MAJOR ARTICLE

Evidence for Louse-Transmitted Diseases in Soldiers of Napoleon's Grand Army in Vilnius

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Background. Many soldiers in Napoleon's Grand Army died of infectious diseases during its retreat from Russia. Because soldiers were commonly infested with body lice, it has been speculated that louse-borne infectious diseases, such as epidemic typhus (caused by *Rickettsia prowazekii*), were common.

Methods. We investigated this possibility during recent excavations of a mass grave of Napoleon's soldiers in Vilnius, Lithuania. Segments of 5 body lice, identified morphologically and by polymerase chain reaction (PCR) amplification and sequencing, were found in earth from the grave that also contained fragments of soldiers' uniforms.

Results. DNA of *Bartonella quintana* (the agent of trench fever) was identified by PCR and sequencing in 3 of the lice. Similarly, PCR and sequencing of dental pulp from the remains of 35 soldiers revealed DNA of *B. quintana* in 7 soldiers and DNA of *R. prowazekii* in 3 other soldiers.

Conclusions. Our results show that louse-borne infectious diseases affected nearly one-third of Napoleon's soldiers buried in Vilnius and indicate that these diseases might have been a major factor in the French retreat from Russia.

Human body lice transmit *Borrelia recurrentis, Bartonella quintana,* and *Rickettsia prowazekii,* the agents of louse-borne relapsing fever, trench fever, and epidemic typhus, respectively [1]. Although these bacterial pathogens have been known for a long time—since 1867, 1919, and 1911, respectively—historical descriptions of the diseases they cause are confusing. Although "famine fevers" and other fevers were associated with conditions that promoted the multiplication of body lice, such as wars, these parasites were only definitively linked with typhus in 1909 by Nicolle [2]. Subsequently, it has been said that they have caused more deaths than weapons during wartime [3]. Historical accounts show that, during the Russian campaign, Napoleon's soldiers were plagued with body lice and that many died with fever [4].

During construction work in late autumn 2001, mass graves were discovered on the site of a former Soviet Army barracks in the northern suburbs of Vilnius (Verkiu Street, Siaures Miestelis Territory). Records in local archives indicated that the graves contained French troops, which were garrisoned in Vilnius from December 1812, during the retreat of Napoleon's Grand Army from Moscow [5].

The remains of 717 individuals were found in the first area studied, at a density of 7 corpses/m^2. Similar densities of corpses were found at other sites along the excavation trench, and this suggested that the site contained a total of 2000–3000 corpses (figure 1). The skeletons were in close proximity to one another (0.2–0.5 m), indicating that they had been buried at the same time. They were not in a position associated with rigor mortis, suggesting that the soldiers had been buried soon after death and that the intense cold had frozen them in the position in which they had been placed. Analysis of fragments of uniforms and buttons revealed that soldiers and officers from 40 different regiments were buried in the trench [5] (figure 2). To identify louse-borne diseases in these remains, we looked for ancient lice and amplified the DNA of the agents of louse-borne diseases in the dental pulp. We performed this search in accordance with the 6 recently published

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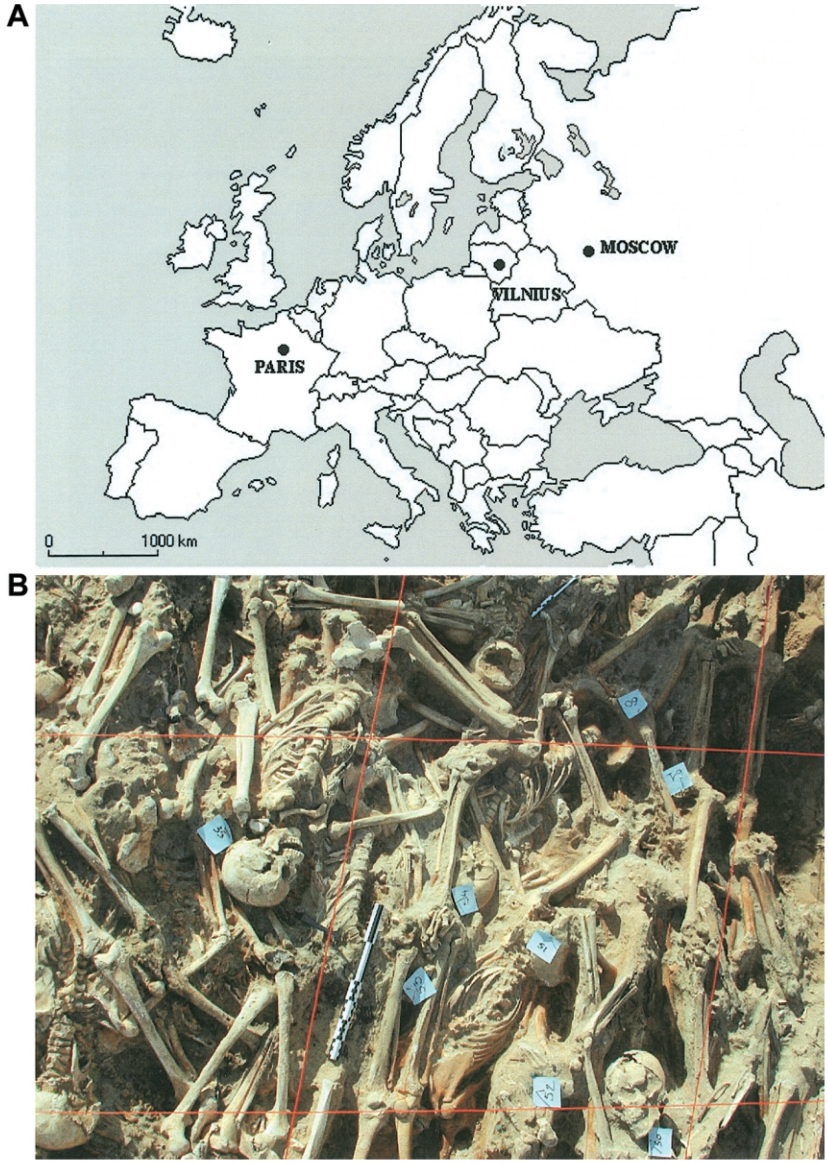

Figure 1. *A,* Map showing the location of Vilnius. The "Grande Armée" retreated form Moscow to Paris. *B,* General view of the grave in Vilnius (photo by P. Adalian, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 6578).

criteria for authentication of molecular data in paleomicrobiology [6]: (1) there should be no positive control; (2) negative controls should test negative (several should be tested that are as similar as possible to the ancient specimens, and the methods used to test the specimens and the negative controls should be as similar as possible); (3) a new primer sequence should be used, one not previously amplified in the laboratory (we named this "suicide polymerase chain reaction [PCR]" [7]); (4) the amplicon should be sequenced; (5) a second target should be amplified and sequenced, to confirm a positive result; and (6) an original sequence that differs from modern homologues should be used, to exclude contamination.

MATERIALS AND METHODS

Extraction and identification of lice. We evaluated 5 techniques for identifying lice in earth samples before we tested our sample from Vilnius. In our preliminary study, we used earth from Marseilles that had a texture similar to that from Vilnius and 25 dead lice (strain Orlando; 15 days to 2 months of age) from our rabbit-fed *Pediculus humanus humanus* colony. The lice were dried at 37°C for 10 days before being mixed with 500 g of earth [8].

In the first assay, we slowly mixed the earth into 5 L of distilled water in a polyethylene 900-cm^2 square dish. After 1

Figure 2. Imperial-type button found in the grave in Vilnius (photo by P. Adalian, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 6578).

h, we carefully searched the debris on the water surface for 0.5–2-mm insect fragments with a binocular magnifying glass. In the other 3 assays, we used paraffin, olive oil, or groundnut oil instead of water. The fifth assay was derived from extraction techniques developed for fossilized insects [9]. Sand was slowly mixed into 2 L of kerosene in a steel 900-cm^2 square dish. Two liters of cold distilled water was then mixed with the kerosene and left to settle before the water-kerosene interface was examined for insects. DNA was extracted from the insect fragments after they were washed in sterile, distilled water for 2 days with gentle rocking at room temperature.

Amplification of louse DNA. DNA was extracted using the QIAamp Tissue Kit (QIAGEN), in accordance with the manufacturer's instructions. Insect specimens were confirmed to be *P. h. humanus* by use of a nested PCR with primer pairs phND4F1/phND4R1 and phND4F2/phND4R2 (table 1), purchased from Eurogentec. A negative control (water) was tested with the 5 lice.

PCRs were performed in a PTC-200 automated thermal cycler (MJ Research), using Blue Taq DNA Polymerase (Gentaur). Measures were taken to prevent PCR carryover contamination, and each PCR step was performed in a different room. For amplification, the 25-μL reaction mixture consisted of 1.25 μL of each primer (1 pmol), 0.8 μL of $MgCl_2$ (final concentration, 1.6 mmol/L), 2.5 μL of dNTP (dATP, dCTP, dGTP, and dTTP; 2 mmol/L each), 2.5 μL of Blue Taq buffer, 2.5 μL of bovine serum albumin, 0.2 μL of DNA polymerase enzyme, 9 μL of sterile water, and 5 μL of the DNA sample. For reamplification, 2.5 μL of each primer (1 pmol), 1.6 μL of $MgCl_2$ (final concentration, 1.6 mmol/L), 5.0 μL of dNTP, 5.0 μL of Blue Taq buffer, and 0.4 μL of DNA polymerase enzyme were added to the product from the first amplification. PCR amplification was performed under the following conditions: an initial 3 min of denaturation at 95°C was followed by 44 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 58°C for the first amplification and 44 cycles at 48°C for the reamplification, and extension for 90 s at 72°C. To enable complete extension of the PCR products, amplification was completed by holding the reaction mixture at 72°C for 7 min. PCR products were separated by electrophoresis on 3% agarose gels and were visualized by staining with ethidium bromide. The products were purified using the QIAquick PCR purification Kit (QIAGEN), in accordance with the manufacturer's instructions, and were sequenced in both directions by use of the dRhodamine Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer), in accordance with the manufacturer's instructions, and an ABI 3100 automated sequencer (Perkin Elmer). The sequences obtained were compared with those available in GenBank, using BLAST [10].

Tooth samples and DNA extraction. Radiographs of the mandible were used to select unerupted teeth to be studied (data not shown). Five negative control teeth were extracted from 5 skeletons excavated from an 18th century grave in Briançon, France. Each skeleton was in an individual coffin in a single grave, and there was no anthropological evidence of an outbreak. One negative control DNA sample was used for every 7 samples. Testing was performed in a blinded manner.

Seventy-two unerupted teeth, identified by radiography, were extracted from 35 skeletons in Vilnius. After thorough washing in PBS, the teeth were fractured longitudinally, and the remnants of the dental pulp were removed aseptically into sterile tubes. DNA was extracted from the samples as described elsewhere [11] and was stored at 4°C for 4 days until use as templates in PCR assays. To avoid contamination, DNA was extracted in 1 building (Marseilles Dental School), and PCR assays were performed in a second building (Timone Hospital microbiology laboratory, where bartonellae and rickettsiae have not been cultured or amplified by PCR previously [12]). To test for other pathogens, including the agents of plague (*Yersinia pestis*), anthrax (*Bacillus anthracis*), and typhoid (*Salmonella typhi*), we attempted to amplify DNA of these organisms by use of previously reported primers [13, 14] in dental pulp remnants, obtained as described above, from 9 teeth from another soldier.

Detection of DNA in teeth. Louse-borne pathogens were detected by suicide PCR, so named because the primers for the target genes selected are used only once. Such primers must never have been used previously in the laboratory and must also nev-

Table 1. Primers used in this study.

Organism, primer name	Target gene (GenBank accession no.)	Primer position relative to target sequence, nt	Primer sequence (5′→3′)	Fragment size, bp	Hybridization temperature, °C
P. h. humanus					
phND4F1	*ND4* (AY316847)	2–23	TTGTTGTGCTTTTGACTTCTTG	168	58
phND4R1	*ND4* (AY316847)	217–191	CCCTGATTTGAAGTATTAAAGAAACTC		
phND4F2[a]	*ND4* (AY316847)	57–77	GAATTTCCTTATTTGTTTAGC	64	48
phND4R2[a]	*ND4* (AY316847)	158–142	CCGAAATAAGAGCCCGT		
R. prowazekii					
Rp601F1	*dna*A (AJ235272)	162115–162139	TGGATAAAATCCAAATATGCTATGG	279	58
Rp601R1	*dna*A (AJ235272)	162438–162418	TCCACCTCCGCCATATAGAAA		
Rp601F2[a]	*dna*A (AJ235272)	162209–162229	CTGGAACAACACAAGCAGTGA	141	56
Rp601R2[a]	*dna*A (AJ238272)	162390–162371	TGATGATTCTGCCACAGCTC		
Rp778F1	*dna*E (AJ238756)	5996–6017	TTTTGTGCTATGCGTAATCACA	246	55
Rp778R1	*dna*E (AJ238756)	5750–5732	CAAGGCAGGTTGTTTTGATTG		
Rp778F2[a]	*dna*E (AJ238756)	5927–5946	ATACAACGGCTTAACCGCAG	77	53
Rp778R2[a]	*dna*E (AJ238756)	5844–5825	AACGAAAAGCAAGAGGAGCA		
B. quintana					
hbpEF1	*hbp*E (AY126675)	231–251	GAGAGTGCTTCACCTAAATAG	429	55
hbpER1	*hbp*E (AY126675)	700–681	CCACCAATCTGTCCTCCAAA		
hbpEF2[a]	*hbp*E (AY126675)	297–316	GAGACGAGTATTAAAGTTTC	282	48
hbpER2[a]	*hbp*E (AY126675)	617–599	CTGAGGAACTATTACATCT		
htrAF1	*htr*A (AY548753)	1–20	AAAGCTGGTATCAAGGCAGG	192	56
htrAR1	*htr*A (AY548753)	233–213	TCATTTGAATCATTGCGCCCA		
htrAF2[a]	*htr*A (AY548753)	52–70	ATTAATGATGTCCGTGATC	113	48
htrAR2[a]	*htr*A (AY548753)	203–184	TTTGAGTCTTCTTTCATAAC		
B. recurrentis					
Br-glpQF1	*glp*Q (AF247155)	21–42	GTTTGCAATAAGTACTGTTCTT	229	53
Br-glpQR1	*glp*Q (AF247155)	290–271	TCTCTAGCTCTTCCTGGAAA		
Br-glpQF2[a]	*glp*Q (AF247155)	86–105	CAGCATTAATTATAGCTCAC	136	49
Br-glpQR2[a]	*glp*Q (AF247155)	261–241	AACATTTGTTGTTGTATCTAG		

NOTE. Primer positions were numbered relative to the *ND4* gene of *Pediculus humanus humanus* (GenBank accession no. AY316847); to the *dna*A (AJ235272) and *dna*E (AJ238756) genes of *Rickettsia prowazekii;* to the *hbpE* (AY126675) and *htr*A (AY548753) genes of *Bartonella quintana;* and to the *glp*Q gene of *B. recurrentis* (AF247155).

[a] Primer used for sequencing.

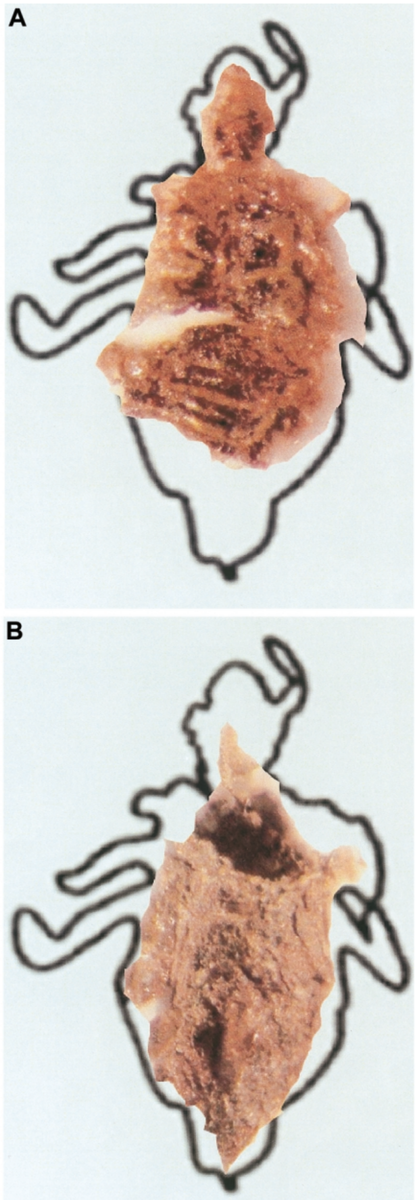

Figure 3. Morphological identification of body lice, using binocular magnification. The outline of a modern dry body louse is included to illustrate the size and positions of the fragments.

er be used subsequently [7, 15]. Specimens were tested for the presence of *R. prowazekii* by use of the double pair of primers Rp601F1/Rp601R1 and Rp601F2/Rp601R2 for the nested amplification. A second suicide PCR was performed for *R. prowazekii,* using the primers Rp778F1/Rp778R1 and Rp778F2/Rp778R2 for the nested amplification. To detect *B. quintana,* we used the primers hbpEF1/hbpER1 and hbpEF2/hbpER2 for the nested amplification. Primer pairs Br-GlpQF1/Br-GlpQR1 and Br-GlpQF2/Br-GlpQR2 were used for detection of *B. recurrentis.*

All PCRs were performed in a PTC-200 automated thermal cycler (MJ Research), as described above, using hybridization temperatures for each primer pair as detailed in table 1. PCR products were sequenced as described above, and the sequences obtained were compared with those available in GenBank.

RESULTS

Identification of lice in the grave. Two kilograms of earth containing bone fragments and remnants of clothing were obtained from Vilnius and examined for lice. In preliminary studies, the water-kerosene interface method enabled us to recover all of the lice placed in a soil sample; using the technique on the sample from Vilnius, we recovered parts (0.4–1.2 mm in cross section) of the abdomen [3] or the dorsum [2] of 5 lice. These were identified as *P. h. humanus* on the basis of gross morphology and appearance (figure 3) and by means of scanning electron microscopy (data not shown). The 5 remnants are the size of lice, and all match perfectly in form and size with drawn outlines of modern lice. This identification was further confirmed by PCR amplification and sequencing.

PCRs targeting the NADH dehydrogenase 4–encoding gene *(ND4)* (table 1) were positive for DNA extracted from all 5 louse parts (figure 4), and the sequences of the amplicons were very similar to those of *P. h. humanus* that are available in GenBank: 100% similarity (64/64) with accession number AY860502, 98.5% (63/64) with AY860506, 100% (60/60) with AY860503, 100% (61/61) with AY860504, and 98.3% (58/59) with AY860505. Although 2 of the 5 lice had a single nucleotide mutation at a nondiscriminant position (figure 4), all 5 were clearly identified as *P. h. humanus* on the basis of the sequence data. Also, they differed from *P. h. capitis* at nt 133, a feature that enables discrimination between the European *P. h. humanus* and *P. h. capitis* louse sequences available in GenBank [16]. Negative controls all tested negative.

Detection of pathogens in lice and teeth. Recently, we detected *Y. pestis* and *B. quintana* in DNA extracted from teeth from human remains [7, 11, 12, 17]. Here, we used suicide PCR to detect louse-borne pathogens in Napoleon's soldiers. Of the 72 teeth tested from 35 skeletons, 4 teeth from 3 soldiers were found to be positive by suicide PCR using primers for the *dna*A gene of *R. prowazekii* (table 2 and figure 5). No positive results were obtained with DNA from the louse parts. All 4 amplicons were identified as *R. prowazekii* on the basis of nucleotide similarity rates of 99.3% (140/141) (GenBank accession number AY860510), 100% (135/135) (AY860507), 100% (141/141) (AY860508), and 100% (138/138) (AY860509) with a reference strain of *R. prowazekii* (AJ235272). The presence of *R. prowazekii* in the specimens was confirmed by a second nested PCR using the Rp778 primers.

Amplification of the *hbp*E gene of *B. quintana* was possible with DNA from 10 teeth from 7 skeletons and from 3 lice. Tooth amplicon sequences from 1 tooth from each skeleton

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louse1                                  TTTTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
louse2                                  TTTTTCTCCTTCTATCCTTCTTTTAGCTCTTCATTCGTCTCCT
louse3                                  --TTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
louse4                                  --TTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
louse5                                  ----CCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
P. h. humanus   GAATTTCCTTATTTGTTTAGCTTTTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
P. h. humanus   GAATTTCCTTATTTGTTTAGCTTTTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
P. h. capitis   GAATTTCCTTATTTGTTTAGCTTTTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
P. h. capitis   GAATTTCCTTATTTGTTTAGCTTTTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
P. h. capitis   GAATTTCCTTATTTGTTTAGCTTTTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
P. h. capitis   GAATTTCCTTATTTGTTTAGCTTTTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
P. h. capitis   GAATTTCCTTATTTGTTTAGCTTTTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
P. h. capitis   GAATTTCCTTATTTGTTTAGCTTTTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
P. h. capitis   GAATTTCCTTATTTGTTTAGCTTTTTCTCCTTCTATCTTTCTTTTAGCTCTTCATTCGTCTCCT
                     phND4F2           *           *

louse1          TTTGGCGGGTGACTTGGATGT
louse2          TTTGGCGGGTGACTTGGATGT
louse3          TTTGGCGGGTGACTTGGAT--
louse4          TTTGGCGGGTGACTTGGATG-
louse5          TTTGGCGGGTGACTTGGATG-
P. h. humanus   TTTGGCGGGTGACTTGGATGTACGGGCTCTTATTTCGG
P. h. humanus   TTTGGCGGGTGACTTGGATGTACGGGCTCTTATTTCGG
P. h. capitis   TTTGGCGGGTGACCTGGATGTACGGGCTCTTATTTCGG
P. h. capitis   TTTGGCGGGTGACCTGGATGTACGGGCTCTTATTTCGG
P. h. capitis   TTTGGCGGGTGACCTGGATGTACGGGCTCTTATTTCGG
P. h. capitis   TTTGGCGGGTGACCTGGATGTACGGGCTCTTATTTCGG
P. h. capitis   TTTGGCGGGTGACCTGGATGTACGGGCTCTTATTTCGG
P. h. capitis   TTTGGCGGGTGACCTGGATGTACGGGCTCTTATTTCGG
P. h. capitis   TTTGGCGGGTGACCTGGATGTACGGGCTCTTATTTCGG
                             *          phND4R2
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Figure 4. Alignment of *ND4* nucleotide sequences from the 5 tested lice with those from *Pediculus humanus humanus* and *P. h. capitis.* Boxed nucleotides denote primer positions. Asterisks indicate divergent nucleotides. Green nucleotides are divergent among tested louse strains. The discriminant nucleotide allowing differentiation between *P. h. humanus* and *P. h. capitis* is shown in blue (*P. h. humanus;* GenBank accession nos. AY316847 and AY316839) or red (*P. h. capitis;* GenBank accession nos. AY316867, AY316866, AY316865, AY316852, AY316855, AY316856, and AY316857). The GenBank accession nos. of the 5 louse sequences are AY860502, AY860503, AY860504, AY860505, and AY860506.

were obtained. They were identified as *B. quintana* on the basis of nucleotide sequence similarity rates of 99.6% (279/280) (GenBank accession number AY860520), 99.6% (278/279) (AY860511), 100% (252/252) (AY860512), 99.6% (276/277) (AY860513), 99.6% (277/278) (AY860514), 99.3% (274/276) (AY860515), and 98.2% (273/278) (AY860516) with a reference strain of *B. quintana* (AY126675). Louse amplicon sequences were also identified as *B. quintana* on the basis of nucleotide sequence similarity rates of 99.4% (172/173) (GenBank accession number AY860517), 99.1% (222/224) (AY860518), and 99.3% (274/276) (AY860519) with GenBank accession number AY126675 (figure 6). The presence of *B. quintana* was confirmed using the *htr*A-based nested PCR on DNA from 7 skeletons.

All negative controls tested negative. No amplification products were obtained from the teeth or lice with primers for *B. recurrentis*. Similarly, we could not identify *Y. pestis*, *B. anthracis*, or *S. typhi* DNA in the 9 teeth we tested from 1 soldier.

DISCUSSION

The majority of the 500,000 soldiers who started the Russian campaign died of dysentery, pneumonia, or fever. It is estimated [3] that, of the 25,000 soldiers who reached Vilnius, only 3000 survived. On the basis of the results of our study, we believe that many were infected with louse-transmitted diseases. Similar large outbreaks of disease have occurred after many wars in the past. Their etiologies are mostly controversial, however,

Table 2. Positive specimens in soldiers' dental pulp.

		PCR result			
		Bartonella quintana		*Rickettsia prowazekii*	
Soldier	Tooth no.	*hbpE*	*htrA*	*Rp601*	*Rp778*
PL3K19	33	+	+	–	–
PL3K26	35	+	+	–	–
PL3K80	37	+	+	–	–
PL3K84	11	+	+	–	–
PL3K128	28	+	+	–	–
PL2K225	23	+	+	–	–
PL3K2	35	+	+	–	–
PL3K170	45	–	–	+	+
PL3K38	22	–	–	+	+
PL3K158	17	–	–	+	+
PL3K158	43	–	–	+	+

NOTE. PCR, polymerase chain reaction.

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AY860507                        ---AATCACCTACTAAGACTTTTGCCGATATCGGCAACAGTGCA
AY860510                        CAGAATCACCTACTAAGACTTTTGCCGATATCGGCAACAGTGCA
AY860509                        --GAATCACCTACTAAGACTTTTGCCGATATCGGCAACAGTGCA
AY860508                        TAGAATCACCTACTAAGACTTTTGCCGATATCGGCAACAGTGCA
AJ235272   CTGGAACAACACAAGCAGTGATAGAATCACCTACTAAGACTTTTGCCGATATCGGCAACAGTGCA
NC_006142                       TAGAATTACCTACTAAAACTTTTTCCAATATTAGCAACAGTGCA
NC_003103                       CAGAATTACCTACTAAGACTTTTGCCGATATCGGCAGCAGTGAG
                 Rp601F2              *         *      *  *    **   *     **

AY860507        CTAAATTCAGAAAATATCGTTTCAACACTTGATGTACGTTTTACTTTCGATAATTTTGTG
AY860510        CTAAATTCAGAAAATATCGTTTCAACACTTGATGTACGTTTTACTTTCGATAATTTTGTG
AY860509        CTAAATTCAGAAAATATCGTTTCAACACTTGATGTACGTTTTACTTTCGATAATTTTGTG
AY860508        CTAAATTCAGAAAATATCGTTTCAACACTTGATGTACGTTTTACTTTCGATAATTTTGTG
AJ235272        CTAAATTCAGAAAATATCGTTTCAACACTTGATGTACGTTTTACTTTCGATAATTTTGTG
NC_006142       CTTAATTCAGAAAATACCATTTCAACACTTGATTTACGTTTTACTTTCGATAATTTTGTG
NC_003103       CTTAATTCGGAAAATATCTTTTCAACCCTTGATGTACGTTTTACTTTCGATAATTTTGTG
                  *     *       * *       *      *

AY860507        GTTGGAGTACCAAATGAACTAGCTTATGCAGCAG---
AY860510        GTTGGAGTACCAAATGAACTAGCTTATGCAGCAGCAA
AY860509        GTTGGAGTACCAAATGAACTAGCTTATGCAGCAGCA-
AY860508        GTTGGAGTACCAAATGAACTAGCTTATGCAGCAGCAA
AJ235272        GTTGGAGTACCAAATGAACTAGCTTATGCAGCAGCAAGAGCTGTGGCAGAATCATCA
NC_006142       GTTGGAGCACCGAATGAGCTAGCTTATGCAGCGGCAA
NC_003103       GTTGGAGCCCCAAATGAGCTAGCCTATGCAGCCGCAA
                       *   *     *     *        *

                                                            Rp601R2
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Figure 5. Alignment of *dna*A nucleotide sequences from the 4 polymerase chain reaction (PCR)–positive teeth with those of *Rickettsia prowazekii* (GenBank accession no. AJ235272), *R. typhi* (NC_006142), and *R. conorii* (NC_003103). Boxed nucleotides denote primer positions. Asterisks indicate divergent nucleotides. Green nucleotides are divergent among PCR-positive soldiers' teeth. The discriminant nucleotides allowing differentiation between *R. prowazekii* and other *Rickettsia* species are shown in blue (*R. prowazekii*) or red (*R. typhi* and *R. conorii*). The GenBank accession nos. of the sequences from the 4 PCR-positive teeth are AY860507, AY860510, AY860509, and AY860508.

because diagnoses made at the time were largely based on unreliable clinical and epidemiological data. For example, in Napoleon's time, lice were not recognized as vectors of disease [18], and typhus was first recognized as a distinct disease only in the 19th century [18]. We consider our results to be valid, because they fulfil the current recommendations for paleomicrobiology. The negativity of our negative controls supports the validity of our positive tests, as does the obtaining of original sequences in lice and in *B. quintana*. Positive tests were confirmed by a second target gene amplification and sequencing.

We identified parts of 5 body lice in the earth from the mass grave in Vilnius. Previously, lice from the 1st century have been identified morphologically in Israel [19], and ancient head lice have been collected in Europe from Herculanum [20] and from pre-Columbian South American mummies [21]. We have recently shown that body and head lice of European origin can be differentiated by sequencing [16], and we identified the DNA signature of the body louse in that study.

B. quintana was reported to occur in lice at the end of World War I [22] and is now recognized to be the most commonly found louse-borne pathogen [23]. We have now identified the organism in body lice from a century earlier and from the dental pulp of 7 of Napoleon's soldiers. We believe that these findings provide firm evidence that the soldiers had trench fever. Previously, we reported the presence of *B. quintana* genes in 4000-year-old teeth [17] and the presence of *Bartonella henselae* genes in naturally infected cats [24] from the present day and from the Middle Ages [25].

Although we could not identify DNA of *R. prowazekii* in the lice we studied, we could detect the DNA of the organism in the dental pulp of 3 soldiers, indicating that Napoleon's soldiers also had epidemic typhus. Although finding bacterial DNA in teeth does not necessarily mean that the organism was the cause of death, when the DNA of a deadly agent such as *R. prowazekii* is present, it is very likely that the organism was the cause of death. Previously, we were unable to detect the DNA of *R. prowazekii* in teeth from people who died during the Black Death [7] but could detect DNA of *Y. pestis* in teeth from the suspected plague victims [11, 12]. In the present study, we were unable to find DNA of *Y. pestis* in 9 teeth from 1 of Napoleon's soldiers [7, 17].

The critical issue in the use of PCR is the prevention of DNA contamination [17]. In our study, we followed recent recommendations to ensure that our results were not influenced by contamination. We did not use positive controls, which can be a considerable source of contamination, and we ensured that our negative controls [7, 26] produced no amplicons. We used separate rooms for sample preparation and for PCR amplification, and we used primers for sequences that had not previously been studied in the laboratory in which the

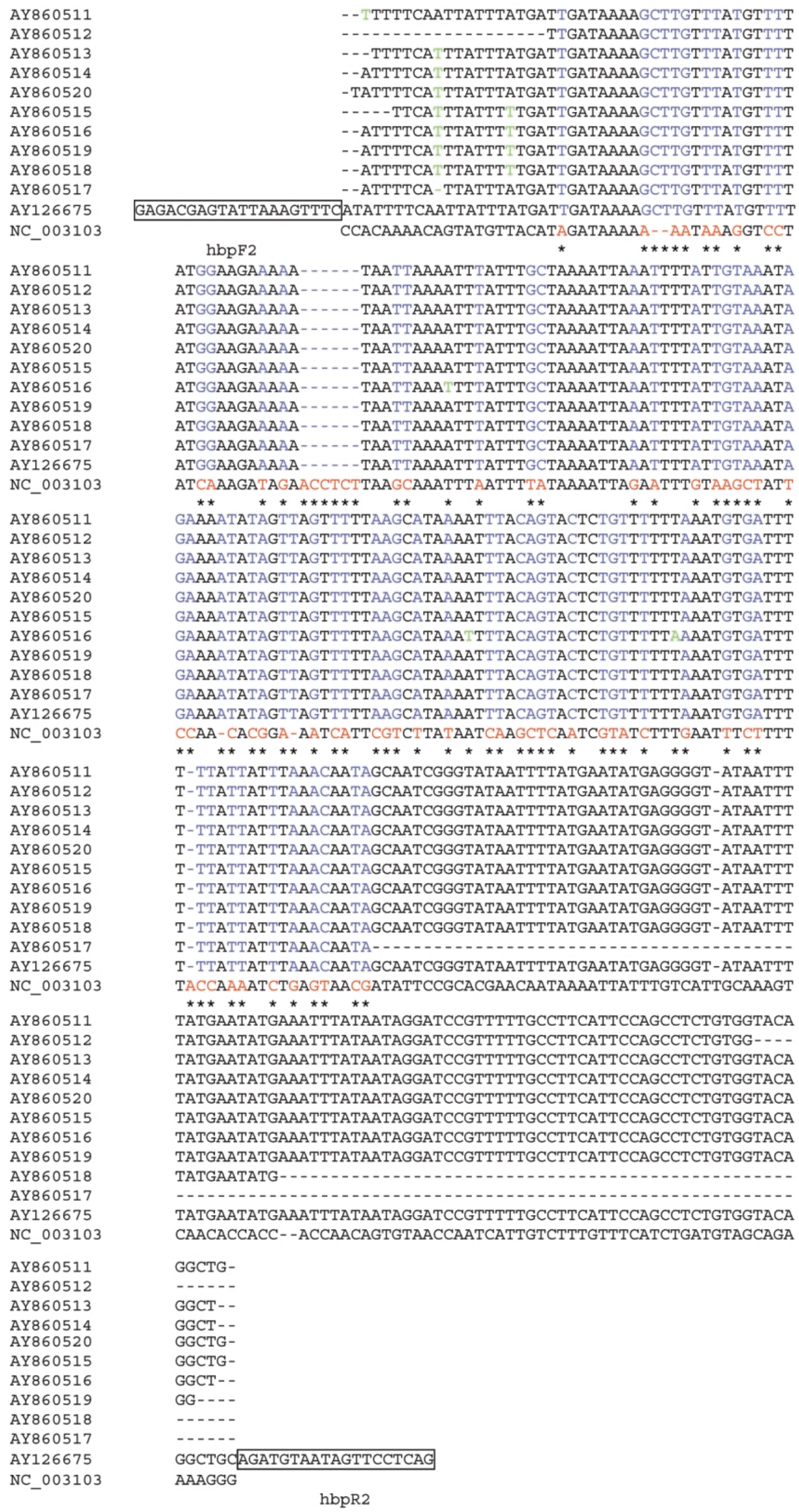

Figure 6. Alignment of *hbp*E nucleotide sequences from the 7 polymerase chain reaction (PCR)–positive teeth and the 3 PCR-positive lice with those of *Bartonella quintana* (GenBank accession no. AY126675) and *B. henselae* (NC_003103). Boxed nucleotides denote primer positions. Asterisks indicate divergent nucleotides. Green nucleotides are divergent among PCR-positive soldiers' teeth and lice. The discriminant nucleotides allowing differentiation between *B. quintana* and *B. henselae* are shown in blue (*B. quintana*) or red (*B. henselae*). The GenBank accession nos. of the sequences from the 7 PCR-positive teeth are AY860511, AY860512, AY860513, AY860514, AY860515, AY860516, AY860520, and those of the sequences from the 3 PCR-positive lice are AY860517, AY860518, and AY860519.

amplifications were performed. Using primers only once in a laboratory, a technique named "suicide PCR" [7, 15], is particularly useful in preventing contamination and is facilitated by the growing number of pathogens for which the complete genome is known. Amplification of a second target and originality of the ancient sequence are also critical [17].

In conclusion, our work shows that Napoleon's soldiers in Vilnius were exposed to body lice containing *B. quintana* and that 10 (29%) of the soldiers had evidence of infection with either *R. prowazekii* or *B. quintana.* Our study also adds to the growing evidence that searching for the DNA of infectious agents in dental pulp is an important tool for investigating the history of infectious diseases [27].

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