Body Lice, *Yersinia pestis* Orientalis, and Black Death

To the Editor: Wild rodent fleas are the most common vectors of *Yersinia pestis*, the plague agent (1). The human body louse (*Pediculus humanus*) has been proposed as a probable additional vector during historical epidemics (2) because human cases of louse-borne plague have been suspected (3) and body louse–borne plague has been demonstrated experimentally with rabbits (4). Using rabbits, we tested the ability of the 3 *Y. pestis* main biotypes to produce a successful rabbit-louse-rabbit-louse cycle of transmission (4).

Two New Zealand White (*Oryctolagus cuniculus*) rabbits were inoculated intravenously with phosphate-buffered saline alone (negative controls) or phosphate-buffered saline containing $10^9$ CFU of *Y. pestis* biotype Nairobi-Rattus Antiqua, biotype 14–47 Medievalis, or biotype 6/69M Orientalis. PCR ensured detection of the virulence factor–encoding plasmids. The rabbits inoculated with biotypes Antiqua, Medievalis, or Orientalis had septicemia of $\approx 2 \times 10^2$ CFU/mL of blood 4 days postinfection and died 1 day later. In contrast, the rabbits bitten by Antiqua-infected or Medievalis-infected lice looked healthy and lacked septicemia 3 weeks after challenge. New groups of 150 uninfected lice fed for 1 hour daily on Orientalis-infected rabbits started to die earlier than did lice fed on Antiqua, Medievalis (1 vs. 2–3 days after blood meal), and uninfected rabbits. Furthermore, 21 days after their first blood meal, lice fed on Orientalis-infected rabbits had a significantly higher death rate (90%) than did control (3%) (p<0.0001), Antiqua-infected (16%), and Medievalis-infected (10%) lice; the latter values were significantly higher than that of the negative controls (p = 0.046). *Y. pestis* could be cultured only from lice and their feces if the lice were fed on rabbits previously bitten by Orientalis-infected lice (online Appendix Figure, www.cdc.gov/EID/content/16/5/892-appF.htm).

Our observation that body lice effectively transmitted *Y. pestis* through a complete cycle of transmission confirms previous experimental (4) and field observations of experimental transmission that used body lice collected from plague patients from the same family in the absence of any other ectoparasite (3). Transmission of Orientalis but not Antiqua or Medievalis organisms did not result merely from experimental bias because negative controls remained negative, data were duplicated, rabbits exhibited equivalent bacteremia, and lice took equivalent blood meals regardless of biotype. Our observations shed new light on the Black Death, a medieval epidemic of plague (5). Historical records indicate that persons with the Black Death had bubonic plague, indicating an ectoparasite-borne transmission (1). *Pulex irritans* fleas were documented in a medieval setting in Viking Greenland (6). However, their poor competence (7) and the Black Death that swept Russia and Scandinavia are not fully compatible with flea-borne transmission alone. Ten infected lice are sufficient for plague transmission (4), and our observation that one third of infected lice remained alive 3 days after infection indicates that an index plague patient carrying as few as 30 body lice could be a source for plague up to 3 days after dying. This figure was highly plausible during the Black Death because body lice currently infest almost 85% of homeless persons, with a mean of 57 lice per person (8). Although the role of fleas as vectors of *Y. pestis* from rodents to humans is undisputed, this tabulation sustains the potential role of body lice as an additional vector of plague from human to human during the Black Death (2).

Paleomicrobiology suggested that most historical cases in Europe resulted from Orientalis (5). This observation challenged the scenario that Antiqua, Medievalis, and Orientalis were responsible for ancient, medieval, and modern plague pandemics, respectively (9). The latter scenario had been hypothesized after the biotypes were observed to have a geographic reparation matching that of the hypothetical sources of the 3 historical pandemics (10) and was further propagated as dogma without further confirmation.

Our data support an alternative scenario of the historical plague epidemics transmitted by body lice, with Orientalis being the only such louse-borne transmissible biotype. This point justifies studies during ongoing epidemics in cold countries, keeping in mind the need to understand and control re-emerging plague in modern populations exposed to body lice.

Acknowledgments

We thank Philippe Hoest for help in handling *Y. pestis* isolates in the biosafety laboratories and Claude Nappez for help with the animal experiments.

Lice fed on septicemic rabbits further fed on 2 uninfected rabbits for 1 hour daily for up to 6 days. The rabbits bitten by Orientalis-infected lice had $2.7 \times 10^2$ CFU/mL of blood for 4 days postinfection and died 1 day later. In contrast, the rabbits bitten by Antiqua-infected or Medievalis-infected lice looked healthy and lacked septicemia 3 weeks after challenge. New groups of 150 uninfected lice fed for 1 hour daily on Orientalis-infected rabbits started to die earlier than did lice fed on Antiqua, Medievalis (1 vs. 2–3 days after blood meal), and uninfected rabbits. Furthermore, 21 days after their first blood meal, lice fed on Orientalis-infected rabbits had a significantly higher death rate (90%) than did control (3%) (p<0.0001), Antiqua-infected (16%), and Medievalis-infected (10%) lice; the latter values were significantly higher than that of the negative controls (p = 0.046). *Y. pestis* could be cultured only from lice and their feces if the lice were fed on rabbits previously bitten by Orientalis-infected lice (online Appendix Figure, www.cdc.gov/EID/content/16/5/892-appF.htm).

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**Salmonella Senftenberg Infections and Fennel Seed Tea, Serbia**

To the Editor: The first documented outbreak of salmonellosis linked to consumption of plant products in the Autonomous Province of Vojvodina, Serbia, occurred from March 2007 through September 2008. Fourteen cases of *Salmonella enterica* serotype Senftenberg infection were reported.

The yearly incidence of salmonellosis in Vojvodina during 2003–2007 ranged from 25/100,000 inhabitants to 70/100,000 inhabitants; 34 outbreaks were reported in 2007, caused predominantly by *S. enterica* serotype Enteritidis (1). Most outbreaks were associated with consumption of food of animal origin (1,2). *Salmonella* spp. were isolated from seeds in 2004, when *S. enterica* serotype Mbandaka and *S. enterica* serotype Virchow were isolated from sesame seeds (3).

Before 2007, S. Senftenberg had rarely been identified in Vojvodina. During 2003, 3 cases were reported. In 2004–2005, no S. Senftenberg cases were reported. In 2006, 8 cases of S. Senftenberg infection were reported among infants <12 months of age. An outbreak investigation did not reveal the source of infection. Common to all of those infected was their age and their consumption of infant formula. Nonetheless, laboratory analysis of samples of the various formulas did not show any pathogens. Two additional cases occurred in 2007 among patients who were <12 months of age. These cases confirmed suspicion that the infections had a source other than formula. Further investigation led to the consideration of tea consumption as a possible factor.

In April 2008, a total of 3 infants <12 months of age with salmonellosis came to the attention of investigators. *S. Enteritidis* was first identified in the samples of their feces. One month later, feces samples from the 3 infants were tested again, and S. Senftenberg was isolated from all 3 specimens.

After these findings, the Institute of Public Health of Vojvodina conducted an outbreak investigation in collaboration with institutes of public health at the district level. A case was defined as the presence of a laboratory-confirmed S. Senftenberg infection during 2007–2008. All case-patients (or their parents) were interviewed by using a standard questionnaire for salmonellosis, which was expanded to include questions regarding tea consumption.

A standardized method of enterobacterial repetitive intragenic consensus (ERIC)–PCR, based on the method of Versalovic et al. (4), with ERIC-PCR with ERIC2 primer (5′AAGTAAA GTGACTCGGGGTGACGG-3′), was applied. DNA was isolated by using the InvitrogenPure Link Genomic DNA purification kit (Invitrogen, Carlsbad, CA, USA). Gene sequences were amplified in a Perkin/Elmer thermal cycler (model 9600) (PerkinElmer, Waltham, MA, USA). A DNA ladder was constructed by using Gene Ruler 100-bp DNA Ladder Plus (Fermentas, Glen Burnie, MD, USA).

Exploratory interviews with parents showed that all 3 infected infants had consumed commercially manufactured baby tea during the previous