Letters

During a survey of parasitic helminths of wild vertebrates from Tres Palos Lagoon, in Guerrero, Mexico, we found *Gnathostoma* sp. advanced third-stage larvae (AdvL$_3$) in the skeletal muscle of several fish species. Fish were caught from March to August 1999 in Tres Palos Lagoon (16° 41' to 16° 50'N and 99° 37' to 99° 47'W), Acapulco Municipality, 25 km south of Acapulco Bay (5). Fish muscle was ground individually, compressed between glass plates, and examined with a magnifying glass and a lamp. The infection was characterized as by Margolis et al. (6).

Of nine fish species examined, five were positive for *Gnathostoma* AdvL$_3$: Eleotridae: *Dormitator latifrons* ("popoyote," n = 83), *Gobiomorus maculatus* ("guavina," n = 66), *Eleotris pictus* ("alahuate," n = 22); Cichlidae: *Cichlasoma trimaculatum* ("charra," n = 62), and Ariidae: *Cathorops caerulescens* ("cuatete," n = 62). The highest prevalence and mean abundance values (number of larvae per fish) were found in *E. pictus* (31.81%, 0.82 ± 1.99); in the other host species values were ≤7.22 and 0.072 ± 0.26, respectively. *E. pictus* mean abundance values differed significantly from those of the other host species (nonparametric Kruskal-Wallis test, H = 27.125, 4 g.l., n = 337, p <0.0).

The intermediate host transmitting the infection to humans in Mexico had previously been identified only in the Rio Papaloapan Basin, in Veracruz and Oaxaca (7,8). The presence of *Gnathostoma* AdvL$_3$ in the muscle of fish species frequently eaten by humans in Acapulco suggests that these fish may have been the main source of infection in the 98 recorded cases of gnathostomosis (3,4). The popularity of "ceviche" (raw fish marinated in lime juice), prepared with the most commonly caught fish (including the three species of eleotrids studied), strongly supports this possibility. The identification of the source of human infection allows local health authorities to implement public information campaigns about the risk of eating raw or undercooked fish (in the form of sushi or ceviche) in this region. After this initial step in the study of this parasitic disease, the worm species must be accurately identified. In addition, understanding the parasite's life cycle is important for control of a parasitic disease.

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First Report of Human Granulocytic Ehrlichiosis from Southern Europe (Spain)

To the Editor: Human granulocytic ehrlichiosis (HGE) is a tickborne zoonosis described in the United States several years ago (1) and in Europe recently (2). Several hundred cases

have been reported in the United States (3). In Europe, nine cases have been reported, six in Slovenia (2,4-6), and three in Sweden (I. Eliasson, http:\\www.healthnet.org/programs/promed.html). We report a serologically confirmed case of HGE in La Rioja, a Lyme disease-endemic area in northern Spain (7-9).

On August 7, 1999, a 16-year-old man from La Rioja, who had been bitten by a tick 15 days before, was seen in an emergency room and treated with 100 mg of doxycycline twice a day. On August 9, he was hospitalized with a 3-day history of malaise, myalgias, headache, and fever (39ºC). The fever abated in the next 36 hours. The patient had not noticed any signs of inflammation or skin rash, and no signs of neurologic injury were evident. He had abdominal pain when the liver was palpated. Chest radiographs were normal, and abdominal ultrasonography showed no abnormalities. Laboratory studies showed a level leukocyte count (3,001/mm^3 [normal range, 4,500-11,000] with 4.3% band forms, 72.3% neutrophils, 4.7% monocytes, 16.7% lymphocytes, and a platelet count of 114,000/mm^3 [normal, 160,000-410,000]). The hemoglobin level was normal. No inclusions (morulae) suggestive of *Ehrlichia* or *Babesia* spp. were seen on blood smears. The erythrocyte sedimentation rate was normal. The aspartate aminotransferase level was 72 U/L [normal, 5-40]; alanine aminotransferase, 65 U/L [normal, 5-40]; and lactodehydrogenase, 637 U/L [normal, 100-250]. All serologic assays were performed by the same, widely experienced microbiologist, in one laboratory. Serologic test results were negative for *Borrelia burgdorferi* (by enzyme-linked immunosorbent assay [ELISA]); *Rickettsia conorii* (indirect fluorescent-antibody assay [IFA]; *Coxiella burnetti* (IFA); *Ehrlichia chaffeensis* (IFA); the agent of HGE (IFA); and hepatitis A, B, and C viruses (ELISA); and indicated immunity for Epstein-Barr virus. Four weeks later, the aminotransferase levels were normal, and the patient was asymptomatic. A new serum determination showed an HGE antibody titer of 1:64 (HGE IFA IgG MRL Diagnostics, California, USA); the serum tested negative for the other microorganisms tested, including with a new test for *E. chaffeensis*. Another serum sample from the patient taken 8 weeks later showed a titer of 1:256 to the HGE

agent. An EDTA-treated sample of whole blood obtained from the patient on day 4 after start of doxycycline treatment was negative for the *E. phagocytophila* genogroup by polymerase chain reaction (PCR). We used a set of primers based on the published sequence of the 16s rNA of *E. phagocytophila* (E1: 5'- GGC ATG TAG GCG GTT CGC TAA GTT - 3' and E2: 5'- CCC CAC ATT CAG CAC TCA TCG TTT A -3') (7). Multiple water samples and a positive blood sample from an experimentally infected lamb were used as controls for PCR amplicon contamination. Doxycycline was administered for 14 days, and the patient's clinical and laboratory abnormalities resolved.

Many tickborne diseases are present in La Rioja. The prevalence of *E. phagocytophila* genogroup in the tick *Ixodes ricinus* is high (24.1% of nymphs, determined by PCR) in La Rioja, and evidence of HGE infection in patients at risk has been reported (10,11). This patient's history of previous tick bite, flulike symptoms, seroconversion to HGE agent, aminotransferase elevation, and response to doxycycline suggest the diagnosis of HGE. As in other reported cases in Europe, no morulae suggestive of *Ehrlichia* infection in the acute phase were visible, the clinical manifestations were moderate, and the fever abated quickly with treatment. Also, as in other cases, the negative PCR result can be explained by the prior treatment with doxycycline.

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Phylogenetic Analysis of the Chinese *Rickettsia* Isolate BJ-90

To the Editor: Five species of tick-associated rickettsiae have been identified in China; of these, three are human pathogens and two are of unknown pathogenicity (1). In 1990, one isolate, BJ-90, was first obtained from a *Dermacentor sinicus* tick, a newly recognized vector collected in a Beijing suburb, an atypical location for *Rickettsia sibirica* (2). Several taxonomic studies of the phenotype, antigenicity, and genotype of BJ-90 have been performed, with inconsistent results (2-6). Recently, phylogenetic analysis based on several gene comparisons has enabled the phylogenetic classification of this rickettsial species (7-11). To confirm the phylogenetic relationships between the BJ-90 strain and other rickettsiae, the 16S rRNA, *gltA*, and OmpA encoding genes were amplified and sequenced. Phylogenetic relationships between the BJ-90 strain and other rickettsia in the GenBank database were inferred by the parsimony and neighbor-joining methods (9). Bootstrap analyses were used to assess the reliability of the phylogenetic analysis.

Both methods showed a high degree of similarity between BJ-90, *R. sibirica* and "*R. mongolotimonae*," which were grouped in the same cluster in three inferred dendrograms. The data from the 16S rRNA and *gltA* sequences showed low statistical significance in the cluster (bootstrap values for the nodes 50% and 33%, respectively). However, data from the *rompA* gene sequence showed highly significant similarity in the cluster (bootstrap value 100%), confirming the reliability of the phylogenetic analysis. The results of this phylogenetic analysis are consistent with those of previous phenotypic, genotypic, and phylogenetic analyses (2,3,5-11), as well as taxonomy derived from direct antigenic comparison of the species (4). The sequences of 16S rRNA, *gltA*, and OmpA have been assigned the following GenBank accession numbers: AF178036 for 16S rRNA, AF178035 for *gltA*, AF179365 for the 611-bp sequence of ompA, and AF179367 for the 3174-bp sequence of ompA. According to previous genotypic and antigenic studies and our phylogenetic analysis, in which the BJ-90 strain is closer to *R. sibirica* than *R. mongolotimonae* in the dendrogram inferred from comparison of the ompA encoding gene sequences, the BJ-90 strain should be considered a variant of *R. sibirica*.

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