

Evidence of Maternal-Fetal Transmission of *Parachlamydia acanthamoebae*

To the Editor: *Parachlamydia acanthamoebae* is a recently identified agent of pneumonia (1–3) and has been linked to adverse pregnancy outcomes, including human miscarriage and bovine abortion (4,5). *Parachlamydia* sequences have also been detected in human cervical smears (4) and in guinea pig inclusion conjunctivitis (6). We present direct evidence of maternal-fetal transmission of *P. acanthamoebae*.

We tested 78 amniotic fluid samples from patients who delivered prematurely (defined as spontaneous delivery before 37 weeks of gestation) at the Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, from 2003 to 2006. DNA was extracted by using the QIAampDNA Mini kit (QIAGEN, Hilden, Germany) and was tested by using a specific *Parachlamydia* real-time PCR (7). One positive sample (threshold cycle of 34.2) was confirmed by using the 16SigF-Rp2Chlam PCR, which targets a large DNA segment of the 16S rRNA gene (8). Because these 2 PCRs target different DNA segments, the positive PCR results were not due to PCR contamination with amplicons. The sequence exhibited 99.6% similarity with *P. acanthamoebae* strain Hall's coccus (1) (GenBank accession no. AF366365).

The sample was obtained from a 29-year-old woman during her second pregnancy, at 16 weeks of gestation. Amniocentesis was performed because a first-trimester test suggested a Down syndrome risk of 1/100. At the time of amniocentesis, the patient had cough and flu-like symptoms of 3 weeks' duration, which resolved spontaneously in a few weeks. Cytogenetic analysis showed a normal 46XX karyotype; amniotic fluid culture remained sterile.

The pregnancy ended prematurely at 35 weeks and 6 days with the vaginal delivery of a 2,060-g newborn (<5th percentile). The mother and child had an uneventful hospital course.

The role of *Parachlamydia* as the etiologic agent of premature labor and intrauterine growth retardation is likely because 1) all vaginal, placental, and urinary cultures were negative; 2) results of routine serologic tests were negative; and 3) only *Parachlamydia* was detected in the amniotic fluid. Intrauterine infection caused by *Parachlamydia* spp. may be chronic and asymptomatic until adverse pregnancy outcomes occur (4).

The infection of this pregnant woman might have occurred through zoonotic contact through her work as a butcher in a rural area known for cattle breeding. Of interest, a recent study of 482 healthy Swiss men found 3 who were seropositive for *Parachlamydia* sp., and all 3 came from the same rural area near Lausanne (within <20-km radius) (9). Moreover, the patient owns 2 guinea pigs, potential vectors of the bacterium (6). Other modes of transmission are possible, e.g., contaminated water (free-living amoebae may serve as hosts for *Parachlamydia* spp. and are widespread in water networks) and ingestion of undercooked meat or contaminated cow milk.

A plausible pathogenic scenario for this case of possible maternal-fetal transmission of *P. acanthamoebae* might include bacteremia in the context of a lung infection. This could have resulted in intrauterine infection and intrauterine growth restriction.

Acknowledgments

We warmly thank Gabrielle Gerelle and Philip Tarr for reviewing the manuscript and Sandrine Gremlich-Irrausch and Françoise Damnon for technical help.

Research performed at the Center for Research on Intracellular Bacteria is mainly supported by grants from the Swiss National Science Foundation (no. FN3200B0-116445), the Faculty of Biology and

Medicine of the University of Lausanne (Prix FBM 2006), and Coopération Européenne dans le Domaine de la Recherche Scientifique et Technique action 855. G.G. is supported by the Leenards Foundation through a career award entitled "Bourse Leenards pour la relève académique en médecine clinique à Lausanne." This study was approved by the ethics committee of the University of Lausanne.

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DOI: 10.3201/eid1501.080911

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Emerging *Mycobacteria* spp. in Cooling Towers

To the Editor: The importance of nontuberculous mycobacteria (NTM) in various clinical situations recently has increased. Members of the *Mycobacterium avium* complex (MAC) cause a high percentage of infections in persons with acquired immunodeficiency syndrome. Some species are considered emerging pathogens, particularly those of the *M. chelonae*–*M. abscessus* group. They occur not only in immunocompromised persons but also in persons without predisposing conditions. Although sources of infection are considered to originate from the human environment, until now cooling towers were not clearly demonstrated to be one of these contamination sources, despite being suspected (1). Natural streams, ground-

water, brook waters, and swamps already were reported to contain different species of NTM. Constructed environments, such as hospital hot water systems, aerosols from showers, ice machines, swimming pools, dental unit water, endoscopes, and bronchoscopes are other sources (2). Other studies have shown members of MAC in drinking-water distribution systems (3).

A 1999 study in South Africa reported the presence of mycobacteria in cooling towers but reported no details about species (4). In 2003, cooling towers were reported to be a potential source of slow-growing mycobacterial species (1). Some mycobacterial species recently were shown to survive within amoebae (5). We aimed to study the population of amoeba-associated mycobacterial species in 3 cooling towers using a co-culture method.

These cooling towers (E, H, and O), located in downtown Paris, France, were sampled in May 2006. Water was taken in a sample point of the cooling circuit located just before the entrance of the tower basins. Because these cooling towers are regularly treated by oxidizing biocide BCDMH (1-bromo-3-chloro-5, 5-dimethylhydantoin) to prevent development of *Legionella* spp., no *Legionella* spp. or *Legionella*-like amoebal pathogens were isolated.

Two liters of water samples were filtered through 0.22- μ m pore-sized filters that were injected onto amoebal microplates as previously described (6) and incubated at 32°C. We screened amoebal microplates by examination under inverted microscope and by Ziehl-Nielsen, Gram, and Gimenez staining. Positive wells were subcultured on ax-

enic medium and incubated at 32°C for 10 days. Acid-fast isolates were identified by using partial *rpoB* gene amplification and sequencing with Myco-F and Myco-R primers (7). These primers did not allow amplification of partial *rpoB* gene for the 6 *M. phocaicum* strains. We thus used Myco-F/Myco-Rbon (5'-AGCGGCTGCTGGGTGATCAT-3') primer pair for *M. phocaicum* (8). We compared these sequences with the *rpoB* gene sequence of *Mycobacterium* type strains available in the GenBank database by using BLASTn on the NCBI website (www.ncbi.nlm.nih.gov).

We observed bacteria growing in amoebae. Subculture on axenic media led to polymicrobial cultures and allowed isolation of 33 mycobacterial strains (Table). All these strains were submitted to molecular identification. The 33 isolates corresponded to 5 mycobacterial species: *M. fortuitum*, *M. conceptionense*, *M. chelonae*, *M. chimaera*, and *M. phocaicum* (Table). Some of these mycobacteria already had been shown to survive in free-living amoebae and to be implicated in human diseases, such as *M. chelonae* (5). The same author demonstrated recently that 26 environmental mycobacteria survived in the trophozoites and cysts of *Acanthamoeba polyphaga* (5). The recently described species *M. phocaicum* was isolated only in samples from humans and was associated with chronic pneumonia (9). However, the natural source of this species is still unknown. *M. fortuitum* was described as an opportunistic *Mycobacterium* associated with disseminated infections in a leukemia patient or in furunculosis after footbath in nail

Table. Identification of isolated nontuberculous mycobacteria according to their *rpoB* gene sequence similarity and GenBank accession numbers

Closest officially described species (<i>rpoB</i>)	Isolates (no.), N = 33	Range in % gene similarity to type strain (GenBank accession no.)	Accession numbers of sequences of isolates
<i>Mycobacterium chelonae</i>	O (2), E (2)	99.7 (AY147163)	EU770577
<i>M. conceptionense</i>	O (12)	99.2–99.9 (AY859695)	EU770583, EU770584
<i>M. fortuitum</i>	E (2), H (5), O (3)	99.5–99.7 (AY147165)	EU770578, EU770579, EU770580
<i>M. chimaera</i>	H (1)	99.7 (EF521908)	EU770576
<i>M. phocaicum</i>	E (5), O (1)	98.3–98.6 (AY859693)	EU770581, EU770582