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other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition.

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References

1. McQuiston JH, Childs JE. Q fever in humans and animals in the United States. Vector Borne Zoonotic Dis. 2002;2: 179–91.
2. Maurin M, Raoult D. Q fever. Clin Microbiol Rev. 1999;12:518–53.
3. Stoker MGP, Marmion BP. The spread of Q fever from animals to man: the natural history of a rickettsial disease. Bull World Health Organ. 1955;13:781–806.
4. Spicer AJ. Military significance of Q fever: a review. J R Soc Med. 1978;71:762–7.
5. Severe acute pneumonitis among deployed U.S. military personnel—southwest Asia, March–August, 2003. MMWR Morb Mortal Wkly Rep. 2003;52:857.
6. Rubertone MV, Brundage JF. The Defense Medical Surveillance System and the Department of Defense serum repository: glimpses of the future of public health surveillance. Am J Public Health. 2002;92:1900–4.
7. Bishay FK. Towards sustainable agricultural development in Iraq: the transition from relief, rehabilitation, and reconstruction to development [monograph on the Internet]. Rome: Food and Agriculture Organization of the United Nations. 2003 [cited 2005 Jun 2]. Available from http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/006/Y9870E/Y9870E00.HTM

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Anaplasma phagocytophilum, Sardinia, Italy

To the Editor: *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*), a tick-transmitted pathogen that infects several animal species, including humans (involved as accidental "dead-end" hosts), is the causative agent of human granulocytic anaplasmosis (HGA). It is a pathogen of veterinary importance responsible for tickborne fever of ruminants and for granulocytic anaplasmosis of horses and dogs (1,2). HGA was first described in the United States in 1994 (2) and is emerging in Europe (3). Although only 2 human cases have been reported in Italy (4), serologic and molecular findings have shown *A. phagocytophilum* infections in dogs and *Ixodes ricinus* ticks (5). Incidence, prevalence, and public impact of HGA and horse granulocytic anaplasmosis are, therefore, unknown for this geographic area. From 1992 to 1996, an average rate of 13.4 cases/year/100,000 inhabitants of tick bite–related fever of unknown etiology has been reported on the island of Sardinia, Italy, which is considerably higher than the corresponding national average value of 2.1 cases/year/100,000 inhabitants. Moreover, 117 cases of tick bite–related fever, whose etiology remains obscure, have been reported from 1995 to 2002 in the central west coast area of the island. Local newspapers occasionally report deaths as a result of tick bites, although no HGA-associated deaths have been documented in Europe.

This study investigated *A. phagocytophilum* in Sardinia. From 2002 to 2004, veterinarians based on the central west coast of the island were instructed to collect EDTA blood samples when a suspected case of tick bite–related fever was found at their clinics. A total of 70 blood samples were collected from 50 dogs and 20 horses that showed tick infestation and symptoms consistent with tickborne disease, such as fever, anorexia, jaundice (only in horses), anemia, myalgia, and reluctance to move. Genomic DNA was extracted from the buffy coat obtained by centrifugation of 2 to 4 mL of blood, as previously described (6). Furthermore, DNA was extracted from 50 *Rhipicephalus sanguineus* ticks removed from 30 dogs. Primers EphplgroEL(569)F (ATGGTATGCAGTTTGATCGC), EphplgroEL (1193)R (TCTACTCTGTCTTTGCGTTC), and EphgroEL(1142)R (TTGAGTACAGCAACACCACCGGAA) were designed and used in combination to generate a heminested polymerase chain reaction (PCR) for the selective amplification of 573 bp of the *groEL* gene of *A. phagocytophilum*. The final 50 μL PCR volume of the first PCR round contained 5 μL of the DNA extraction, primers EphplgroEL (569)F and EphplgroEL(1193)R, and HotMaster Taq DNA polymerase (5u/μL, Eppendorf) according to the manufacturer's basic protocol (Eppendorf AG, Hamburg, Germany). Heminested PCR was performed by using 5 μL of each of the first PCR products and primer EphgroEL (1142)R. To confirm the PCR diagnosis, amplicons were digested with the *Hin*dIII restriction endonuclease (predicted digestion pattern: 3 fragments of 525 bp, 21 bp, and 27 bp). *Anaplasma phagocytophilum* DNA was obtained from strain NCH-1 and used as positive control in PCR reactions. Sequences were obtained by cloning the PCR products into the pCR2.1-TOPO vector (Invitrogen S.R.L., Milan, Italy) and using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA), according to the protocols supplied by the manufacturers. Sequences (AY848751, AY848747) were aligned to the corresponding

region of other species belonging to *Rickettsiales* by using ClustalX (7). Genetic distances among species were computed by the Kimura 2-parameters method by using MEGA, and were used to construct bootstrapped neighbor-joining trees (8).

Of 120 DNA samples, 1 tick, 3 dog, and 3 horse samples generated the predicted band of 573 bp representative of the *groEL* gene of *A. phagocytophilum*. *Hin*dIII digestions confirmed PCR diagnosis (see Appendix Figure, available at http://www.cdc.gov/ncidod/eid/vol11no08/05-0085_app.htm). Two different *groEL* sequence types were obtained from 1 dog and 1 horse and confirmed by BLAST (http://www. ncbi.nlm.nih.gov/Education/BLASTinfo/information3.html) queries as *A. phagocytophilum groEL* sequences (average identity 99%; average E value = 0), indicating that sequences did not reflect contamination. Bootstrapped neighbor-joining trees confirmed the identity of the new sequences obtained, which are closely related to HGA strains isolated in Europe and the United States (Figure).

The molecular approach applied in this study established *A. phagocytophilum* in an area of Sardinia characterized by a high prevalence of tick bite–related fever in humans and animal species. To our knowledge, this is the first evidence of *A. phagocytophilum* in Sardinian dogs and horses and the first documentation of infection in Italian horses caused by pathogenic strains. Therefore, these findings suggest the emergence of *Anaplasma phagocytophilum* in Italy. *Ixodes ricinus* ticks are indicated as vectors transmitting *A. phagocytophilum* in Europe. Although only 0.3% of 4,086 ticks collected in 72 sites of Sardinia (9) have been identified as *Ixodes*, other tick species are better represented on the island (*Rhipicephalus*, 67.2%; *Haemaphysalis*, 24.1%; *Dermacentor*, 4.9%). *A. phagocytophilum* in 1 *Rhipicephalus sanguineus* could indicate a role of this tick in the epidemiology of HGA. Finally, these data indicate the presence of a potential threat to human and animal health and suggest activation of further epidemiologic surveillance and controls.

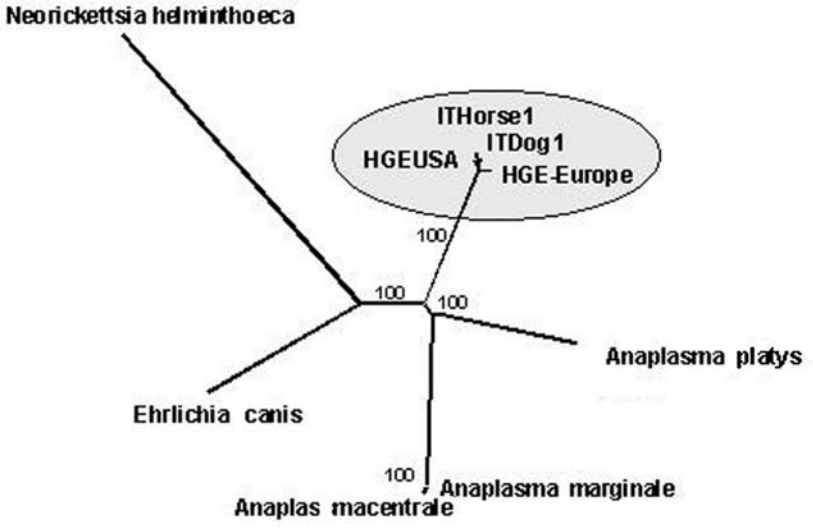

Figure. Bootstrapped neighbor-joining tree of several species belonging to Rickettsiales and identification of the strains isolated during the study as *Anaplasma phagocytophilum.* Strains associated to Sardinian *groEL* variants are closely related to European and American pathogenic human granulocytic anaplasmosis strains. Numbers indicate statistically supported bootstrap values.

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References

1. Dumler JS, Barbet AF, Bekker CPJ, Dasch GA, Palmer GH, Ray SC, et al. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia*, and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGA agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int J Syst Evol Microbiol. 2001;51:2145–65.
2. Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. J Clin Microbiol. 1994;32:589–95.
3. Parola P. Tick-borne rickettsial diseases: emerging risks in Europe. Comp Immunol Microbiol Infect Dis. 2004;27:297–304.
4. Ruscio M, Cinco M. Human granulocytic ehrlichiosis in Italy: first report on two confirmed cases. Ann NY Acad Sci. 2003;990:350–2.
5. Manna L, Alberti A, Pavone LM, Scibelli A, Staiano N, Gravino AE. First molecular characterisation of a granulocytic *Ehrlichia* strain isolated from a dog in South Italy. Vet J. 2004;167:224–7.
6. Pusterla N, Huder J, Wolfensberger C, Litschi B, Parvis A, Lutz H. Granulocytic ehrlichiosis in two dogs in Switzerland. J Clin Microbiol. 1997;35:2307–9.
7. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acid Res. 1997;25:4876–82.

8. Kumar S, Tamura K, Jakobsen IB, Nei M. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics. 2001;17: 1244–5.
9. Di Todaro N, Piazza C, Otranto D, Giangaspero A. Ticks infesting domestic animals in Italy: current acarological studies carried out in Sardinia and Basilicata regions. Parassitologia. 1999;41(Suppl 1): 39–40.

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Williamsia muralis Pulmonary Infection

To the Editor: Bacteria of the genus *Williamsia* are mycolic acid–containing actinomycetes of the suborder Corynebacterineae (1). This suborder also includes the genera *Gordonia*, *Mycobacterium*, *Nocardia*, *Corynebacterium*, *Rhodococcus*, *Dietzia*, *Skermania*, *Tsukamurella*, and *Turicella* (2,3). Within the genus *Williamsia*, only 2 species have been reported: *Williamsia muralis*, isolated from a daycare center (4), and *W. maris*, isolated from the Sea of Japan (5). One important aspect shared by both species is their apparent lack of pathogenicity, since they have been isolated only from environmental samples.

An 80-year-old woman, whose medical history included allergy to penicillin and high blood pressure, was admitted to the cardiothoracic intensive care unit at Juan Canalejo Hospital Complex in La Coruña, Spain, because of a loss of consciousness following an aortic valve replacement. Physical examination showed a systolic murmur and an echocardiogram showed aortic stenosis. Transaortic peak pressure was 100 mm Hg, and the aortic valvular area was 0.3 cm^2. A biologic valve prosthesis (Mitroflow 21, Sorin Group Canada, Ltd., Burnaby, British Columbia, Canada) was inserted under the cardiopulmonary bypass.

Forty-eight hours later, the patient had paroxysmal atrial fibrillation and a temperature of 39°C, with severe hemodynamic and respiratory impairment. She was intubated and intravenous drugs were administered. Blood and urine cultures were requested. Central venous pressure lines were changed, and cultures were obtained. Empiric treatment with levofloxacin, amikacin, and teicoplanin was started for the patient. One of 2 blood cultures was positive for *Staphylococcus epidermidis*, as were cultures from femoral and jugular venous lines. Although considered a contaminant, we observed that *S. epidermidis* was susceptible to empiric antimicrobial drugs.

One week later, a chest radiograph showed bilateral alveolar infiltrates suggestive of pulmonary edema (Figure). To rule out infection, bronchoscopy and protected specimen brush were conducted. An unidentified gram-positive bacillus was cultured from the brush sample. Urine cultures were positive for *Candida kefyr*, but the patient showed no evidence of candidemia. An echocardiogram showed no evidence of infective endocarditis. Since the patient's condition did not improve, levofloxacin was replaced with imipenem, and treatment with fluconazole was initiated. However, the patient developed septic shock, adult respiratory distress syndrome, and oliguric acute renal failure, and died of multiple organ failure.

On direct examination, a Gram stain of the protected specimen brush sample showed numerous gram-positive bacilli. After incubation for 48 h in either an aerobic or capnophilic atmosphere, >1,000 CFU/mL were observed on Columbia agar plates containing 5% sheep blood (BD Stacker Plates, BBL, Franklin Lakes,

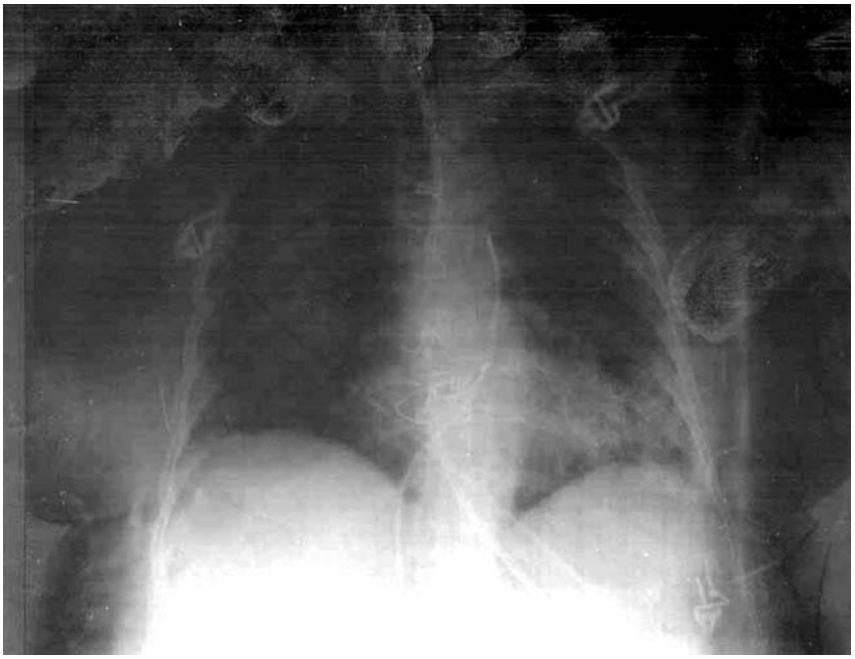

Figure. Chest radiograph of the patient showing bilateral alveolar infiltrates. Although pulmonary edema was the initial diagnosis, an infectious cause should be considered and, on the basis of sepsis, appropriate treatment initiated.