ORIGINAL ARTICLE

Enzootic plague foci, Algeria

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Abstract

In Algeria, PCR sequencing of *pla, glpD* and *rpoB* genes found *Yersinia pestis* in 18/237 (8%) rodents of five species, including *Apodemus sylvaticus,* previously undescribed as pestiferous; and disclosed three new plague foci. Multiple spacer typing confirmed a new Orientalis variant. Rodent survey should be reinforced in this country hosting reemerging plague.

Keywords: Algeria, Molecular typing, North Africa, Plague, *Yersinia pestis*
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Introduction

Plague, a deadly infection caused by the bacterium *Yersinia pestis,* is reemerging in some North African countries, including Libya and Algeria [1–3]. In Algeria, plague reappeared after 50 years of silence with two consecutive episodes in 2003 in Oran [1] and in 2008 in a small camp of nomads in the Thait El Maa area in Laghouat province [2]. In both outbreaks, patients originated from rural areas where they raised animals. Confirmation of the two Algerian outbreaks was made by using molecular investigations of the presence of *Y. pestis* in rodents and in rodents' fleas [1,2]. When the disease broke out in the Oran area in 2003, no plague focus had been described for decades in Algeria after rodent surveys were dropped.

Therefore, in an effort to depict the current activity of plague foci in Algeria, we initiated a rodent study and molecular investigations of rodents captured in nine regions of Algeria.

Methods

Yearly field missions were conducted in 2009 to 2012, primarily in northern Algeria (Fig. 1). These missions aimed to better understand the diversity of small mammals, including rodents maintaining *Y. pestis* in zoonotic foci throughout the country. All catches were made on private farms from November 2009 to February 2012 by using BTS (Besançon Technique Service; INRA, Montpellier, France) and Sherman Trap (H. P. Sherman Traps, Tallahassee, FL, USA). After morphological identification, rodents were humanely killed; the spleen was extracted and stored individually in a sterile Eppendorf tube in ethanol (70%) before being tested in Marseille, France, in a biological security level 3 laboratory. Ethanol-preserved spleens were rinsed with sterile distilled water for 2 minutes, and total DNA was extracted by using the NucleoSpin DNA purification tissue kit according to the manufacturer's instructions (Macherey and Nagel, Düren, Germany). Real-time PCRs were performed by using a CFX 96 Real Time PCR System (Applied Biosystems, Coignières, France). Negative controls, consisting of noninfected Balb/c mice spleen total DNA, were introduced every five samples in all PCR experiments. In a first step, a 98 bp fragment of the plasminogen activator gene (*pla*) was amplified as previously described [4]. Confirmation was done by further

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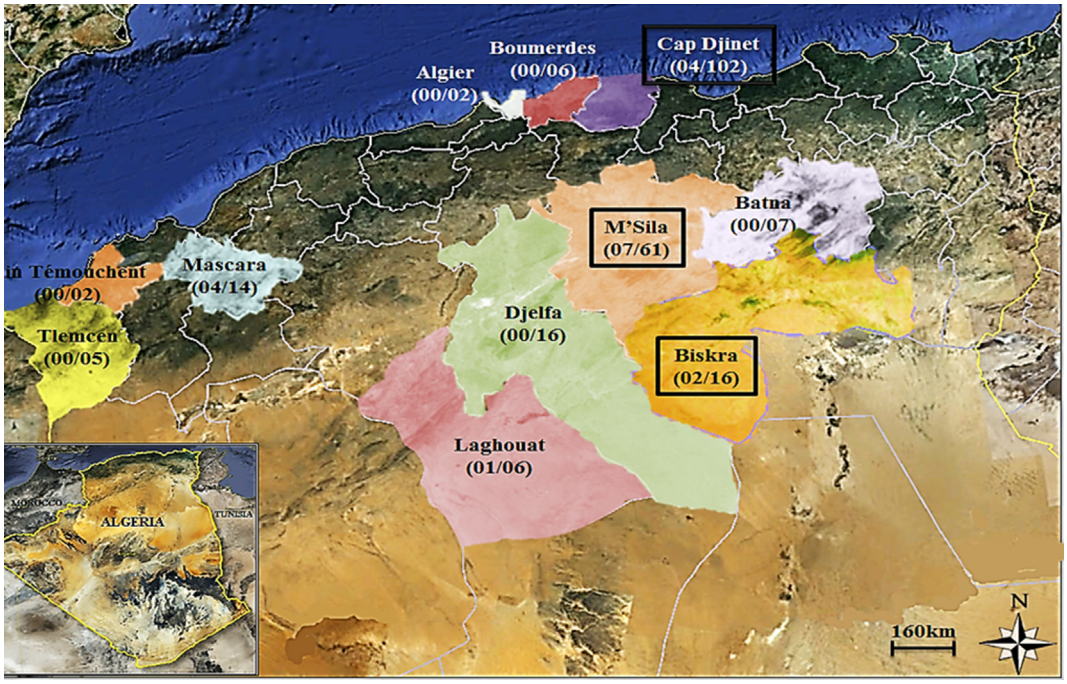

FIG. 1. Map of Algeria indicating number of *Yersinia pestis*–positive captured rodents in 12 areas. Tlemcen: five *Rattus norvegicus*. Aïn Témouchent: one *Rattus rattus* and one *Meriones shawii*. Mascara: 14 *R. rattus*. Laghouat: six *M. shawii*. Djelfa: eight *R. rattus*, four *M. shawii* and four *Mus spretus*. M'Sila: 22 *M. shawii*, 12 *Psammomys obesus*, 11 *R. rattus*, ten *Mus spretus*, five *Jaculus jaculus* and one *Atelerix algirus*. Biskra: 16 *M. shawii*. Batna: four *P. obesus*, two *R. rattus* and one *Mus spretus*. Algiers: two *R. rattus*. Boumerdès: three *A. algirus* and three *R. rattus*. Cap Djinet: 58 *Mus musculus*, 24 *Crocidura russula*, 13 *Apodemus sylvaticus*, six *Lemniscomys barbarus*, one *R. rattus*.

partial PCR amplification and sequencing of the *glpD* gene encoding the glycerol-3-phosphate dehydrogenase [5] and on positive specimens by partial PCR amplification and sequencing of a 100 bp fragment of *rpoB* gene that encodes the β subunit of RNA polymerase [6]. Positive specimens were further genotyped by multiple spacer typing (MST) by sequencing PCR-amplified spacers YP1, YP3, YP4, YP5, YP7 and YP8, as previously described [7]. Gene sequences obtained with an ABI 3130Xl Genetic Analyzer (Applied Biosystems) were compared with those available in GenBank by using the nucleotide–nucleotide BLAST (blastn) program (available from http://www.ncbi.nlm.gov/BLAST/), and spacer sequences were compared with those previously reported [7].

Results

A total of 237 rodents were captured in the geographical area indicated in Fig. 1. While negative controls remained negative, *pla* fragments were amplified in 44/237 (18.5%) spleen specimens, with a cycle threshold value ranging from 27.64 to 34.35. *Pla*-positive specimens were collected from two *Rattus norvegicus* in Tlemcen; six *Rattus rattus* in Mascara; one *Meriones shawii* in Laghouat; two *M. shawii* in Biskra; one *R. rattus* in Batna; four *M. shawii*, three *Psammomys obesus*, one *Mus spretus* and two *R. rattus* in M'Sila; and 13 *Mus spretus*, six *Apodemus sylvaticus* and three *Crocidura russula* in Cap Djinet. Among 44 *pla*-positive specimens, 18 (41%) were further positive for both the *glpD* gene and for *rpoB* amplification in four *R. rattus* from Mascara; one *M. shawii* from Laghouat; two *M. shawii* from Biskra; two *M. shawii*, two *P. obesus*, two *R. rattus* and one *M. spretus* from M'Sila; and two *C. russula*, one *M. spretus* and one *A. sylvaticus* from Cap Djinet. These specimens were regarded as definitely positive for *Y. pestis*. *glpD* sequences exhibited 100% identity to the reference sequence for biovar Orientalis (GenBank accession numbers AL590842 and YPO3937) characterized by a 93 bp deletion. Multispacer sequence typing yielded the same profiles in all the specimens, including spacer YP1 type 1; spacer YP3, type 5; spacer YP4, type 1; spacer YP5, type 1; and spacer YP8, type 2. YP7 spacer was sequenced in only nine specimens as a result of a limitation of the materials, and yielded a type 9. Altogether, MST data indicated a new MST type 20 in the Orientalis biovar.

Discussion

Here, we achieved a renewed picture of plague enzooty in Algeria by using PCR sequencing of *Y. pestis* in field rodents