major biovars of *Brucella* spp. by four pairs of primers.
Lane 1, BA/B5; 2, JPF/JPR-ab; 3, JPF/JPR-ca; 4, 1S/1AS; M, Size marker.

B. canis, respectively, fragments were obtained by PCR using either the JPF/JPR-ab or JPF/JPR-ca primers. As shown in Fig. 1, PCR using a pair of primers, JPF/JPR-ab, amplified a 186-bp fragment from *B. abortus*, *B. melitensis* and *B. suis*, but not from *B. canis*, while the primers JPF/JPR2-ca amplified a 187-bp fragment from *B. canis* and *B. suis*. A pair of primers 1S/1AS was designated to amplify a 249-bp fragment from the *omp31* gene encoding another *Brucella* outer membrane protein. However, due to the presence of a large deletion in the *omp31* gene of *B. abortus* (14,16), primers 1S/1AS did not amplify the fragment from *B. abortus* (Table 1).

These results demonstrated that four species of *Brucella* could be successfully identified by combinatorial PCR using four sets of primers (Fig. 1). *B. abortus* could be identified when the amplification of fragments of *omp2b* and *omp2a* by *B. abortus*-specific primers (JRF/JPR-ab) took place, moreover, *B. abortus* could also be identified based on the amplification of *BCSP31* by B4/B5 primers. *B. melitensis* could be identified by the amplification of fragments of *BCSP31* and *omp31* as well as *omp2b* and *omp2a* by a pair of primers, JRF/JPR-ab, and *omp31*. In contrast, identification of *B. canis* could be achieved if *BCSP31* and *omp31* were amplified and if the amplicons of *omp2b* and *omp2a* were detected by *B. canis*-specific primers (JRF/JPR-ca), but not by *B. abortus*-specific primers (JRF/JPR-ab). In cases when specific amplifications occurred using all pairs of primers, the strain was identified as *B. suis*. In this study, we included 23 bacteria belonging to genera other than *Brucella* spp. Since the PCR series reported here did not amplify any fragments from these 23 non-*Brucella* bacteria, this method appears to be highly specific for the genus *Brucella*. Moreover, this PCR also amplified specific sequences from mouse tissue homogenates and blood experimentally spiked with *B. abortus* or *B. canis* (data not shown). The PCR detection limit was observed to be approximately 1 pg of DNA (data not shown).

Since multiplex PCR has been used for the simultaneous detection of several pathogens, we also attempted to establish a multiplex PCR for the detection of *Brucella* spp. However, the detection limit of that multiplex approach was inferior to that of the PCR reported here, most likely due to the competitive consumption of ingredients among amplicons (data not shown). Additionally, multiplex PCR using the primer pairs, B4/B5, JPF/JPR-ab and 1S/1AS, did distinguish *B. abortus* and *B. canis* from other *Brucella* spp., but *B. melitensis* could not be differentiated from *B. suis*. In contrast, using the primer pairs, B4/B5, JPF/JPR-ca and 1S/1AS, we were able to distinguish *B. abortus* and *B. melitensis* from the other species by multiplex PCR, although *B. canis* and *B. suis* could not be differentiated in this manner (Table 1). From these results, we concluded that it was more practical to perform combinatorial PCR rather than a multiplex PCR to identify the genus *Brucella* at the species level.

Although we tested a limited number of biovars belonging to each *Brucella* spp., it appears likely that the method reported here will enable the reliable identification of the four major species of the genus *Brucella* which infect human beings.

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IASR

ブルセラ症(1999年4月～2007年3月31日現在)

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ブルセラ症(brucellosis)はブルセラ属菌(Genus *Brucella*)による人獣共通感染症である。ヒトに感染する菌種は病原性の強い順に、*B. melitensis*(自然宿主:ヤギ、ヒツジ)、*B. suis*(ブタ)、*B. abortus*(ウシ)である。これら家畜の持つブルセラ菌のヒトへの感染は、感染動物の加熱(殺菌)処理していない生乳およびそれから作ったチーズ、食肉の喫食や、死体・流産時の汚物・汚染物などとの接触や、それらからのエアロゾルの吸入による。授乳、性交などによるヒトーヒト感染もありうるが、極めてまれである。潜伏期は通常1～3週間であるが、時に数カ月に及ぶこともある。軽症の場合、単なる感冒様症状のこともある。通常、症状は他の熱性疾患と似ているが、筋・骨格系への影響が強く、全身的な疼痛・倦怠感や、間欠熱・波状熱といった特徴的な熱型を示すこともある。これらの症状は数週間～数カ月、数年に及ぶこともある。*B. canis*(自然宿主:イヌ)もヒトに感染することがあるが一般に症状は軽く、気がつかないケースも多い。感染イヌは流産を起こすが、その流産胎子、胎盤、汚物や、尿、精液などへの接触により感染する。

本疾患は世界中で発生している。特に家畜での対策が不十分な地域では、年間数百～数千症例のヒト患者が報告されているが、実際の患者数はその10～25倍以上と推定されている。地域的には、特に西アジア、中東、地中海沿岸、アフリカ、中南米、カリブ海諸国などに多い。日本では家畜対策(摘発・淘汰)が功を奏し、清浄化していると考えられ、従って家畜から感染する可能性は低い。ただし、イヌでは2～5%前後が*B. canis*の感染歴を持つとされている。

わが国では従来、本疾患は届出の対象ではなかったため、発生状況は正確に把握されていなかった。しかし、1999年4月1日施行の感染症法で4類感染症に指定され、診断したすべての医師に届出が義務づけられた。それ以降、2007年3月31日現在までに届出は8例みられているが、2005年2例、2006年5例と、近年に集中している(表)。これは実際に患者数が増加したことよりも、むしろ診断の際にブルセラ症が考慮されるようになったためと考えられる。

国外を推定感染地域とする4例のうち、血液培養により菌が分離同定されて、*B. melitensis*感染が確定された2例(表中#2、4)は、いずれも海外で感染したものである。1例はシリアでの羊肉の摂食によると考えられ(IASR 26: 273-274, 2005参照)、もう1例はエジプトでの環境からのエアロゾル吸入による可能性が最も疑われている(IASR, 27: 125-126, 2006参照)。*B. abortus*感染が確定された1例(表中#6)は海外で感染・発症し、治療を受けたが、国内で再燃したと考えられており、感染原因としてエジプトでのミルクの摂取が推定されている。このように、本疾患は輸入感染症として注意する必要がある。

国内を推定感染地域とする3例は、いずれも*B. canis*に対する抗体が検出されているが、3例ともに明らかなイヌとの接触歴は認められなかった。

ブルセラ症の症状には特徴的なものがなく、診断には血清抗体測定や菌分離などの病原診断が欠かせない。血清診断は通常、*B. abortus*や*B. canis*を抗原とした試験管内凝集反応が行われ、民間の臨床検査機関でも可能であるが、凝集抗体価がそれぞれ1:40、1:160以上の時に陽性と判断される(従来、抗原がいずれであっても160倍以上の抗体価をもって届出の対象とされていたが、2007年4月に*B. abortus*については40倍以上を対象とすることに変更された)。*B. melitensis*、*B. suis*感染が疑われるときでも、*B. abortus*を抗原とした抗体の検出を行う。菌種の特定には菌分離が必要であり、血液や骨髄の培養が行われるが、抗菌薬がすでに投与されていて分離できないことが多い。これまでの報告でも、特に国内での感染が疑われる3例ではすべて菌が分離されておらず、病原診断は凝集反応陽性によりなされている。しかも、1例(表中#3)を除き、単血清での陽性結果で診断されているが、血清抗体のみで確定診断するにはペア血清を用いることが望ましい。また、PCR法による病原体遺伝子診断も可能であり、国立感染症研究所獣医科学部に依頼が可能である。

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