

on erythromycin (1.0 g/day) and quickly responded to this treatment without administration of diphtheria antiserum. Erythromycin was intravenously administered at 1 g per day for 9 days, then orally administered at 1,200 mg per day for the next 14 days. Throughout the hospitalization, no complication occurred, and no abnormalities were noted in the electrocardiograms or in the patient's neurologic status. The patient was discharged uneventfully, and no serious sequelae were noted for 20 months. History of immunization for diphtheria was not known.

After the hospitalization for this acute illness, a laboratory report showed that *C. ulcerans* was cultured from the thick white coat of the throat. No other bacteria were found. The National Institute of Infectious Diseases in Tokyo later confirmed identification of the bacteria. By using Elek's test, Vero cell toxicity, and polymerase chain reaction for toxigenic, this strain of *C. ulcerans* was proven to produce diphtheria toxin identical to *C. diphtheriae* (2-4). Although administering appropriate antibiotics as well as antitoxin is a standard of care for patients with diphtheria, antitoxin was not given to this patient because of her rapid response to the erythromycin therapy.

*C. ulcerans* infections in humans occur after drinking unpasteurized milk or coming in contact with dairy animals or their waste (5,6). However, person-to-person transmission of *C. ulcerans* has not been reported, and in some cases, the route of transmission is not clear (7). Recently, *C. ulcerans*-producing diphtheria toxin was isolated in the United Kingdom from cats with nasal discharge (8).

Our patient did not have direct contact with dairy livestock or unpasteurized dairy products; however, more than 10 dairy farms are scattered around her home. Moreover, she kept nearly 20 cats in her house and had been scratched by a stray cat a week

before illness developed. This stray cat, which had rhinorrhea and sneezing, had wandered into her house. The stray cat died before the patient became ill, and no further investigation could be made. Stray cats might well be one of the possible carriers of *C. ulcerans* and might have transmitted the bacteria to this patient. To our knowledge, a case of human infection caused by *C. ulcerans* has never been reported in Japan. On the basis of current experience, this bacterium does exist in Japan and can potentially cause a serious diphtheria-like illness in humans.

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## **Salmonella in Birds Migrating through Sweden**

**To the Editor:** To determine how common *Salmonella* infection is in the migrating wild bird population, we considered the biology of the bacterium and that of its avian hosts. Previous studies have attempted to determine in which stages wild birds become infected, how infections are acquired, and how this information should be translated into epidemiologic risk assessments for human and animal health. For instance, most published studies originate from small epizootics and are of either dead birds at feeding stations (1) or infected birds in or around barns where the livestock has *Salmonella* infection (2). This bias has important consequences, as the natural prevalence of *Salmonella* in the non-epizootic situation likely is overestimated. Finding infected birds close to a barn with infected cattle does not prove that transmission occurred from the birds to the animals. In addition, an epizootic at a feeding station does not prove that *Salmonella* normally occurs in the inflicted bird species, as the birds could have become infected through proximity to the infected animals, or in the case of the bird feeder, through feed contaminated from an unknown source. We need baseline surveillance data on the prevalence of *Salmonella* in non-epizootic situations, in healthy bird communities and in different

stages of a bird's life (e.g., during breeding, molting, and migration), to better understand *Salmonella* epidemiology in relation to wild birds.

We focused on the migratory bird fauna of the North Western Palearctic, where most of the birds migrate south to spend the nonbreeding season in continental Europe and Africa. In these areas, certain species, such as gulls, corvids, starlings, and thrushes, may overwinter in agricultural and urban areas where domestic animals are present. We sampled apparently healthy birds trapped on active migration at Ottenby Bird Observatory (56°12'N, 16°24'E), on the southernmost tip of the island Öland, southeast Sweden, during the migration periods July–November 2001, March–May 2002, and July–December 2002. We used a standardized trapping and sampling scheme, previously used at the same site for large-scale screening of *Campylobacter* infections in wild birds (3). To apply a random procedure in selection of species and persons, every 10th bird banded during the migration periods was sampled for *Salmonella* spp. We did not sample recaptured birds previously banded by us.

In total, 2,377 samples from 110 species of migratory birds (1,086 samples in autumn 2001, 777 in spring 2002, and 514 in autumn 2002) were analyzed for *Salmonella* infections. We applied routine procedures for isolation of putative *Salmonella* isolates, with enrichment in Rappaport-Vassiliadis broth and injection into xylose-lysine-desoxycholate (XLD) agar. On this media, most *Salmonella enterica* serotype Enterica appears as red transparent colonies with black centers. Colonies with growth characteristics of *Salmonella* were observed in 236 samples, and full phenotypic identification was performed on these isolates by using standard biochemical and serologic testing. By using the

API system (4), the isolates were identified as *Citrobacter youngae*, *C. braakii*, *C. freundii*, *Escherichia vulneris*, *E. coli*, *Hafnia alvei*, *Klebsiella pneumoniae ozaenae*, *Acinetobacter baumannii*, *Providencia stuartii/rettgeri*, and *Yersinia kristen senii*. Only one of the isolates, obtained from a Mistle Thrush (*Turdus viscivorus*) and sampled during the spring migration 2002, carried *Salmonella*. This isolate was characterized by serotyping according to the Kauffman-White serotyping scheme (5) at the reference laboratory of the Swedish Institute for Infectious Disease Control. The thrush isolate was identified as *S. Schleissheim*, a rare *Salmonella* serotype. Human salmonellosis caused by this serotype has been previously reported only in Turkey (6). No reservoir of *S. Schleissheim*, in animals or in humans, has been reported in Sweden in the last 10 years covered by the current epidemiologic records.

The failure to find *Salmonella* was probably not caused by technical problems. The sampling methods used, with fecal samples from fresh droppings or cloacal swabs, are well-established techniques for studying *Salmonella* prevalence in birds (2,7,8). The laboratory methods used, with enrichment in Rappaport-Vassaliadis broth and subsequent culturing on XLD-agar, are extremely sensitive for detecting *Salmonella*, even for samples highly contaminated with other *Enterobacteriaceae* (9). Thus, in this large dataset, only one *Salmonella* isolate was found, representing a serotype rarely observed in clinical or veterinary samples. In particular, one serotype, *S. Typhimurium* DT40, has been associated with epizootics in wintering passerine birds (10), but this serotype was not found in any of our samples.

Results from our study indicate that the prevalence of this serotype in the healthy wild bird population is low. Our dataset was composed of

many different species, but the number of tested individual birds for each species was low in many cases. Earlier studies have pointed to certain species (gulls and corvids) in which the prevalence of *Salmonella* is sometimes high (2% to 20%), and argued that concern should be strong about epidemiologic disease transmission with these birds (7,8). These species have the capability to live in an opportunistic manner in close proximity to humans and can base their diet on waste products and garbage. Most bird species, however, have little or no niche overlap with humans or domesticated animals; virtually no data exists on the occurrence of *Salmonella* in this major group of migrating birds during a non-epizootic situation. Our results suggest that the natural occurrence of *Salmonella* in healthy birds during migration in Sweden may be low. Therefore, the *Salmonella* incidence is probably also low for most wild bird species. We suggest that researchers consider analyzing the non-epizootic natural occurrence of *Salmonella* in wild birds. Accumulated knowledge from many different regions, over many years, is a prerequisite for thorough risk assessment of the importance of *Salmonella* carriage in wild birds.

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## 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'-TAGTGATCTGCTACGGGATTT, 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 μM 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 *Chlamydomydia pneumoniae* and *Chlamydia psittaci* 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/mm<sup>3</sup> with 80 CD4 cells/mm<sup>3</sup>; microbiologic investigations (in which the bronchoalveolar lavage was examined for cytomegalovirus, *Chlamydomydia 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*,