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Detection of the Tetanus Toxin Gene by Polymerase Chain Reaction: A Case Study

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Approximately 50 tetanus cases are reported every year in Japan. Although the incidence is low, the mortality rate is high (1). We report here a tetanus case in which the tetanus toxin gene was detected by the polymerase chain reaction (PCR).

A 60-year-old male suffered a laceration injury of 5 cm on the left parietal region of his head on August 12, 2005, but left the injury untreated. On August 15, he developed gait and respiratory disturbances, in addition to head and neck pain. He was immediately sent to the emergency unit of a hospital. On admission, he showed clear consciousness but had difficulty in speaking. Due to difficulties in respiration, he had a tracheal intubation and his respiration was then maintained by a respirator. He was clinically diagnosed with tetanus and was treated with anti-tetanus human γ-globulin (total, 6,000 units). On August 18, generalized convulsions, trismus, and spastic paralysis of the upper extremities and trunk appeared. Typical Gram-positive drumstick-shaped bacilli were detected in samples from the injury site. The administration of penicillin G (20,000,000 units/day) was started together with muscle relaxing and anticonvulsion therapies. On August 26, since the tetanus bacilli continued to be detected in the injury site, the site was washed with oxydol and human γ-globulin (total, 3,000 units) was administered.

Beginning on September 10, the convulsions and spasms lessened and the patient was taken off the respirator on September 14. He was able to seat himself on September 17. Because he continued to have difficulty opening his mouth,

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he was transferred to another hospital for rehabilitation.

Microscopic examination of the patient's specimens that our laboratory received on August 31 showed Gram-positive cocci and bacilli. *Clostridium tetani* infection was suspected, and the specimen was streaked on the periphery (in order to take advantage of its high mobility) of 5% sheep red blood cell agar that had been incubated under anaerobic conditions. After anaerobic culture for 24 h, the tips of the colonies were examined by Gram and Moeller spore stainings, and were found to show nearly pure cultures of *Clostridium*-like bacilli.

For detection of the tetanus toxin (tetanospasmin) gene, colonies on an agar plate were suspended in distilled water and lysed by incubation at 95°C for 10 min. After centrifugation (15,000 rpm for 60 sec), the supernatant of the lysate was used for DNA extraction. PCR detection of the tetanospasmin gene was carried out following the method described by Kato et al. (2). The PCR amplification conditions were 35 cycles of 95°C for 20 sec and 55°C for 20 sec, and the PCR product was separated on 2% agarose gel (TAKARA L0-3; Takara Bio, Inc., Otsu, Japan). The 331-bp and 229-bp bands expected for tetanospasmin gene fragment were obtained (Fig. 1).

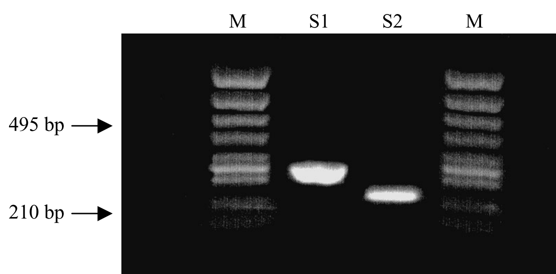

Fig. 1. Detection of tetanospasmin gene by PCR. Primer pairs were GAT1 and GAT2 for S1, and GAT5 and GAT6 for S2. The expected size for tetanospasmin gene is 331 bp for S1 and 229 bp for S2. M, molecular weight size marker (Marker 5; Nippon Gene, Tokyo, Japan).

Although tetanus is usually diagnosed clinically, PCR detection of tetanospasmin can facilitate the rapid definitive diagnosis of this infection. In the present case, we obtained the final results on September 2, only 3 days after receipt of the specimen.

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