peat analysis (MLVA) as described (*1*,*3*–*5*). Multilocus sequence typing profiles of these strains were identical to the type strain *B. microti* CCM 4915^T and strain CCM 4916. MLVA showed that these strains also clustered with *B. microti* strains CCM 4915^T and CCM 4916, with identical panel 1 and panel 2A genotypes but a different panel 2B genotype.

In summary, we successfully isolated *B. microti* from soil samples collected at the same site 7 years after primary isolation of this novel species from common voles. *B. microti* could still be isolated from the same soil samples 6 months after storage at 4°C. This finding indicates long-term survival of *B. microti* in soil; thus, soil might function as a reservoir of infection. Identification of *B. microti* as a potential soil bacterium is consistent with *Brucella* spp. whole genome sequencing data, in particular with the genome sequence of *B. suis*, which exhibits fundamental similarities with plant pathogens such as *Agrobacterium* spp. and *Rhizobium* spp. (*6*). Whether soil is the primary habitat of *B. microti* or other vectors, such as nematodes, remains to be investigated.

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Holger C. Scholz,*
Zdenek Hubalek,†
Jirina Nesvadbova,†
Herbert Tomaso,*
Gilles Vergnaud,‡§
Philippe Le Flèche,‡§
Adrian M. Whatmore,¶
Sascha Al Dahouk,#
Monika Krüger Csilla Lodri,***
and Martin Pfeffer*

*Bundeswehr Institute of Microbiology, Munich, Germany; †Academy of Sciences, Brno, Czech Republic; ‡Centre d'Etudes du Bouchet, Vert le Petit, France; §Université Paris-Sud 11, Orsay, France; ¶Veterinary Laboratories Agency, Addlestone, UK; #University of Aachen, Aachen, Germany; and **Veterinary University of Leipzig, Leipzig, Germany

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Address for correspondence: Holger C. Scholz, Bundeswehr Institute of Microbiology, Neuherbergstrasse 11, D-80937 Munich, Germany; email: holger1scholz@bundeswehr.org

Plasmodium falciparum in Ancient Egypt

To the Editor: Malaria is a disease caused by parasites of the genus *Plasmodium.* The infection is transmitted to humans through the bites of female flies of the genus *Anopheles.* Four species of *Plasmodium* are pathogenic to humans, and each leads to different clinical features: *P. falciparum* causes severe malaria with undulating high fever (malaria tropica); *P. malariae*, *P. vivax,* and *P. ovale* cause less severe clinical courses of disease with the manifestations of malaria quartana (*P. malariae*) and malaria tertiana (*P. vivax* and *P. ovale*). Literary evidence for malaria infection dates back to the early Greek period when Hippocrates described the typical undulating fever (*1*), highly suggestive of plasmodial infection. Although it is believed that malaria widely affected early pre-Hippocrates populations, until now only 1 study, which used molecular analysis, clearly identified *P. falciparum* in a Roman infant dating back to the 5th century AD (*2*). Two other studies used molecular analysis to identify more recent plasmodial DNA in ancient human remains, i.e., from 100–400 years ago (*3*,*4*). A substantial number of nonspecific amplifications in these previous studies raised concerns as to the specificity of current molecular markers for ancient malaria (*3*,*4*).

In this report, we describe the unambiguous identification of ancient DNA (aDNA) for *P. falciparum* in ancient Egyptian mummy tissues from ≈4,000 years ago. We analyzed 91 bone tissue samples from ancient Egyptian mummies and skeletons. The Egyptian material derived from the Predynastic to Early Dynastic site of Abydos (n = 7; 3500–2800 BC), a Middle Kingdom tomb in Thebes West (n = 42; 2050–1650 BC), and various tomb complexes in Thebes West, which were built and used between

the Middle and New Kingdom until the Late Period (n = 42; c. 2050–500 BC). All samples were first tested for *Plasmodium* spp. DNA by using the heminested PCR for the 18S rDNA primer targets usually used for malaria identification (*5*). Direct sequencing was performed on those with positive amplification products. Thereby, a high number of amplification products of various sizes (including the expected size) were detected. However, on sequencing, all amplicons provided nonspecific products. Consequently, in a second set, all material was tested for the *P. falciparum* chloroquine-resistance transporter gene (*pfcrt* gene) (*6*,*7*), which was also further characterized by direct sequencing.

In this second set of experiments, 2 of the 91 ancient Egyptian samples tested positive for the 134-bp fragment of the pfcrt region of *P. falciparum* (Figure). The specificity of the amplification was verified by sequencing, which showed 99% sequence concordance. The result was verified by parallel analysis in 2 independent laboratories; observations were fully concordant. The 2 positive samples originated from 2 different tomb complexes dating from the New Kingdom until Late Period (1500–500 BC). Each sample was obtained from adults who had osteopathologic evidence of chronic anemia. No positive results were found for the earlier samples from the Predynastic to Early Dynastic or Middle Kingdom periods.

Previously, immunologic tests have been used to investigate the presence and incidence of malaria in ancient Egyptian mummies (*8*,*9*). Because >40% of all samples and 92% of samples from persons with bone lesions suggestive of chronic anemia tested positive for the *P. falciparum* histidine-rich protein-2 antigen, doubts as to the specificity of those tests have been raised.

Our study unambiguously identified *P. falciparum* aDNA in Egyptian mummy samples, thereby proving a specific infection by falciparum malaria in ancient Egypt. With respect to the infection incidence, our molecular analysis suggests a more realistic frequency than had been previously suggested by paleoimmunologic methods. Consequently, the aDNA analysis is superior with respect to the reaction specificity, so that the latter should not further be used for that purpose.

This report adds another infectious disease to the spectrum of paleomicrobiology in ancient Egypt, thereby further explaining the previously postulated influence of infectious diseases on the low life expectancy for ancient Egyptian populations (*10*). Molecular detection of pathogen aDNA can be used not only to identify a certain disease, but it may also provide information on disease frequency, evolutionary origin, and pathways.

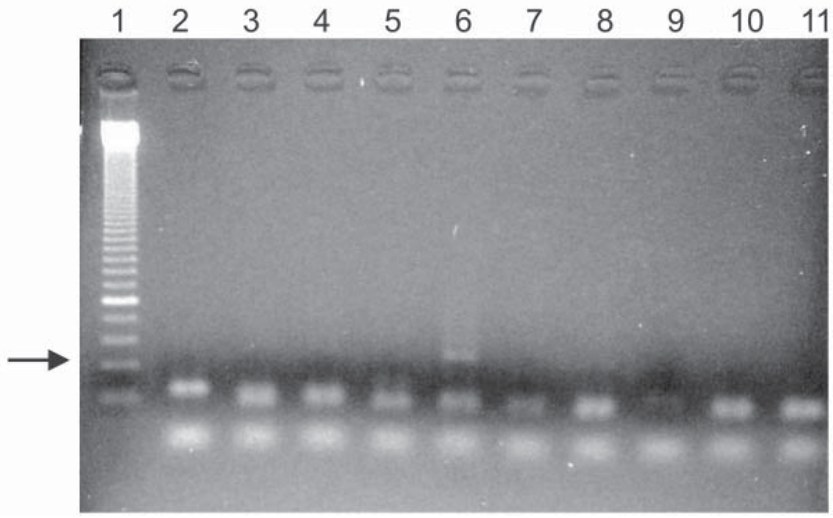

Figure. PCR amplification of a 134-bp fragment of ancient DNA of *Plasmodium falciparum* in Egyptian mummies. Lane 1, molecular marker; lanes 10 and 11, 2 negative controls. One (lane 6) of 8 samples shows a positive amplification product (arrow). Specificity of the product was verified by sequencing.

Andreas G. Nerlich,*
Bettina Schraut,*
Sabine Dittrich,†
Thomas Jelinek,‡
and Albert R. Zink*[1]

*Academic Teaching Hospital München-Bogenhausen, Munich, Germany; †University of Manchester, Manchester, UK; and ‡Institute of Tropical Medicine, Berlin, Germany

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[1]Current affiliation: Eurac, Bolzano, Italy.

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Address for correspondence: Andreas G. Nerlich, Division of Paleopathology, Institute of Pathology, Academic-Teaching Hospital München-Bogenhausen, Englschalkingerstr 77 D-81925, Munich, Germany; email: andreas.nerlich@extern.lrz-muenchen.de

Brucellosis in Infant after Familial Outbreak

To the Editor: Brucellosis is a known cause of small household outbreaks (*1*,*2*), usually attributed to exposure of all infected family members to the animal/animal product pathogen source. Although the means of disease transmission is well delineated (*3*), in certain cases the pathogen's entry into the human body cannot be clearly defined; this has led to suggestions of direct human-to-human transmission and also to the increasing recognition of airborne brucellosis, which is important in the context of the role of *Brucella* spp. as potential biological weapons (*4*). Another understudied transmission route is entry by direct contact through skin and mucosal abrasions. We report a case of infantile brucellosis in which airborne transmission in the context of familial brucellosis or indirect contact with the animal source through other family members was considered the only possible means of infant infection.

In 2006, a 2.5-month-old girl was admitted to the Pediatric Department of the University Hospital of Ioannina, in a region of northwestern Greece where animal and human infection from *Brucella melitensis* is still common (*5*,*6*). She had a 2-week history of poor feeding and a 5-day history of swelling of the right wrist. She was born after 38 weeks' gestation with a birthweight of 3,050 g and was fed formula milk exclusively. Results of the physical examination were normal except the finding of a tender swelling of the right wrist. The infant came from a family of shepherds, and her father and paternal grandfather had been treated for brucellosis 10 and 22 months ago, respectively. At that time, the whole family was screened for additional cases; screening was also often repeated at the patients' followup examinations. On admission, laboratory tests showed characteristic relative lymphocytosis (leukocytes 10.5 × 10^9/L, 65.3% lymphocytes) and an increase in inflammatory markers (C-reactive protein, 22 mg/L, and erythrocyte sedimentation rate, 47 mm/h). Results of a wrist x-ray were normal. Because brucellosis was suspected, serum agglutination test, ELISA, and blood PCR for *B. melitensis* were performed. Agglutination titer was 640; ELISA immunoglobulin M (IgM) antibodies and PCR results were positive. No organisms were grown in blood culture.

All family members were rescreened. The father and paternal grandfather had negative serum agglutination and ELISA IgM and positive IgG serologic results, indicating past infection. The mother and paternal grandmother were again negative. Veterinary investigation showed active disease in a few sheep of the family's herd. The infant was treated with a combination of oral trimethoprim-sulfamethoxazole and rifampin for 6 weeks. The course of the illness was uneventful, and she recovered completely. Followup PCR results were negative for *B. melitensis.* Six months later, only an ELISA IgG had positive results; IgM and IgA antibody and agglutination test results were negative. The patient remains without relapse 2 years after treatment.

Awareness of brucellosis is low in disease-endemic areas, including knowledge of its transmission potential and its medical consequences.. As a consequence, familial clusters of brucellosis are the norm. Recognition of a human case should prompt investigation of other family members so that early recognition and treatment for other household case-patients are possible. However, limitations in eradicating the initial animal disease source may lead to continuous exposure and appearance of new cases after a protracted period, or to infection of new household members.

Our case raises the need for awareness of the transmission dynamics of *Brucella* spp. because the disease emerged in a household member who did not have any direct contact with the animal source or any related products. The baby was not breastfed and had not digested raw milk. Her feeding bottle was specifically used for formulated milk and for feeding her only. Ingestion of breast milk from an infected mother (*7*) and vertical transmission transplacentally or during delivery are acknowledged means of transmission (*8*), but in this case the mother had never had brucellosis (she had been repeatedly screened during her husband's initial disease and followup). Neither previously infected household member had any clinical or laboratory sign of relapse or residual disease. The infant was never in contact with the infected animals and