Jorge Hernandez,*†
Jonas Bonnedahl,*
Jonas Waldenström,*§¶
Helena Palmgren,‡ and Björn
Olsen*‡¶

*Research Institute for Zoonotic Ecology and Epidemiology (RIZEE), Färjestaden, Sweden; †Kalmar County Hospital, Kalmar, Sweden; ‡Umeå University, Umeå, Sweden; §Lund University, Lund, Sweden; and ¶Ottenby Bird Observatory, Degerhamn, Sweden

References

1. D'Aoust PY, Busby DG, Ferns L, Goltz J, McBurney S, Poppe C, et al. Salmonellosis in songbirds in the Canadian Atlantic provinces during winter-summer 1997–1998. Can Vet J 2000;41:54–9.
2. Cízek A, Literák I, Hejlícek K, Treml F, Smola J. *Salmonella* contamination of the environment and its incidence in wild birds. Zentralbl Veterinarmed B 1994;41:320–7.
3. Waldenström J, Broman T, Carlsson I, Hasselquist D, Achterberg RP, Wagenaar JA, et al. Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. Appl Environ Microbiol 2002;68:5911–7.
4. Brooks KA, Sodeman TM. A clinical evaluation of the API microtube system for identification of *Enterobacteriaceae*. Am J Med Technol 1974;40:55–61.
5. Kauffmann F. Das Kauffmann-White-Schema. Ergebn. Mikrobiol 1957;30:160–216.
6. Aksoycan N, Meco O, Ozsan K, Tekeli ME, Saganak I, Ozuygur B. First isolation of a strain of *Salmonella schleissheim* in Turkey from a patient with enteritis. Mikrobiyol Bul 1983;4:257–8.
7. Hubálek Z, Sixl W, Mikulásková M, Sixl-Vogel B, Thiel W, Halouzka J, et al. *Salmonella* in gulls and other free-living birds in the Czech republic. Cent Eur J Public Health 1995;3:21–4.
8. Palmgren H, Sellin M, Bergström S, Olsen B. Enteropathogenic bacteria in migrating birds arriving in Sweden. Scand J Infect Dis 1997;29:565–8.
9. Isenberg HD. Interpretation of growth culture for stool samples. In: Isenberg HD, editor. Essential procedures for clinical microbiology. Washington: American Society for Microbiology; 1998. p. 90–4.
10. Prescott JF, Poppe C, Goltz J, Campbell GD. *Salmonella typhimurium* phage type 40 in feeder birds. Vet Rec 1998;142:732.

Address for correspondence: Björn Olsen, Department of Infectious Diseases, Umeå University, SE-901 87 Umeå, Sweden; fax: +46-90-13 30 06; email: BjornOl@LTKALMAR.SE

Parachlamydiaceae as Rare Agents of Pneumonia

To the Editor: Members of the *Parachlamydiaceae* family are emerging intracellular bacteria living in amoebae (1,2). Serologic studies have suggested that *Parachlamydia acanthamoeba* might be an agent of community-acquired pneumonia transmitted from a water source (3,4). In a single occasion, 16s rRNA of a member of the *Parachlamydiaceae* family was amplified and sequenced from a bronchoalveolar lavage sample (5). Thus, to specify the role played by the *Parachlamydiaceae* as agents of lower respiratory tract infection, we developed a real-time polymerase chain reaction (PCR) assay and applied it to 1,200 bronchoalveolar lavage samples, taken mainly from patients with pneumonia of unknown cause and received in our diagnostic microbiology laboratory between 1997 and 2002.

DNA extraction was performed by using the MagNA Pure LC instrument and the MagNA Pure LC DNA Isolation Kit III (Roche Molecular Biochemicals, Mannheim, Germany). Real-time PCR was performed by using TaqMan technology and targeting the gene encoding for a nonmitochondrial ATP/ADP translocase (GenBank accession no. AF490592). This energy parasite gene is present only in rickettsiae, chlamydiae, and plant plastids (6). The master mixture was prepared from the TaqMan Universal Master Mix kit (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions, and included 200 nM of each primer (Adp81F 5′- TAGTGATCTGC-TACGGGATTT, Adp84R 5′-TTG-GATTAGGATATTGCAATTT) and 200 nM of the fluorescent labeled probe (6-FAM-5′-AACCTTGTA-G A A G T A A C C T G G A A - GAACCAGC-3′-TAMRA, where 6-

FAM is 6-carboxyfluorescein and TAMRA is 6-carboxytetramethylrhodamine). Amplification was carried out on the ABI 7700 sequence detection system (TaqMan system, Applied Biosystems), by running 45 cycles, with annealing temperature of 52°C and polymerization temperature of 60°C. To prevent carryover, 200 μ M of uracil triphosphate was part of the master mixture, and uracil-N-glycosylase was used systematically. *Parachlamydia acanthamoeba* strain Hall coccus (kindly provided by T.J. Rowbotham) (3) and sterile water were used as positive and negative controls, respectively. In addition, PCR was tested on *Chlamydophila pneumoniae* and *Chlamydia psitacci* and four strains of *Rickettsia*. All but one (*Rickettsia montana*) was negative, as were 64 sterile water controls.

Of the 1,200 bronchoalveolar lavage samples tested, 5 (0.42%) were positive. When PCR was repeated for those five samples, four were negative for *P. acanthamoeba* DNA, and only one was a true positive, confirmed by sequencing the product of the additional PCR. The sequence shared 100% DNA homology with *P. acanthamoeba* strain Hall coccus (GenBank accession no. AF490592). The patient, a 31-year-old man who was HIV-positive, had pneumonia, cough, and no fever. Chest x-ray examination showed an opacity in the right lung and a bilateral infiltrate. Leukocyte count was 5,000/mm3 with 80 CD4 cells/mm3; microbiologic investigations (in which the bronchoalveolar lavage was examined for cytomegalovirus, *Chlamydophila pneumoniae*, *Legionella pneumophila*, *Pneumocystis carinii*, *mycobacteria*, and *Toxoplasma gondii*) did not identify a causal agent.

We developed a highly sensitive PCR, which could amplify as few as 10 bacteria. The assay results in a relatively high specificity (1,195/ 1,199; 99.67%) because it uses a target gene found only in *Rickettsiae*,

Chlamydiae, and plant plastids, and uses a specific DNA probe. We considerably decreased the risk of horizontal and vertical contamination of the PCR reaction by using uracil and uracil-N-glycosylase and by keeping reaction cups closed since the first amplification cycle.

More importantly, our study showed that *Parachlamydia* DNA is rarely found in bronchoalveolar lavage samples (0.083%). This suggests that persons are infrequently exposed to *Parachlamydia* organisms and, consequently, members of the *Parachlamydiaceae* seldom cause pneumonia in humans. In the only positive sample, whether *Parachlamydia* originated from bacteria in the oropharynx, from water, or from a colonization of the lower respiratory tract was not known; whether they caused the patient's pneumonia is also not known. That two strains of *Parachlamydia* found in amoebae were recovered from the nasopharynx of healthy volunteers (7) favors the first hypothesis. However, that the positive broncholaveolar lavage specimen was taken from an HIV-positive patient with community-acquired pneumonia suggests that *Parachlamydia* might occasionally play a pathogenic role in AIDS patients. Moreover, any amoebae-associated bacteria should be considered as a potential emerging pathogen because intra-amoebal growth may lead to the selection of virulence traits and to the adaptation to professional phagocytes, such as alveolar macrophages (1,2). Further studies are warranted to determine whether *Parachlamydiaceae* causes community-acquired pneumonia, particularly in HIV-infected persons.

Acknowledgments

Gilbert Greub,* Pierre Berger,*
Laurent Papazian,†
and Didier Raoult*

*Université de la Méditerranée, Marseille, France; and †Hôpital Sainte Marguerite, Marseille, France

References

1. Greub G, Raoult D. Parachlamydiaceae, potential emerging pathogens. Emerg Infect Dis 2002;8:625–30.
2. Greub G, Raoult D. Crescent bodies of *Parachlamydia acanthamoeba* and its life cycle within *Acanthamoeba polyphaga*: an electron micrograph study. Appl Environ Microbiol 2002;68:3076–84.
3. Birtles RJ, Rowbotham TJ, Storey C, Marrie TJ, Raoult D. Chlamydia-like obligate parasite of free-living amoebae. Lancet 1997;349:925–6.
4. Marrie TJ, Raoult D, La Scola B, Birtles RJ, de Carolis E. Legionella-like and other amoebal pathogens as agents of community-acquired pneumonia. Emerg Infect Dis 2001;7:1026–9.
5. Corsaro D, Venditti D, Le Faou A, Guglielmetti P, Valassina M. A new chlamydia-like 16s rDNA sequence from a clinical sample. Microbiology 2001; 147:515–6.
6. Wolf YI, Aravind L, Koonin EV. *Rickettsiae* and *chlamydiae* evidence of horizontal gene tranfer and gene exchange. Trends Genet 1999;15:173–5.
7. Amann R, Springer N, Schonhuber W, Ludwig W, Schmid EN, Muller KD, et al. Obligate intracellular bacterial parasites of *Acanthamoebae* related to *Chlamydia* spp. Appl Environ Microbiol 1997;63:115–21.

Address for correspondence: Didier Raoult, Unité des Rickettsies, CNRS UPRESA 6020 Faculté de Médecine, Université de la Méditerranée, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France; fax: 00-33-491-83-03-90; email: didier.raoult@medecine. univ-mrs.fr

Hantaviruses in the Czech Republic

To the Editor: Infections caused by hantaviruses have been known for a long time, but their causative agent was not detected until 1976 (1). These viruses of the genus *Hantavirus*, family *Bunyaviridae*, have >20 genotypes. Hantaviruses circulate in wild rodents within natural foci over Euroasia and North and South America. They cause asymptomatic persistent infections in these small mammals. Humans may acquire infection accidentally from inhalation of virus-contaminated aerosols of rodent excreta. Hantavirus genotypes may be nonpathogenic for humans or cause serious diseases with high death rates. In Eurasia, these pathogens involve primarily the kidney and cause hemorrhagic fever with renal syndrome; in North and South America, these pathogens involve primarily the lung and cause hantavirus cardiopulmonary syndrome.

First reports on the occurrence of hantaviruses in central Europe originated from former Czechoslovakia (2,3) and Germany (4) and date back to 1984 and 1985, respectively. The first cases of hantavirus disease in humans in the Czech Republic were reported in 1992 (5). This flulike disease accompanied by microhematuria was recorded in southern Moravia. Another severe imported case was described in a soldier on active military duty in the Balkans (6). The first isolation of nonpathogenic hantavirus Tula was reported in the Czech Republic (7). Currently, several hantavirus infections have been recorded in humans, manifesting mainly as interstitial nephritis. One fatal case was also reported in a patient who had never travelled outside the Czech Republic.

We conducted studies of hantavirus ecology in the Czech Republic and hantavirus seroprevalence in the Czech population. As in neighboring Slovakia (8), hantaviruses of three genotypes, i.e., Dobrava, Puumala, and Tula, were identified in the Czech Republic. Most serious infections are caused by the Dobrava genotype; Tula genotype