

## Laboratory and Epidemiology Communications

### 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 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 $\gamma$ 3 '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<sub>2</sub>, *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). Phylogenetic 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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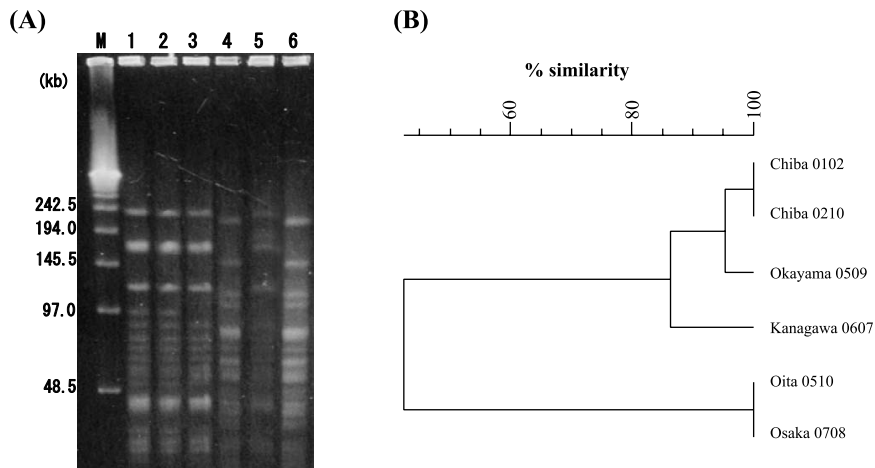


Fig. 1. (A) PFGE profiles of *Corynebacterium ulcerans* isolated from humans and a dog in Japan. PFGE of *Sfi*I-digested genomic DNA was performed as described elsewhere (15,16) with a slight modification. M, Molecular size marker (sizes are indicated on the left); lane 1-5, culture isolates from 5 humans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