CT 画像で両下葉の背側部分に浸潤影が認められた。入院当日の喀痰より口腔内常在菌および *C. diphtheriae* が検出され，毒素非産生株であった。患者血清中のジフテリア毒素抗体価は検出限界以下であった。

SBT/ABPC で治療を行い，肺炎は軽快し10月18日退院。

細菌学的検討

塗抹及び培養検査

痰の前処理（洗浄）後，検体塗抹標本のグラム染色（フェイバー法），および培養をおこなった。培養はトリ・ソイ血液寒天培地（ヒツジ）No.2（極東），およびチョコレート寒天培地 No.2（極東）を用い炭酸ガス（ローソク）培養を行った。

1 症例目の検体は膿性痰で，グラム染色では多くの好中球とともに長めで細く，やや湾曲したグラム陽性桿菌が多数見られた。また貪食像も顕著に認められた。（9 月の再発時のグラム染色でも同様の所見が見られた。）（図 1）

2 症例目の塗抹標本では有意な菌は見られなかったが，3症例目の標本では誤嚥性の炎症を示唆する所見の中に 1 症例目と同様なグラム陽性桿菌が雑多な菌とともに多数見られた。

3 例とも一夜培養後，血液寒天培地上に透過光による観察で確認できる程度の弱い β 溶血を示す 0.5mm 程度の小さな白色コロニーが発育した。これらの菌株を RapID CB Plus（アムコ）で検査を行い，3 菌株とも *C. diphtheriae*（コード5304511，確率 99.89%）と同定された。

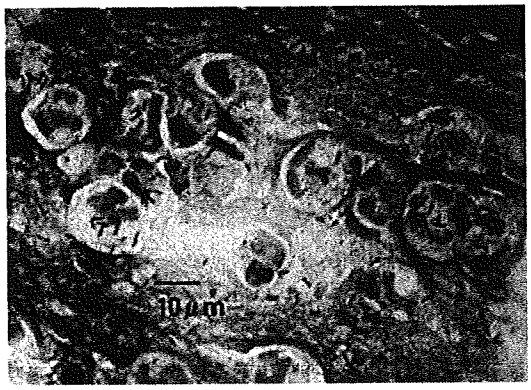

図 1　喀痰の直接塗抹標本（グラム染色）×1000

荒川培地と同等の濃度（0.04%）になるよう亜テルル酸 K を添加したハートインフュジョン寒天培地に一夜培養した分離菌のコロニーでは，亜テルル酸 K の還元による黒色のスムース型のコロニーが観察された（図 2）。菌株のナイセル染色では異染小体が認められた（図 3）。

DNase 試験（塩酸法）は DNA 培地（栄研）に一夜培養後，1N 塩酸を培地全体に注ぎ反応させたのち判定した。各症例から分離された 3 株とも発育した菌の周辺部に透明帯が確認され，陽性であった（図 4）。

これらの菌株は国立感染症研究所で精査し，16s rRNA による遺伝子解析を行うとともに生物学的性状（表 1）から *C. diphtheriae*（生物タイプ mitis 型）であることが確認された。

また分離菌株のジフテリア毒素遺伝子の有無（図 5）と，ジフテリア毒素産生能の検査，および患者血中ジフテリア抗体価（抗毒素価）を測定した結果，

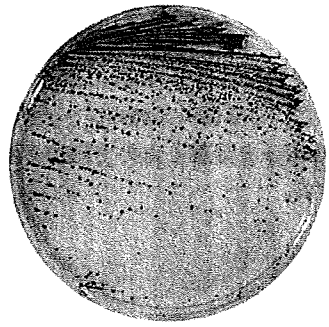

図 2　亜テルル酸 K 加ハートインフュジョン寒天培地

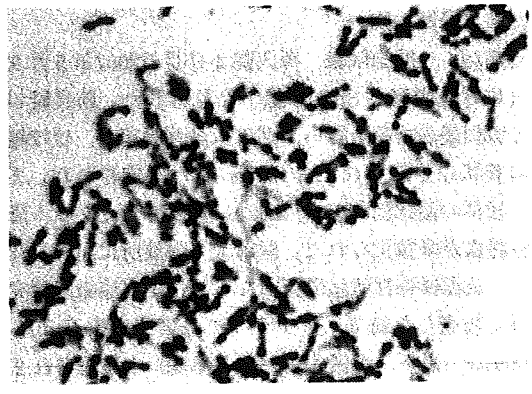

図 3　ナイセル染色

3例の分離菌株は，いずれも毒素非産生株と確認された。

抗菌薬感受性試験はドライプレート（栄研）に，ストレプトヘモサプリメントを添加したミュラーヒントンブロスを用い35℃，20時間培養後判定。CPFX，LVFXについてはミュラーヒントンSヒツジ血液寒天培地（栄研）を用い，E-testによる感受性試験を追加した。

β-ラクタム薬，ミノサイクリン，アミノグリコシド，などには低いMIC値を示したが，フルオロキノロンのCPFX，LVFXは共に>32μg/mlと高いMIC値を示した。

また，2症例目および3症例目の菌株では，EMやCLDMでも高いMIC値を示した（表2）。

上記3症例から分離された3菌株についてパルスフィールドゲル電気泳動による遺伝子解析を行ったところ2症例目と3症例目の泳動パターンが一致した。また1症例目については別の泳動パターンを示した（図6）。

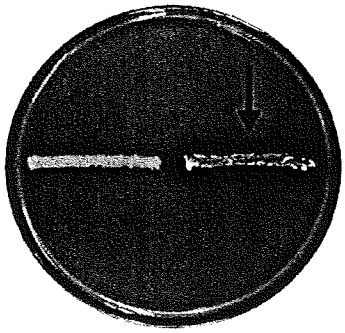

図4　DNA培地
左：陰性対照　*C.striatum*　陰性
右：分離菌株　陽性
菌を接種し，35℃，24時間培養後，1N塩酸を培地全体に注ぐ。
陰性対照は培地が白濁するが，分離菌株は，DNaseによって，コロニーの周囲に透明帯が見られた。

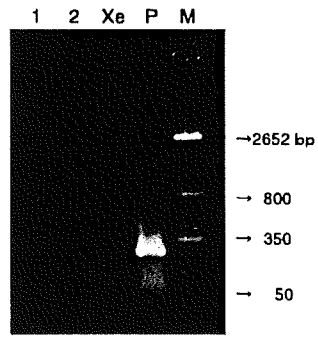

図5　ジフテリア毒素遺伝子PCR

	結果
1：レフレル培養株	陰性
2：ハートインフュージョン培養株	陰性

Xe：陰性コントロール Corynebacterium xerosis
P：陽性コントロール Corynebacterium diphtheriae PW8
M：50bp DNAラダーマーカー

表1　生物学的性状と毒素産生の有無

検査項目	結果
グラム染色	陽性　桿菌
異染小体染色（ナイセル染色）	陽性
レフレル培地	増殖（+）
チンスダール培地	増殖（+）黒色コロニー
エレク試験	陰性
アピコリネ同定試験	*C. diphtheriae* mitis コード0010324 確率　99.4%
ジフテリア毒素活性（培養細胞法）	陰性
ジフテリア毒素遺伝子PCR（248bp）	陰性

表2　薬剤感受性成績　MIC　μg/ml

	1症例目	2症例目	3症例目
SBT/ABPC	≦0.06	0.12	0.12
CEZ	≦0.12	0.25	0.25
IPM	≦0.06	≦0.06	≦0.06
MEPM	≦0.06	≦0.06	≦0.06
EM	≦0.06	4	4
CLDM	0.25	＞4	＞4
MINO	≦0.06	≦0.06	≦0.06
VCM	≦0.5	≦0.5	≦0.5
TEIC	≦0.5	≦0.5	≦0.5
FOM	>16	>16	>16
TFLX	>32	>32	>32
LVFX	>32	>32	>32

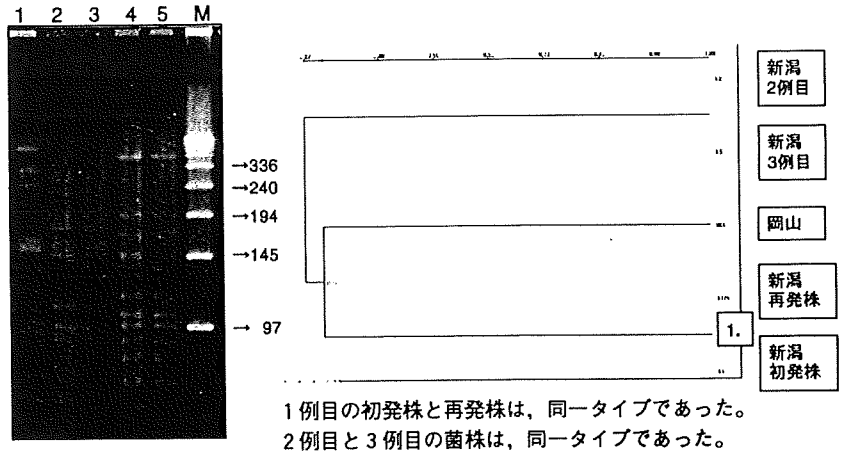

図6　PFGE解析結果（UPGAMA法）

No.
1　岡山県2006年分離株（gravis型）
2　新潟県　初発株（mitis型）
3　新潟県　再発株（mitis型）
4　新潟県　2症例目（mitis型）
5　新潟県　3症例目（mitis型）
M　DNAラムダラダーマーカー
1.5%　ゲル
BIO-RAD CHEF DR Ⅱ泳動装置
14℃
5－20秒　18時間
1－5秒　14時間
制限酵素：Sfi Ⅰ

考　察

3症例とも毒素非産生株であったことや，患者血清のジフテリア毒素抗体価が低値であることから，患者はジフテリア毒素に感作されていないと思われた。

C. diphtheriae のおもなバイオタイプ（gravis, mitis, intermedius）は，Andersonらにより1931年に記載されており[5]その後，多くの著書にその特徴が記載されている[6][7]。

今回の分離菌は血液寒天上でのβ溶血やスムース型のコロニー，生化学的性状などからバイオタイプはmitis型に分類された。

Diamondらは，*C. diphtheriae* がDNase陽性，他の *Corynebacterium* spp. は陰性であることを報告[8]している。また *C. diphtheriae* とDNaseの関係を示した論文・著書も多い[6][9][10][11]。今回分離された菌株もDNase陽性であった。

ジフテリア症状がある場合や *C. diphtheriae* を強く疑う場合はDNase試験の結果にかかわらず詳細な検討が必要と思われるが，毒素非産生株などジフテリア症状を呈しない感染や保菌者などのスクリーニングとして，DNase試験は安価で簡便な方法であり，どこの検査室でも実施可能なため同定試験のひとつとして有用な検査法と思われた。

C. diphtheriae に対する薬剤感受性試験では，各系統の薬剤で感性が保たれている菌株が多いものの，一部にマクロライド，ミノサイクリンなどに耐性化（MIC値の上昇）が見られるとの海外の報告がある[12][13][14][15]。

今回の分離菌について3菌株ともフルオロキノロン系抗菌薬であるCPFX，LVFXのMIC値が高く，2症例目，3症例目の菌株ではさらにエリスロマイシン，クリンダマイシンにも高いMICを示したこと

から抗菌薬選択に注意が必要と思われた。

我が国ではトキソイドワクチンの普及によりジフテリア症，ならびに *C. diphtheriae* に遭遇する機会はほとんどなくなっている。

しかしジフテリアトキソイドワクチンは接種後10年余りで抗体価が低下するといわれており[16]，国立感染症研究所などの調査でも日本人成人での抗体価の低下が指摘されている[1)17)]。

2007年5月から10月までの半年間に3例の *C. diphtheriae* が分離されたことは，国内においても *C. diphtheriae* が潜在的に増加している可能性があると思われた。

国外においては，1990年代にロシアをはじめとする旧ソ連圏の各国でジフテリア症の大流行が発生している。また現在でも東南アジアでは多くの感染が報告[18]されており，その他，世界各地で発生が見られている。

さらに毒素非産生株による感染例も英国など世界各地から多数報告され[4)15)19)]，ウィルソンらはジフテリア菌の増加に警鐘を鳴らしている[20)]。

我が国においても2006年1月，岡山県で血液培養から毒素非産株が検出されている[21)]。

今回分離された菌株のパルスフィールドゲル電気泳動では，2症例目と3症例目からの菌株で泳動パターンが一致した。しかし2人の患者間に共通の接点がなく，1症例目の菌株では異なる泳動パターンを示したこともあり感染経路は不明であった。今後は疫学調査を含めた対応が必要と思われた。

また現時点では2類感染症に指定されていないが，ジフテリア症状を呈する diphtheria-like toxin 産生 *C. ulcerans* による感染例が国内において2001年～2006年の間に5例発生している[22)]。

このことから *C. diphtheriae* や人畜共通感染症としての *C. ulcerans* などについて医療機関での検査体制を含め，再認識することが必要と思われた。

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Laboratory and Epidemiology Communications

A Case of Afebrile Pneumonia Caused by Non-Toxigenic *Corynebacterium diphtheriae*

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Afebrile pneumonia is a relatively unusual disease and may be caused by certain pathogens such as *Chlamydia trachomatis*, *Ureaplasma urealyticum*, respiratory syncytial virus, and *Pneumocystis jirovecii* (1,2). However, the specific causative agents of afebrile pneumonia have not yet been fully identified to date. Non-toxigenic *Corynebacterium diphtheriae* and toxigenic *C. diphtheriae* are partly responsible for respiratory infections including pneumonia, although the incidence of these infections may be very low (3,4). Here, we describe a case of afebrile pneumonia caused by non-toxigenic *C. diphtheriae*.

A 60-year-old Japanese female had been diagnosed with amyotrophic lateral sclerosis (ALS) at the age of 47 years. Two years after onset, she underwent tracheotomy and was provided with a mechanical ventilator. Aside from an occa-

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sional history of urinary tract or respiratory infections, she had no previous history of chronic or other infectious diseases. On May 9, 2007 (hospital day 1), she presented with respiratory distress due to elevated airway intrapressure, and purulent sputum (non-bloody, white-yellow color) was aspirated using a respiratory catheter, but no fever was noted (36°C). Chest radiography showed many spots of consolidations in the bilateral lung, pleural effusion in the left intrathoracic space, and infiltrations with partial atelectasis in the left lower lobe of the lung (Fig. 1A). On the day of radiologic examination, her clinical data were as follows: leukocyte count, $1.06 \times 10^4/\mu L$ (reference values, 3.5 to $9.0 \times 10^3/\mu L$); platelet count, $1.87 \times 10^5/\mu L$ (reference values, 1.5 to $3.0 \times 10^5/\mu L$); and C-reactive protein level, 1.8 mg/dL (reference values, <0.3 mg/dL). On the basis of these findings, she was diagnosed with afebrile pneumonia and was treated with levofloxacin (200 mg/day, day 1), panipenem/betamipron (500 mg/day, days 2 to 10), and intravenous fluids for 12 days. The lung lesion improved after hospital day 5 and the patient was afebrile during the clinical course.

Aspirated sputum samples were collected and examined by bacterial culture using 5% sheep blood agar. Many small and optically identical colonies (diameter, 0.5 to 1 mm) grew on the blood agar. Gram and Neisser staining of the colonies suggested *Corynebacterium* (Fig. 1B). In addition, phagocytosis of bacteria was clearly observed in the sputum smear (data not shown). Confirmation of the bacterial pathogen *Corynebacterium* was performed using the RapID CB Plus kit (Kyokuto Pharmaceutical, Tokyo, Japan). We also amplified the diphtheria toxin gene using a PCR technique (5,6); however, the gene was not detected (Fig. 1C). Moreover, the nucleotide sequence of the 16S rRNA gene of the present strain completely matched that of the *C. diphtheriae* prototype strain (7). The results confirmed the pathogen to be non-toxigenic *C. diphtheriae* (biotype: mitis). *C. diphtheriae* was no longer detected after hospital day 3. The isolate was susceptible to erythromycin, clarithromycin, clindamycin, benzylpenicillin, sulbactam/ampicillin, cefazolin, cefmetazole, cefpirome, imipenem, meropenem, vancomycin, teicoplanin, and minocycline, while the isolate was resistant to levofloxacin, ciprofloxacin, and fosfomycin. The pathogen's infection route could not be precisely elucidated in the present case.

It has been suggested that non-toxigenic *C. diphtheriae* may be associated with various infections such as pharyngitis, myocarditis, polyneuritis, and pneumonia (3,4). The present patient with ALS was confirmed to have afebrile pneumonia caused by this pathogen. In general, high fever is an important clinical sign in patients with pneumonia; however, few patients are diagnosed with afebrile pneumonia (1,2). The epidemiology of non-toxigenic *C. diphtheriae* in many countries, including Japan, remains poorly understood, despite the fact that this infection seems to be increasingly reported and has been found to occur in vaccinated individuals in other countries (3). ALS is a rare progressive neurodegenerative disease with an incidence of about 1/50,000, and it is known that respiratory complications in ALS patients may involve respiratory failure, pneumonia, and pulmonary embolism (8,9). Various bacteria may be associated with pneumonia in ALS patients, and this disease may be life-threatening (8,9). Here, we encountered a single case of afebrile pneumonia associated with ALS. From this case alone, we could not definitively determine whether *C. diphtheriae* easily causes afebrile pneumonia or not in ALS patients. However, it is possible that non-toxigenic *C. diphtheriae* is an etiologic pathogen of afebrile pneumonia, although it may be rare.

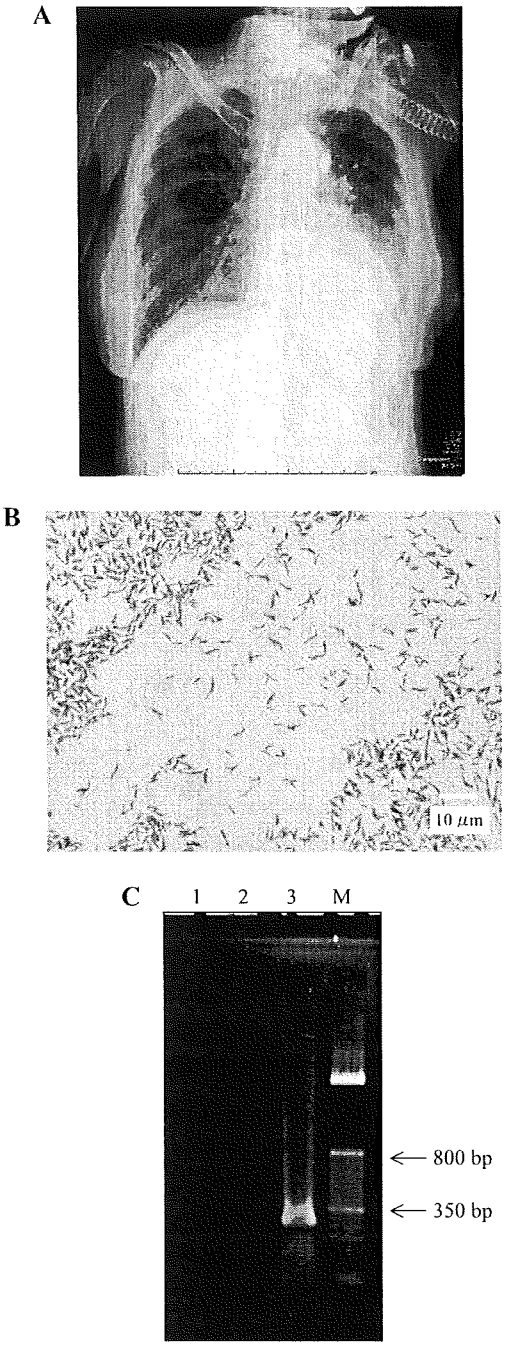

Fig. 1. (A) Chest radiography of the present case. (B) Gram staining of the isolate. Var indicates 10 μm. (C) Detection of diphtheria toxin gene. Amplicons were electrophoresed on a 1.5%-agarose gel. Lane 1, the present strain; lane 2, negative control (an amplicon derived from a non-toxigenic *Corynebacterium* [*C. xerosis*]); lane 3, positive control (an amplicon derived from toxigenic *C. diphtheriae*); M, marker.

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Toxigenic *Corynebacterium ulcerans* Isolated from the Domestic Dog for the First Time in Japan

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Corynebacterium ulcerans is known as a pathogen that causes purulent inflammation such as mastitis, lymphadenitis, dermatitis, and respiratory infections in various kinds of animals (1). The organism can carry a beta-corynephage similar to that which codes for the diphtheria toxin. Toxigenic strains of *C. ulcerans* cause infectious disease in humans, also similar to that caused by *Corynebacterium diphtheriae* (2). Five cases of human *C. ulcerans* infections have been reported since 2001 in Japan, and toxigenic *C. ulcerans* has been detected in each of these patients. Although a relationship between infected companion animals and human infection have been suspected in some cases, the source of infection has remained unidentified in all of these cases (3-7).

To obtain additional information on the distribution of toxigenic *C. ulcerans* among companion animals and to monitor for the prevalence of *C. ulcerans* infection, we investigated 65 healthy dogs that were under the care of the Osaka Prefectural Government from December 2006 to September 2007 for various reasons. Throat swabs were collected and kept in preservation medium (SEEDSWAB γ3 'Eiken'; Eiken Chemical, Tokyo, Japan) at 4°C until examined. Each specimen was inoculated on sheep blood agar and selective medium of a new formulation, Katsukawa medium, which contained heart infusion agar, potassium tellurite (0.03%), sheep blood (10%) and activated charcoal (0.05%). After 48 h of cultivation on Katsukawa medium at 35°C in 5% CO_2, *C. ulcerans*-like coryneform organisms were detected in the cultivated specimen that had been collected on August 7, 2007. However, none of these organisms were detected on the sheep blood agar. The dog from which the sample had been taken was a female mongrel that weighed approximately 20 kg. This dog had been kept by a family until it was sent to Osaka Prefectural Government. The dog did not externally exhibit any signs of illness. The Gram-positive coccobacilli isolate was positive for glucose fermentation and negative for sucrose utilization on DSS medium; the isolate also showed positive reactions to catalase and urease. Identification of the isolate was performed using API Coryne (bioMérieux, Marcy-l'Etoile, France). However, the API results (code 0011326) were insufficient for an unequivocal identification (*C. ulcerans* 87.2%, *Corynebacterium pseudotuberculosis* 12.5%). Moreover, because 16S rDNA sequencing is unsuitable for discrimination between *C. ulcerans* and *C. pseudotuberculosis* due to very high sequence similarity, *rpoB* and *hsp65* partial sequencing was carried out (8,9). Based on the nucleotide sequence from both loci, the organism was identified as *C. ulcerans*. PCR analysis of the diphtheria toxin gene, a modified Elek test, and VERO cell cytotoxicity and neutralization assays were used to demonstrate the toxigenicity of this strain of *C. ulcerans* (10-13). The entire nucleotide sequence of the diphtheria toxin gene (*tox*) of this strain was determined and compared with that of *C. diphtheriae* (GenBank accession no. BX248354). The two sequences differed from each other by 29 amino acids (homology, 94.8%).

The isolate was genetically compared with five isolates from Japanese human patients by pulsed-field gel electrophoresis (PFGE) (Fig. 1). The locations of cities in which six isolates of *C. ulcerans* have been reported are shown (Fig. 2). Phylogenic analyses revealed that the isolates could be divided into two groups as follows. One group contained strain 0102 (isolated in Chiba) (3), 0210 (Chiba) (7), 0509 (Okayama) (4), and 0607 (Kanagawa) (6). The other group contained an isolate from the dog in Osaka (0708) and the isolate 0510 (Oita) (5). Notably, PFGE revealed that the genotype pattern of the dog isolate was identical to that of the human isolate 0510 in Oita. Two isolates were identified in places that were geographically distant from each other, and therefore their relationship remains equivocal.

Few reports have clearly indicated a relationship between *C. ulcerans*-infected companion animals and human cases of infection (14). To the best of our knowledge, this report is the first epidemiologic study of dogs infected with *C. ulcerans*. Because the dog isolate was identical to an isolate from a human patient, as demonstrated by molecular epidemiology using PFGE, it is possible that the infection route was mediated by dogs. It will be necessary to investigate in large-scale studies any potential association between human disease and dog carriers of *C. ulcerans*.

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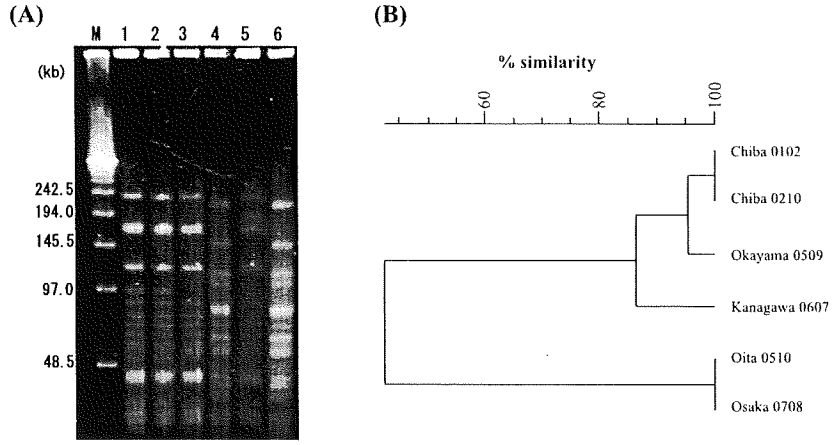

Fig. 1. (A) PFGE profiles of *Corynebacterium ulcerans* isolated from humans and a dog in Japan. PFGE of *Sfi*I-digested genomic DNA was performed as described elsewhere (15,16) with a slight modification. M, Molecular size marker (sizes are indicated on the left); lane 1-5, culture isolates from 5 humans (lanes 1 and 2, Chiba; lane 3, Okayama; lane 4, Oita; lane 5, Kanagawa); lane 6, isolates from the dog in Osaka. (B) Phylogenic analysis of *C. ulcerans* isolates. PFGE patterns were analyzed by using diversity database software with UPGMA algorithm.

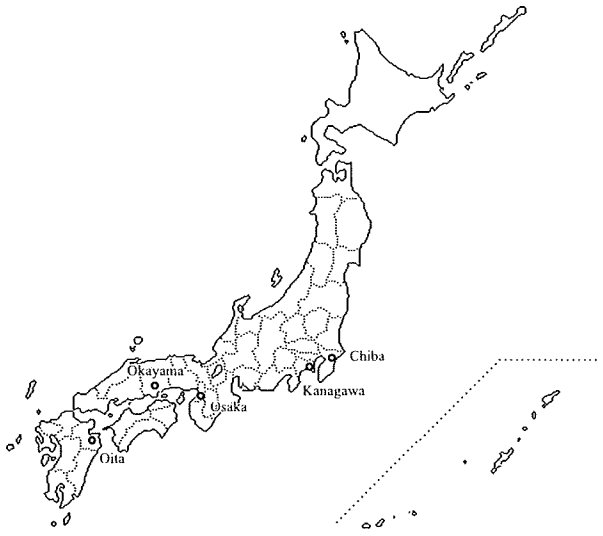

Fig. 2. Location of cities/prefectures in which 6 isolates of *C. ulcerans* were reported, including 5 human patients and a dog infected. First and second cases in humans were reported in Chiba, third case in Okayama, fourth case in Oita, and fifth case in Kanagawa. The dog origin was isolated in Osaka.

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