trophoresis (PFGE) analysis 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 seafood 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.

Jaime Martinez-Urtaza,* Lourdes Simental,* David Velasco,† Angelo DePaola,‡ Masanori Ishibashi,§ Yoshitsugu Nakaguchi,¶ Mitsuaki Nishibuchi,¶ Dolores Carrera-Flores,# Carmen Rey-Alvarez,# and Anxela Pousa#

*Universidad de Santiago de Compostela, Santiago de Compostela, Spain; †Complexo Hospitalario Universitario Juan Canalejo, A Coruña, Spain; ‡US Food and Drug Administration, Dauphin Island, Alabama, USA; §Osaka Prefectural Institute of Public Health, Osaka, Japan; ¶Kyoto University, Kyoto, Japan; #Consejería de Sanidade, Santiago de Compostela, Spain

References

1. Mead PS, Slutsker L, Dietz V, McCAig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis. 1999;5:607–25.
2. Joseph SW, Colwell RR, Kaper JB. Vibrio parahaemolyticus and related halophilic vibrios. Crit Rev Microbiol. 1982;10:77–124.
3. Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on Vibrio vulnificus and Vibrio parahaemolyticus. European Commission. 2001.
4. Lozano-Leon A, Torres J, Osorio CR, Martinez-Urtaza J. Identification of tdh-positive Vibrio parahaemolyticus from an outbreak associated with raw oyster consumption in Spain. FEMS Microbiol Lett. 2003;226:281–4.
5. Martinez-Urtaza J, Lozano-Leon A, DePaola A, Ishihashi M, Shimada K, Nishibuchi M, et al. Characterization of pathogenic Vibrio parahaemolyticus isolates from clinical sources in Spain and comparison with Asian and North American pandemic isolates. J Clin Microbiol. 2004;42:4672–8.
6. Okuda I, Ishihashi M, Hayakawa E, Nishino T, Takeda Y, Mukhopadhyay AK, et al. Emergence of a unique O3:K6 clone of Vibrio parahaemolyticus in Calcutta, India, and isolation of strains from the same clonal group from Southeast Asian travelers arriving in Japan. J Clin Microbiol. 1997;35:3150–5.
7. Daniels NA, Ray B, Easton A, Marano N, Kahn E, McShan Al II, et al. Emergence of a new Vibrio parahaemolyticus serotype in raw oysters: a prevention quandary. JAMA. 2000;284:1541–5.
8. González-Escalona N, Cachicas V, Acevedo C, Rioseco ML, Juan A, Vergara JA, et al. Vibrio parahaemolyticus diarrhea, Chile, 1998 and 2004. Emerg Infect Dis. 2005;11:129–31.
9. Matsumoto C, Okuda J, Ishihashi M, Iwana M, Garg P, Rammanurthy T, et al. Pandemic spread of an O3:K6 clone of Vibrio parahaemolyticus and emergence of related strains evidenced by arbitrarily primed PCR and toxRS sequence analyses. J Clin Microbiol. 2000;38:578–85.

Address for correspondence: Jaime Martinez-Urtaza, Instituto de Acuicultura, Universidad de Santiago de Compostela, Campus Universitario Sur, 15782 Santiago de Compostela, Spain; fax: 34-981-547-165; email: ucmjmur@usc.es

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 required intubation and mechanical ventilation. This investigation involved extensive serologic testing for possible infectious causes of pneumonia, including C. burnetii. Of 19 patients with severe pneumonia tested for C. burnetii, 3 had positive antibody titers by immunofluorescence assay (IFA). No other infectious cause was confirmed for the remaining cases of pneumonia. Although C. burnetii was not determined to be the cause of the pneumonia outbreak, the finding of 3 patients with positive antibody titers launched an effort to ascertain other cases of Q fever among military personnel who served in Iraq during that time.

Approximately 62 cases of pneumonia, both severe and nonsevere, occurred in Iraq from March 1 to August 20, 2003. A pneumonia case 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.

| Patient | IgG     | IgM       |
|---------|---------|-----------|
| 1       | 1:1,024 | Negative  |
| 2       | 1:128   | Negative  |
| 3       | >1:1,024| 1:512     |
| 4       | 1:256   | 1:256     |
| 5       | 1:512   | >1:1,024  |
| 6       | 1:512   | 1:512     |
| 7       | 1:64    | 1:64      |
| 8       | >1:1,024| >1:1,024  |

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

Research was conducted in compliance with the Animal Welfare Act and...
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.

Alicia D. Anderson,* Bonnie Smoak,† Eric Shuping,‡ Christopher Ockenhouse,* and Bruno Petruccelli§

*Walter Reed Army Institute of Research, Silver Spring, Maryland, USA; †Ireland Army Community Hospital, Fort Knox, Kentucky, USA; and ‡US Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, Maryland, USA

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 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 nationwide 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 tick-borne 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 (ATGGTATGCA-GTTTTGATCGC), EphplgroEL (1193)R (TCTACTCTGTTCTTGCCTTC), and EphplgroEL(1142)R (TTGAGTA-CAGCAACACGACCGGAA) 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 (5µL, 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 EphplgroEL (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