

First Report of *Salmonella* Serotype Tilene Infection in Korea

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Salmonellosis is a common food- and water-borne disease and is also a major zoonosis. Currently, the isolation of rare *Salmonella* serotypes is increasing every year in Korea. Among them, the *Salmonella* serotype Tilene was first isolated from two people who visited a hospital located in Andong-si in 2013. Clinical symptoms were weak or non-existent. There was no clear epidemiological connection between the two cases. However, it was assumed that both were independently exposed to a single infectious agent. Perhaps due to their geographical proximity, molec-

ular epidemiological analysis showed the same result between the isolated strains. This serotype has increasingly reported an association with hedgehogs. Recently, the importation of exotic animals, including hedgehogs, as pets has been gradually increasing. Thus, it is recommended that high-risk groups avoid contact with exotic pets. (*Ann Clin Microbiol* 2016;19: 24-27)

Key Words: Exotic animals, Rare serotypes, *Salmonella* Tilene, Zoonosis

INTRODUCTION

Salmonella species is the most well-known food-borne pathogen worldwide. It is also a cause of major zoonosis acquired through various animals [1]. In recent years, several reports have shown that rare serotypes in humans were mainly transmitted by non-traditional pets [2]. For example, *Salmonella* serotype Tilene was very rarely the cause of human infection, but it was generally associated with African pygmy hedgehogs [3]. The first human infection was reported in a child in Senegal, 1960 [4], and *S. Tilene* infection was also documented in Washington State, the United States, 1994 [3]. And then *S. Tilene* was first isolated from two people at Andong-si in Korea, 2013. In this study, we investigated the infection source and characterized a phenotypic and a genetic analysis of the pathogens.

CASE REPORT

The first case was a 10 year-old girl who visited the emergency department in the hospital with non-bloody diarrhea on

April 19, 2013. Her condition was considerably improved 3 days after treatment with symptomatic medicine including a binding medicine. The second case was an asymptomatic 78 year-old woman on April 22, 2013. She visited the hospital to routine check-up in a healthy condition with neither any clinical symptoms nor any treatment. There was no clear epidemiological connection between the two people. They were not members of the same family, had no social relationship, and did not breed any exotic pets, including hedgehogs. However, it was assumed that they lived in a nearby area because they visited the same hospital located in Andong-si, Korea.

Stool samples were collected from the two people and used to investigate the causative pathogens. Microbiological tests were performed at the Gyeongsangbuk-do Institute of Health and Environment, following the laboratory diagnosis guidelines of the National Research Institute of Health. The molecular epidemiological investigation was performed at the Division of Enteric Disease, National Research Institute of Health [5]. In brief, fresh stool was enriched in Tryptic Soy Broth before plating a loopful of the sample on MacConkey agar, salmonella-shigella agar, and Xylose Lysine Deoxycholate agar plate medium

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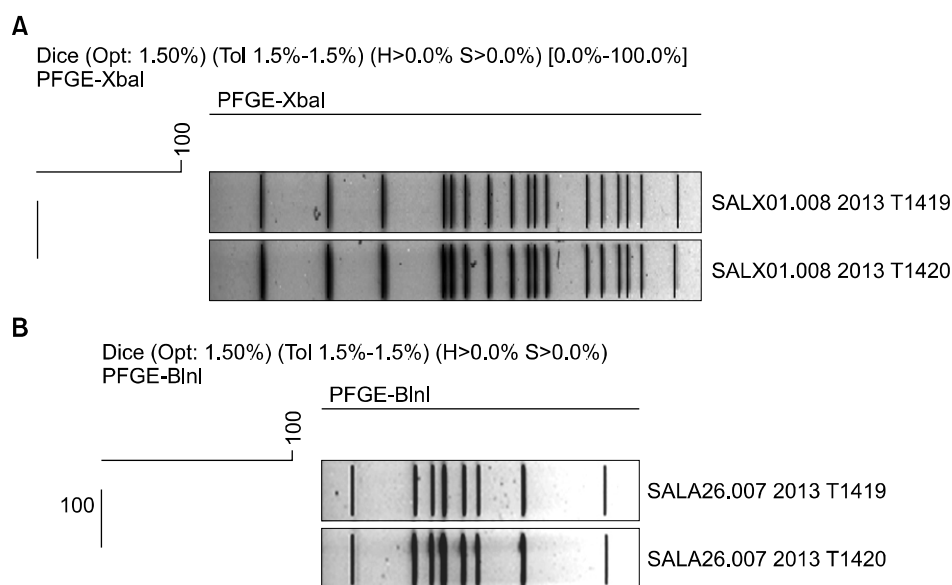


Fig. 1. Pulsed-field gel electrophoresis pattern of *Salmonella* Tilene isolates. Human isolate T1419 (a 10 year-old girl) and T1420 (a 78 year-old woman) show identical band patterns that following digestion of their genomic DNAs with *XbaI* (A) and *BlnI* (B).

(BD diagnostic systems, Sparks, MD, USA). The unique colonies were streaked on Kligler Iron Agar (BD), and their identification was finally confirmed using an API 20E kit (bioMérieux, Marcy-l'Etoile, France) and a serological test [5,6]. The serotypes of *Salmonella* were identified using the combination of somatic O and flagellar H antigens according to the Kauffman-White scheme [6,7]. As a result, serotype of the strains was identified as *S. Tilene* of the R group (Antigenic formulae, [1],40:e,h:1,2). An antimicrobial susceptibility test was performed using a Vitek2 with AST-N169 (bioMérieux). The two isolates were all susceptible to 17 antimicrobial agents (ampicillin, amoxicillin-clavulanic acid, amikacin, chloramphenicol, cephalothin, ciprofloxacin, ceftriaxone, cefotetan, cefotaxime, cefazolin, ceftiofur, gentamicin, imipenem, nalidixic acid, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, and tetracycline) according to the CLSI guideline [8]. Furthermore, pulsed-field gel electrophoresis (PFGE) was performed according to the PulseNet standard protocol (<http://www.pulsenetinternational.org/protocols/>). DNA of each of the isolates was digested with *XbaI* and *BlnI* restriction enzymes and was separated by a CHEF MAPPER (Bio-Rad, CA, Hercules, USA). *Salmonella* Braenderup (ATCC BAA664) was used as a reference marker strain. The PFGE patterns were analyzed using BioNumerics v5.1 software (Applied Maths, Saint-Martens-Latem, Belgium) and showed 100% similarity in both *XbaI* and *BlnI* digestion (Fig. 1).

DISCUSSION

The host range of *Salmonella* infection was affected by clinical syndrome and mortality. Examples include *S. Gallinarum*, for the host-restricted serotype that infects poultry and *S. Enteritidis* for a wide-host range serotype [9]. Zoonotic serotypes, with a wide host range, are often characterized as causing high morbidity, but low mortality and gastro-intestinal symptoms are the predominant clinical manifestation [10]. Gastroenteritis was the main symptom, and mortality was very low, although these results did not confirm that *S. Tilene* is a zoonosis. The absence or weak clinical symptoms of *S. Tilene* infections were shown in this study and the previous cases also reported mild symptoms [11]. However, there is a small but dangerous possibility of sepsis with high fever and diarrhea [3].

We could not clearly demonstrate the infection route because of the restriction of access to private information and the limited epidemiological data on *S. Tilene* infections. However, we assumed that the two people were independently exposed to a single infectious agent at the same time. First, they visited the same hospital over the 3 days interval in the same geographical areas, Andong-si. They might be Andong-si residents, although this could not be confirmed as we could not obtain their addresses due to the policy of private information. Andong is a small city in Gyeongsangbuk-do with a population of 168,910 as of November 2015. Second, molecular epidemiological and antibiogram analysis from the two different isolates revealed identical results. The two isolates reflected a single clone based on PFGE restriction patterns using two restriction enzyme

treatments. Third, hedgehogs were reported to be a major host of *S. Tilene*, and previous infection cases were also closely associated with hedgehog contact [2,3,11]. *Salmonella* isolated from exotic animals has been shown to be generally susceptible to antimicrobial agents [12,13], and these isolates were tested susceptible to all 17 agents. For this reason, the infection source may be an imported hedgehog or another exotic animal that is not common in Korea, especially in a small city like Andong-si.

Recently, isolation of rare *Salmonella* serotypes has been increasing every year, and human infection cases have been continuously increasing in Korea [3,5]. *S. Tilene* is representative of a globally rare serotype. Human infections were strongly associated with hedgehog contact, and there were outbreaks and sporadic cases in the United States and Canada [3,11]. However, the role of domestic animals in infections is currently ambiguous. Several *S. Tilene* strains were isolated from cattle intestines and chicken meat samples but were eventually associated with hedgehogs [14]. The contact with hedgehogs existing in the natural environment is unlikely, but hedgehogs bred domestically as pets offer a more probable opportunity of direct animal contact. According to the Korea Customs Service's statistics, it has shown a gradually increasing trend in the importation of exotic animals, including hedgehogs since 2002 [15]. However, quarantine of exotic animals was not performed, and isolation of *Salmonella* will continue to be reported. The emergence of zoonotic infections underscores the importance of strict regulatory and surveillance policies in the customs and pet industry.

Wild-life and exotic pets clearly represent potential *Salmonella* infection sources and may cause several other zoonotic diseases. Thus, it is recommended that high-risk groups, such as immune-deficient people, children younger than 5 years of age, and pregnant women, avoid contact with exotic pets.

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=국문초록=

국내 최초로 분리된 *Salmonella* Serotype Tilene에 의한 감염 2예

질병관리본부 국립보건연구원 감염병센터 수인성질환과

채수진, 윤영선, 유천권, 정경태, 이덕용

살모넬라균은 수인성·식품매개질환을 일으키는 병원체 중 하나이지만 인수공통감염병의 원인 병원체이기도 하다. 최근 국내에 희귀혈청형의 분리 및 감염사례가 지속적으로 증가하고 있으며, 그 중에서 *Salmonella* serotype Tilene이라는 희귀혈청형이 2013년 경상북도 안동지역에서 국내 최초로 분리되었다. 가족관계가 아닌 10살 여아와 78세 여성은 동일한 병원에 3일 간격으로 내원하였고 각자 미약한 임상증상 및 무증상을 보였다. 감염원에 대한 역학조사 자료가 부족하여 명확한 감염경로를 증명할 수는 없었지만, 두 사람은 비슷한 시기에 각자 독립적으로 단일 감염원에 노출되었다고 추정된다. 그에 대한 근거로 둘은 동일지역에 거주하고, 분리된 균주 간의 분자역학조사 결과에서 100% 상동성을 보였으며, 전 세계적으로 *S. Tilene*은 고슴도치에 의한 감염사례가 대부분이라는 점 등을 들 수 있다. 최근 국내에는 애완동물로서의 고슴도치 수입이 증가하는 추세이다. 따라서 살모넬라 감염으로 심각한 전신성 감염을 일으킬 수 있는 위험군의 경우에는 외래종 애완동물과의 접촉을 주의하는 것이 필요할 것이다. [Ann Clin Microbiol 2016;19:24-27]

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