Poore and colleagues (9) have recently demonstrated these to be a unique clone distinct from Asian and American clinical strains (5).

In July 2004, a *V. parahaemolyticus* outbreak of 80 illnesses occurred in A Coruña, Spain. All the case-patients attended weddings in the same restaurant. *V. parahaemolyticus* was isolated from stool samples of 3 patients. The outbreak isolates were characterized by serotyping, polymerase chain reaction (PCR) for species-specific genes (*Vp-toxR* and *tlh*), virulence-related genes (*tdh* and *trh*), and group specific (GS)-PCR (a PCR method to detect the pandemic clone). Two isolates belonged to the serotype O3:K6, while the remaining isolate was O3:K untypeable. All 3 isolates had the *toxR, tlh*, and *tdh* genes, lacked the *trh* gene, and were positive for the GS-PCR assay to detect pandemic strains. These results unequivocally linked the outbreak isolates to the O3:K6 pandemic clone of *V. parahaemolyticus*. To confirm the relationship with the pandemic clone, the outbreak isolates were additionally subjected to DNA fingerprinting analyses. PFGE and arbitrarily primed PCR analyses showed that these isolates exhibited a pattern indistinguishable from those of pandemic strains from Asia. The epidemiologic investigation associated with the outbreak identified the boiled crab eaten in the restaurant as the most probable source of the infection. Live crabs were imported to Spain from the United Kingdom, processed under unhealthy conditions, and stored at room temperature for several hours before they were eaten. All the seafood eaten at the weddings was harvested in Europe, and no imported food was eaten or handled in the restaurant.

Pandemic O3:K6 clone of *V. parahaemolyticus* appeared in Asia around 1996 (6). Since its emergence, it has accounted for most *V. parahaemolyticus* infections in Asia. It spread to the United States in 1998 (7) and more recently to Chile (8), where it has caused hundreds of infections, resulting in the first *V. parahaemolyticus* pandemic in history (9). We report the first evidence that it has been introduced to Europe. The emergence of this virulent serotype in Europe is a public health concern and emphasizes the need to include *V. parahaemolyticus* in microbiologic surveillance and reexamine control programs for shellfish-harvesting areas and ready-to-eat seafood.

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References


**Q Fever and the US Military**

To the Editor: Q fever is a zoonotic disease caused by the rickettsialike organism Coxiella burnetii. The disease has a worldwide distribution and can infect many different species, although cattle, sheep, and goats are the primary reservoirs (1). Transmission to humans usually occurs by inhaling dust or aerosols from infected animals, and approximately half of infected persons manifest clinical symptoms. In acute Q fever infection, the 3 main sets of symptoms are flulike syndrome, pneumonia, and hepatitis (2,3).
Q fever has military relevance not only in its potential use as a bioterrorism agent, but also because of the risk for natural infection in deployed military personnel. Thousands of cases of Q fever have been seen in military personnel since the disease was first reported in the 1930s (4). Since the most common mode of transmission is airborne, personnel do not need to have direct contact with infected animals to be exposed.

*C. burnetii* was first recognized as an infectious disease threat to US military troops serving in Iraq in 2003 during a pneumonia outbreak investigation. Nineteen cases of severe pneumonia, including 2 deaths, occurred from March 1 to August 20 (5). A case was defined as occurring in a patient with bilateral alveolar infiltrates that was defined as occurring in a patient with a chest radiograph suggesting pneumonia and ≥1 of the following symptoms: fever, cough, or shortness of breath. The Defense Medical Surveillance System (DMSS) was queried to determine how many patients had both predeployment and postdeployment serum samples available for Q fever testing. The Army Medical Surveillance Activity, which operates DMSS, also maintains the Department of Defense Serum Repository and stores serum from service members after mandatory HIV testing and deployment processing (6). Predeployment sera must be collected within the year before deployment.

Twenty-two soldiers had predeployment and postdeployment sera available; samples were tested for phase I and phase II antibody to Q fever by using IFA. Results showed 5 additional soldiers in whom pneumonia was diagnosed while serving in Iraq and who seroconverted to *C. burnetii* before postdeployment serum draws (Table). All predeployment antibody titers for both immunoglobulin (Ig) G and IgM were negative in these 5 soldiers, with an IFA titer of 1:16 as a cutoff.

The initial 3 Q fever patients ascertained through the pneumonia outbreak investigation were extensively interviewed for possible exposures. All 3 patients first experienced symptoms while in northern Iraq and reported contact with domestic animals, including dogs, cats, sheep, goats, and camels. Two of the patients reported tick bites within 30 days before becoming ill, and 1 reported drinking raw sheep’s milk. The 5 other patients who became ill with pneumonia also first sought care while in northern Iraq. Predeployment sera from these 3 patients were also tested for *C. burnetii* by IFA, and all samples were negative for both IgG and IgM.

Extremely limited information is available on Q fever disease prevalence in Iraq, either in animals or humans. Iraq is primarily an agricultural country, and nomadic herding takes place countrywide, except in the northernmost regions and along the eastern border, where adequate land is available for grazing livestock. The most common livestock in Iraq are cattle, sheep, and goats (7). Although herds of infected animals may exist in any region of Iraq, larger concentrations of livestock may exist in northern areas, where land is suitable for ruminants to graze. This concentration could lead to a higher risk for transmission to humans because the chance of contact with infected animals would be greater.

These data indicate the potential importance of *C. burnetii* as an infectious disease threat to US military troops in Iraq. Healthcare providers should include Q fever in their differential diagnosis of community-acquired pneumonia and consider adding doxycycline to a combined antimicrobial drug regimen to presumptively treat severe pneumonia. Future studies to be completed include case ascertainment to locate US troops who were infected with Q fever while in Iraq and in whom pneumonia or other clinical manifestations of illness may have developed.

Research was conducted in compliance with the Animal Welfare Act and

<table>
<thead>
<tr>
<th>Patient</th>
<th>IgG</th>
<th>IgM</th>
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<tbody>
<tr>
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<td>Negative</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
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<td>1:64</td>
</tr>
<tr>
<td>8</td>
<td>&gt;1:1,024</td>
<td>&gt;1:1,024</td>
</tr>
</tbody>
</table>

*All predeployment titers were negative for immunoglobulin (Ig) G and IgM.*

Table. Postdeployment serum antibody titers to phase II antigen for Q fever in 8 US military personnel who served in Iraq, March 1–August 20, 2003.
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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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 theuffy coat obtained by centrifugation of 2 to 4 mL of blood, as previously described (6). Furthermore, DNA was extracted from 50 Rhizopinus sanguineus ticks removed from 30 dogs. Primers EphphgroEL(569)F (ATGGTATGCA-GTGGTATC), EphphgroEL(1193)R (TCTACTCTGTCTTTGCGTTC), and EphphgroEL(1142)R (TTGAGTA-CAGGAAACACCGGAA) 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 EphphgroEL(569)F and EphphgroEL(1193)R, and HotMaster Taq DNA polymerase (5 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 EphphgroEL (1142)R. To confirm the PCR diagnosis, amplicons were digested with the HindIII 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