

all 29 patients, a history of eating raw oysters or other raw or undercooked seafood before illness onset was uncommon and was only reported by 1 patient. Although *V. vulnificus* has not been proven as the cause of gastroenteritis, Hseuh et al. have suggested that such results might have occurred because patients with diarrhea seldom sought care from a large teaching hospital or saved stool samples for investigation (7).

V. vulnificus infection was first reported in humans in 1979 (1). Since then, most case reports have focused on immunocompromised persons and their risk from eating raw oysters and their risk from eating raw oysters among (4–6). Our study found that a considerable proportion of *V. vulnificus* infections in Hong Kong occur among healthy persons. Furthermore, severe forms of the infection, such as necrotizing fasciitis and septicemia, are relatively common among healthy persons, although they may cause fewer deaths than they do among persons with predisposing medical conditions. Among healthy persons, *V. vulnificus* infection is most likely associated with a history of cutaneous injury caused by handling seafood, which can allow the bacteria to enter the body through an open wound. The risk of exposure is more important in this locality than in other areas where swimming or eating raw oysters and undercooked seafood are the major risk factors (4,6–8), possibly because fresh seafood is widely consumed, and seafood is easily accessible in wet markets in Hong Kong. Our study shows that the risk is higher during the summer, which is consistent with the fact that *V. vulnificus* is more active in warmer temperatures (9). We suggest that all persons, even healthy persons, exercise caution to avoid injury while handling seafood. Physicians should consider possible *V. vulnificus* infection when diagnosing a rapidly progressive skin and soft tissue infection in a healthy person who reports an injury from handling seafood.

**P.H. Chung,* S.K. Chuang,*
Thomas Tsang,* Lai Wai-man,†
Raymond Yung,‡ and Janice Lo‡
for the Collaborative Study Group
on *Vibrio vulnificus* Infection in
Hong Kong**

*Department of Health, Hong Kong Special Administrative Region, People's Republic of China; †Hospital Authority, Hong Kong Special Administrative Region, People's Republic of China; and ‡Centre for Health Protection, Hong Kong Special Administrative Region, People's Republic of China

References

1. Blake PA, Merson MH, Weaver RE, Hollis DG, Heublein PC. Disease caused by a marine *Vibrio*. Clinical characteristics and epidemiology. *N Engl J Med*. 1979;300:1–5.
2. Klontz KC, Lieb S, Schreiber M, Janowski H, Baldy L, Gunn RA. Syndromes of *Vibrio vulnificus* infections: clinical and epidemiologic features in Florida cases, 1981–1987. *Ann Intern Med*. 1988;109:318–23.
3. Mitra AK. *Vibrio vulnificus* infection: epidemiology, clinical presentation, and prevention. *South Med J*. 2004;97:118–9.
4. Gholami P, Lew SQ, Klontz KC. Raw shellfish consumption among renal disease patients. A risk factor for severe *Vibrio vulnificus* infection. *Am J Prev Med*. 1998;15:243–5.
5. Haq SM, Dayal HH. Chronic liver disease and consumption of raw oysters: a potentially lethal combination—a review of *Vibrio vulnificus* septicemia. *Am J Gastroenterol*. 2005;100:1195–9.
6. Potasman I, Paz A, Odeh M. Infectious outbreaks associated with bivalve shellfish consumption: a worldwide perspective. *Clin Infect Dis*. 2002;35:921–8.
7. Hsueh PR, Lin CY, Tang HJ, Lee HC, Liu JW, Liu YC, et al. *Vibrio vulnificus* in Taiwan. *Emerg Infect Dis*. 2004;10:1363–8.
8. Tacket CO, Brenner F, Blake PA. Clinical features and an epidemiological study of *Vibrio vulnificus* infections. *J Infect Dis*. 1984;149:558–61.
9. Shapiro R, Altekruse S, Hutwagner S, Bishop R, Hammond R, Wilson S, et al. The role of Gulf Coast oysters harvested in warmer months in *Vibrio vulnificus* infections in the United States, 1988–1996. *J Infect Dis*. 1998;178:752–9.

Address for correspondence: P.H. Chung, Medical and Health Officer, Field Epidemiology Training Program, Surveillance and Epidemiology Branch, Centre for Health Protection, Department of Health, Hong Kong

Special Administrative Region, People's Republic of China; email: mo_fetp2@dh.gov.hk

Neorickettsia helminthoeca in Dog, Brazil

To the Editor: *Neorickettsia helminthoeca* causes salmon poisoning disease (SPD) in canids. SPD has been described only in the United States and the northwestern Pacific region of Canada (1). This report complements previous pathologic findings (2) and identifies SPD beyond the known disease-endemic region.

From 2001 to 2005, 20 dogs (5 mongrels and 15 beagles) showed pathologic lesions consistent with SPD. All beagles were born in coastal Florianópolis, Santa Catarina, Brazil, and later transferred to Maringá, Paraná, Brazil, for the last 3–4 years of life. Lymph nodes, spleen, liver, and intestines from 10 beagles were aseptically obtained at necropsy in Maringá and frozen at –20°C until used at the Johns Hopkins Medical Institutions in Baltimore, Maryland.

Genomic DNA was extracted from frozen tissues with QIAamp DNA Mini Kits (Qiagen, Valencia, CA, USA). DNA from *N. helminthoeca* and *Anaplasma phagocytophilum* was used as a positive control. Nuclease-free water was used as a negative control. We used gene-specific primers for *Neorickettsia* spp. 16S rRNA (*rrs*) (NeoSH-F; 5'-TAGGCCCGCGTTA-GATTAGCTTGT-3' and NeoSH-R; 5'-TACAACCCAAGGGCCCTTCATCACT-3') and *N. helminthoeca* RNA polymerase β -subunit (*rpoB*) (NH-rpoB-F; 5'-TGTCTTCGAAGGCC-

CAAAGACAGA-3' and NH-rpoB-R: 5'-AGAACCGATAGAGCGGGCAT-GAAT-3') (3) and heat-shock protein *groESL* (NH-*groESL*-F: 5'-AGGC-TACTTCGCAGGCAAATGAGA-3' and NH-*groESL*-R: 5'-CACGCTT-CATTCCGCCCTTTAACT-3') (4,5). Citrate synthase (*gltA*) gene primers (6) were also used. Two PCRs were conducted to maximize sensitivity.

Specificity of *N. helminthoeca*-specific primers was shown by amplification studies of genomic DNA of *A. phagocytophilum*, *Ehrlichia chaffeensis*, *E. canis*, *N. risticii*, *N. sennetsu*, and *N. helminthoeca*. All amplicons were separated by electrophoresis in 1% agarose gels and purified before cloning (pGEM-T and pGEM-T Easy Vector Systems, Promega, Madison, WI, USA) and sequencing. The Maringá sequences obtained were compared with those in GenBank by using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>). Phylogenetic trees, sequence alignments, and identity tables were created by using Vector NTI Advance10 Software (Invitrogen, Carlsbad, CA, USA). GenBank accession numbers of *Anaplasmataceae* and their phylogenetic relationships are shown in the Figure.

Two dogs (N40-05, mesenteric lymph node, Maringá 1 and N20-04, Peyer's patch, Maringá 2) contained *Neorickettsia* spp. *rrs*, *rpoB*, or *groESL* genes. Both samples produced partial sequences for *Neorickettsia* spp. *rrs* gene; a similarity of 99% was observed for the 2 Maringá dog *rrs* sequences with *N. sennetsu*, *N. risticii*, and the *Stellantchasmus falcatus* (SF) agent. However, *N. helminthoeca* *rpoB* and *groESL* partial sequences were obtained only from dog 1. DNA identities of 100%, 82%, and 81% were observed between Maringá dog 1 sequences and *N. helminthoeca*, *N. risticii*, and *N. sennetsu* for the *rpoB* genes, respectively. All dogs were negative when tested with *gltA* gene primers. We observed 100% identity

between the Maringá dog 1 sequence and *N. helminthoeca* *groESL* gene sequences. Similarities of 84%, 80%, and 79% were observed with *N. sennetsu*, the SF agent, and *N. risticii*, respectively. All positive controls showed bands of appropriate sizes, whereas negative controls yielded no products, confirming lack of amplicon contamination.

This study demonstrates that 2 dogs from Maringá, Brazil, with pathologic lesions consistent with SPD (7) were infected with a *Neorickettsia* sp. The partial

sequences from dog 1 were identical to *N. helminthoeca* *rrs*, *groESL*, and *rpoB* genes, confirming infection with this organism (2). To our knowledge, this is the first confirmed description of this organism beyond the known geographic area of SPD. The organism identified in Brazil has been named *N. helminthoeca* Maringá strain.

Because of difficulty in recovering DNA from samples, need for a highly efficient PCR targeting small DNA regions, and limited sensitivity of the amplifications, sequences obtained

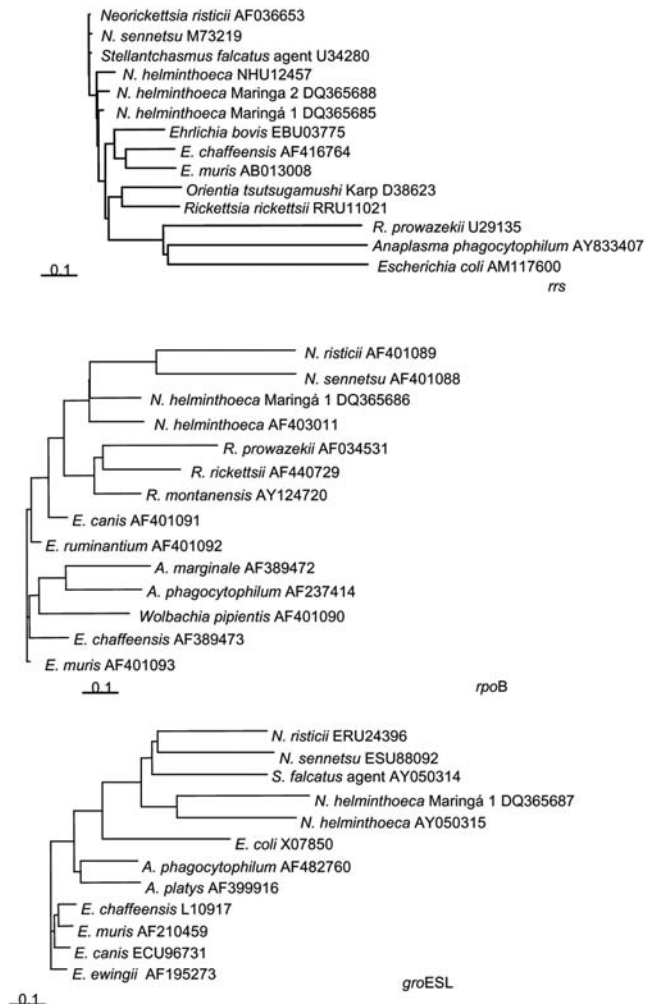


Figure. Neighbor-joining phylogenetic trees of the 16S rRNA (*rrs*), RNA polymerase β -subunit (*rpoB*), and heat-shock protein (*groESL*) gene sequences of *Anaplasmataceae* families. Trees were constructed with Vector NTI Advance10 Software (Invitrogen, Carlsbad, CA, USA). Bars represent substitutions per 1,000 bp. GenBank sequence accession numbers are provided.

for *N. helminthoeca* Maringá dog 1 (112 bp for *rrs*, 92 bp for *groESL*, 143 bp for *rpoB*) were short compared with those in GenBank (*rrs* 1,453 bp, *groESL* 1,914 bp, *rpoB*, 464 bp). Efficiency and sensitivity of targeting small DNA regions was necessary since storage and shipment of frozen samples were not optimal. Small DNA sequences are often suboptimal for delineation of phylogenetic relationships.

Bootstrapping analyses showed poor resolution (<380/1,000 iterations) below the genus level for the short *rrs* region examined. However, both the short *rpoB* and *groESL* regions examined had high bootstrap values (941/1,000 and 995/1,000 iterations, respectively). This finding allowed differentiation of *N. helminthoeca* and the Brazilian dog strain from *N. sennetsu*, *N. risticii*, and other related *Anaplasmataceae* and provided a high degree of confidence in the identification. More work is being implemented to obtain longer sequences to confirm and extend these genotypic comparisons. We propose further study to isolate the pathogen from other dogs for comparative biologic analyses.

Although SPD is caused by *N. helminthoeca*, infections by other *Neorickettsia* spp., including *N. risticii* (Potomac horse fever) and *N. sennetsu* (sennetsu fever), illustrate

the potential of these widely distributed species to infect and cause disease in mammals and humans. Detection of *N. helminthoeca* in Brazilian dogs extends the range of this species and warrants a broad search for infections and spectrum of disease of *Neorickettsia* in animals and humans.

Acknowledgments

We thank Joseph Mankowski for help with the initial studies and Yasuko Rikihisa for *N. helminthoeca* cultures. This study is part of a PhD thesis for S.A.H. at the Universidad Estadual de Londrina.

This study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior Brasília, Brazil (S.A.H.), and the National Institute of Allergy and Infectious Diseases (J.S.D).

Selwyn A. Headley,*

Diana G. Scorpio,†

Nicole C. Barat,† Odilon Vidotto,*

and J. Stephen Dumler†

*Universidade Estadual de Londrina, Londrina, Brazil; and †Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

References

- Dumler JS, Rikihisa Y, Dasch GA. Family II *Anaplasmataceae*. In: Garrity GM, editor. Bergey's manual of systemic bacteriology. 2nd ed. Vol. 2. New York: Springer; 2005. p. 117–43.
- Headley SA, Vidotto O, Scorpio D, Dumler JS, Mankowski J. Suspected cases of *Neorickettsia*-like organisms in Brazilian dogs. *Ann N Y Acad Sci*. 2004;1026:79–83.
- Taillardat-Bisch AV, Raoult D, Drancourt M. RNA polymerase β -subunit-based phylogeny of *Ehrlichia* spp., *Anaplasma* spp., *Neorickettsia* spp. and *Wolbachia pipientis*. *Int J Syst Evol Microbiol*. 2003;53:455–8.
- Dumler JS, Barbet AF, Bekker CP, 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 "HGE agent" as subjective synonyms of *Ehrlichia phagocytophila*. *Int J Syst Evol Microbiol*. 2001;51:2145–65.
- Rikihisa Y, Zhang C, Kanter M, Cheng Z, Ohashi N, Fukuda T. Analysis of p51, *groESL*, and the major antigen P51 in various species of *Neorickettsia*, an obligatory intracellular bacterium that infects trematodes and mammals. *J Clin Microbiol*. 2004;42:3823–6.
- Inokuma H, Brouqui P, Drancourt M, Raoult D. Citrate synthase gene sequence: a new tool for phylogenetic analysis and identification of *Ehrlichia*. *J Clin Microbiol*. 2001;39:3031–9.
- Cordy DR, Gorham JR. The pathology and etiology of salmon disease. *Am J Pathol*. 1950;26:617–37.

Address for correspondence: J. Stephen Dumler, Division of Medical Microbiology, Department of Pathology, Johns Hopkins University School of Medicine, 624 Ross, 720 Rutland Ave, Baltimore, MD 21205, USA; email: sdumler@jhmi.edu

Correction: Vol. 12, No. 4

In "Potential Arbovirus Emergence and Implications for the United Kingdom" by Ernest A. Gould et al., an error occurred on page 549. The first paragraph of the article incorrectly states that African horse sickness virus is circulating in Europe. The sentence should read "Finally, the family *Reoviridae* contains a variety of animal arbovirus pathogens, including bluetongue virus, which is currently circulating in Europe, and African horse sickness virus,

which has been found in Europe but is not currently circulating."

The corrected text appears in the online article at <http://www.cdc.gov/ncidod/EID/vol12no04/05-1010.htm>

We regret any confusion this error may have caused.