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Plague Epidemic in the Kingdom of Naples, 1656–1658

To the Editor: In 1656, an epidemic of plague occurred in the Kingdom of Naples, Italy. Earlier the disease had spread from Algiers to Spain; in June 1647, it appeared in Valencia, and in the spring of 1648, it appeared in Aragon and several other Spanish areas of Valencia, Andalusia, and Catalonia. In 1652, plague had spread to Sardinia and then to the cities and territories of Naples, Rome, and Genoa. Within the Kingdom of Naples, plague first reached the town of Naples in the spring of 1656. Despite measures restricting population movement, by the summer of 1656, the disease had reached several provinces in southern Italy (*1,2*).

Historical records indicate that the epidemic in Barletta, in southern Italy, developed after the arrival of a ship from Naples. On May 26, 1656, the ship Sant' Andrea arrived from Naples at the port of Barletta. However, after sanitary inspection, the ship was prevented from landing and obliged to depart, but this measure was not sufficient to prevent the disease from entering the port. The Barletta epidemic peaked in October, after which the number of cases diminished; and on June 22, 1657, Barletta was declared free of plague. Of this city's original population of 20,000, the disease killed 7,000–12,000 persons. It is hypothesized that throughout the Kingdom, the plague killed ≈1,250,000 persons (*1,2*).

Since the 14th century, noble families of Barletta had been buried in tombs in underground tunnels of Sant' Andrea church. During restoration of the church in 2009, more underground tunnels containing many skeletons were discovered. It has been hypothesized that the church had also been used as a cemetery during the plague epidemic. During an inspection of the skeletons, 5 skulls of young persons were identified and collected. For a negative control, the skull of a person buried in a tomb before the epidemic was also collected.

The skulls were radiographed to identify unerupted teeth (Figure), which were then aseptically extracted. After classification, each tooth was cut along a sagittal line to uncover the dental pulp, which was then hydrated in sterile phosphate-buffered saline (pH 7.2) for 48 h at 37°C. The DNA was extracted by using DNAeasy Blood and Tissue Kits (QIAGEN, Hilden, Germany) and by modifying the first step, which was conducted overnight at 56°C with 600 μL of ATL buffer (QIAGEN) and 50 μL of proteinase K. To verify the presence of inhibiting substance, the control DNA extracts were screened by using a PCR for human mitochondrial DNA (*3*).

To investigate the cause of the deaths, we adopted a PCR suicide method and searched for *Yersinia pestis*. We amplified the *pla* gene for *Y. pestis* by using Sybr green PCR in real time with a modification of a previous protocol (*4*) coupled with conventional PCR according to Drancourt et al. (*5*). Conventional PCRs were adopted for *Bacillus anthracis* by targeting the *pag* and *capC* genes (*6*) and for *Salmonella enterica* serovar Typhi by targeting the *narG* gene (*7*). To prevent cross-contamination, we conducted all PCRs with a negative control and in the absence of positive controls. Melting curve analysis and agarose

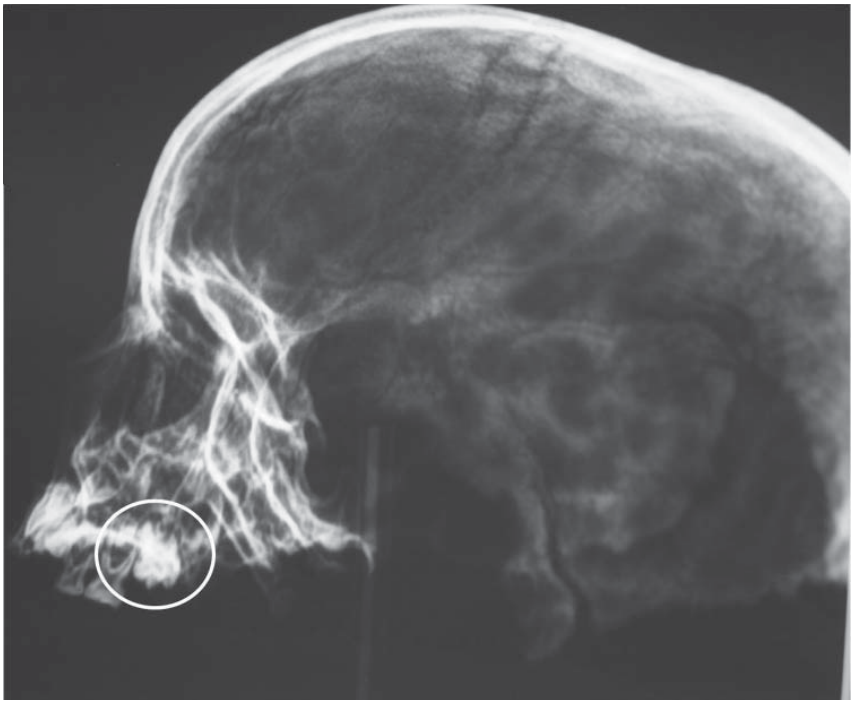

Figure. Radiograph of skull found under Sant' Andrea church in Barletta, Italy, in 2009, showing unerupted teeth (circled) that were later extracted aseptically.

gel electrophoresis of PCR products indicated suspected positive samples. All amplicons relative to conventional and suicide PCR were submitted for sequencing analysis confirmation. The negative DNA was reanalyzed to confirm the results.

From the 26 dental pulp samples analyzed from the 5 skulls of young persons, 7 samples were positive for the *pla* gene of *Y. pestis* by the Sybr green real-time PCR, and 2 of these were positive for this gene by conventional PCR. All were negative for *B. anthracis* and *S. enterica* ser. Typhi. GenBank BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi) results of the 2 sequenced amplicons found a 100% match with the reference sequences (GenBank accession no. AL109969.1); query coverage was 100%. The sequences obtained were deposited in the GenBank sequence database under accession nos. JN208020–1.

In conclusion, the confirmed finding of DNA of *Y. pestis* in 2 skeletons and suspected finding in the remaining 3 suggests that these persons died of plague during the 1656–1658 epidemic in southern Italy. Although it has not been universally agreed upon, several studies have confirmed that the agent of 16th to 18th century "plague" epidemics in Europe were caused by *Y. pestis*. Different methods have documented *Y. pestis* as the agent in 10 Black Death burial sites scattered over 5 countries (*8*). In northern Italy, the presence of *Y. pestis* has been confirmed in Venice (14th–17th centuries) (*8*), Genoa (Bastione dell'Acquasola) (14th century) (*9*), and Parma (16th–17th centuries) (*10*). This study confirms that the plague that infected the Kingdom of Naples, which spanned almost all of southern Italy, was also caused by *Y. pestis*.

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Leprosy, Still Present in La Réunion

To the Editor: During recent decades, a considerable and consistent decrease in worldwide incidence of leprosy has been observed, mainly because of the recommendation to introduce multidrug therapy in 1981 (*1*) and the implementation of free therapy in 1994 (*2*) by the World Health Organization (WHO). The prevalence rate of the disease has been reduced globally by >90% since 1985 (*3*). Since 2000, WHO has recommended the implementation of a leprosy surveillance system in leprosy-endemic countries with indicators for screening, treatment, and monitoring of patients (*4*). From these indicators, WHO establishes an annual official report on the global status of the disease. According to official reports received from 141 countries, the global registered prevalence of leprosy was 211,903 cases in 2010 (*5*).

La Réunion is a French overseas department located in the Indian Ocean 700 km east of Madagascar. La Réunion's health care system is similar to that of continental France. Although new cases of leprosy have been punctually reported by health professionals during the past few years, which suggests that the disease is still present, the situation in La Réunion is poorly documented because of the lack of a specific surveillance system. Thus, the goal of eliminating leprosy as a public health problem (i.e., prevalence <1/10,000) (*6*) cannot be assessed because the goal requires a good knowledge of the epidemiologic status of the disease. Furthermore, the risk of leprosy recrudescence linked to a relapse of patients with autochthonous cases or patients with leprosy migrating from neighboring leprosy-endemic countries, such as Madagascar, Comoros, and Mayotte (*5*), is present. In 2009, a total of 1,572 new cases of leprosy were detected in Madagascar, 319 in Comoros, and 37 in Mayotte (*5*,*7*). If one considers the geographic proximity and the many tourist exchanges between La Réunion and those neighboring islands, the risk of importation, although low, is constant.

In that context, Cire Indian Ocean (the Regional Office of French Institute for Public Health Surveillance), in collaboration with health professionals involved in diagnosis and treatment of the disease, has implemented a specific surveillance system for leprosy in La Réunion. The objectives are to guide potential preventive measures by determining incidence of leprosy, following the disease's evolution, and characterizing the patients affected.

The surveillance system was based on the notification of every case by health professionals likely to diagnose and treat subjects according to the WHO case definition (*8*), i.e., clinicians, private or hospital dermatologists, and infectious disease specialists. The notification was realized through a standardized questionnaire that included sociodemographic, clinical, and microbiological data. Concurrently, the pathology laboratories were consulted to detect any nondeclared cases and to improve the completeness of data.

This surveillance was retrospective for 2005–2010, then prospective for 2011. In total, 17 patients responding to the case definition of leprosy and given a diagnosis during 2005–2010 were reported for an average population of 804,025 inhabitants in La Réunion (data from the National Institute of Statistics and Economics Studies). The mean annual incidence during this period was 3.4 cases/10^6 inhabitants. The male:female sex ratio was 2.2, and the median age was 54 years (range 8–77 years). More than half the patients were born in La Réunion (n = 9); the other patients' birthplaces were Comoros Islands (n = 4), Mayotte (n = 3), and Madagascar (n = 1). Among the patients born in La Réunion, 6 had never left the island, 3 had traveled but had always resided in La Réunion, and 6 patents resided in the same area of a city in the southwestern part of the island.

An active search for other cases in this area was performed by contacting all the health professionals likely to diagnose leprosies; 1 clinician reported a suspected case among his patients. That patient is currently being screened. Of the patients overall, 15 were screened by skin biopsy or smear from the ear.