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SHORT REPORT: DUAL INFECTION OF ANIMAL HOSTS WITH DIFFERENT ECHINOCOCCUS SPECIES IN THE EASTERN QINGHAI-TIBET PLATEAU REGION OF CHINA

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Abstract. The eastern Qinghai-Tibet plateau of China is a highly endemic region of echinococcosis where Echinococcus granulosus sensu stricto (sheep strain), Echinococcus multilocularis, and Echinococcus shiquicus are distributed sympatrically. We developed a polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) method for the identification of the three species in this region. The PCR-RFLP showed the dual infection of animals with different Echinococcus spp. The first case was a domestic dog concurrently infected with adults of E. granulosus and E. multilocularis. The second case was a plateau pika (Ochotona curzoniae) harboring metacestodes of E. multilocularis and E. shiquicus in the liver. The high susceptibility of some mammalian hosts to the parasites and the high prevalence of the three co-endemic species probably increase the chance of mixed infections in the eastern Tibetan plateau.

The three taeniid tapeworms, Echinococcus granulosus sensu stricto (sheep strain), Echinococcus multilocularis, and Echinococcus shiquicus, are distributed in the Qinghai-Tibet plateau of western China.1–3 The former two species are the causes of cystic echinococcosis (CE) and alveolar echinococcosis (AE) to humans, respectively, whereas the latter is a newly described species whose pathogenicity, if any, is still unknown.3,4 On the eastern part of the Tibetan plateau, various mammals are involved in the two host transmission cycles of Echinococcus spp.3,5,6 Dogs, red foxes (Vulpes vulpes), and Tibetan foxes (Vulpes ferrilata) can serve as definitive hosts, whereas sheep, yaks, Tibetan hare (Lepus oiiostolus), pika (Ochotona spp.), and voles (e.g., Microtus spp.) may act as intermediate hosts. Because of the severe environment/weather of high altitude steppe, semi-nomadic pastoralism, entrenched poverty, and traditional customs and beliefs, the Tibetan lifestyle usually results in close contact with both domestic animals and wildlife. Zoonotic diseases will be expected to have a higher prevalence in such communities. Strong religious beliefs, furthermore, do not easily allow for the elimination of stray dogs. These ecological and social factors contribute to the high prevalence and disease burden of both CE and AE in the inhabitants of the plateau.4,7

Previous epidemiologic studies in Shiqu County of Sichuan Province, China, situated on the eastern Tibetan plateau (32°19′–34°20′N, 97°20′–99°15′E), showed that domestic and stray dogs play a key role in the transmission of both E. granulosus and E. multilocularis to humans.7,8 Domestic dogs commonly feed on the offal of slaughtered sheep and other livestock with increased risk of infection with E. granulosus. Moreover, dogs allowed to roam free are at risk of infection with E. multilocularis when they prey on small mammals such as hares, pikas, and voles that live in the periphery of human communities.9,10 Necropsy and purgation surveys of dogs indicated that the prevalence of echinococcal infections fluctuated between 8.4 and 13.2% for E. granulosus and between 12.1 and 17.0% for E. multilocularis.5,9 Despite these high prevalences in dogs, no cases of mixed infections were documented in definitive or intermediate hosts until recently. During the course of canine purgation studies using arecoline hydrobromide, we noticed that some dogs seemed to harbor adult tapeworms of both E. granulosus and E. multilocularis.5,9,10 Furthermore, we found both unilocular and alveolar metacestodes in the liver of a plateau pika (Ochotona curzoniae). In this study, the polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) was developed to differentiate among E. granulosus, E. multilocularis, and E. shiquicus on the plateau. The PCR-RFLP method showed the dual infections of animal hosts with different Echinococcus spp.

Genomic DNA was purified from each metacestode lesion using DNeasy tissue kit (Qiagen, Hilden, Germany). Alkali-lyses from individual adult tapeworms were prepared as described previously.5 The adult lysate or the metacestode DNA was used as a template for PCR. The primer pair, Ech-LSU/F (5′-GGTTATTCTTTGCTTTCTTATCATGTG-3′) and Ech-LSU/R (5′-ATCACGTCAAACCATTCAAACAAGC-3′), was used to amplify an ~570-bp DNA fragment of mitochondrial gene (rrnL, large subunit of ribosomal RNA) containing a species-specific SpI restriction site (Figure 1). These primers were designed from the conserved regions of rrnL sequences among E. granulosus sensu stricto (accession no. AF297617), E. multilocularis (AB018440), and E. shiquicus (AB159140). PCR amplification was performed in a 25-μL reaction mixture containing 1 μL of templates, 200 μmol/L of each dNTP, 0.2 μmol/L of each primer, 0.5 unit of Taq polymerase (ExTag Hot Start Version; TaKaRa Bio-medicals, Tokyo, Japan), and the manufacturer-supplied buffer. Thermal reactions were performed for 35 cycles of 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 60 seconds. Without DNA purification, the amplicons were cleaved with SpI (New England BioLabs, Ipswich, MA) at 37°C for 2 hours. The restriction fragments were electrophoresed in 2% agarose gel and stained with ethidium bromide.

A total of 69 Tibetan Echinococcus isolates collected from Sichuan, Qinghai, and Gansu Province of China (5 adults and
The partial 18S rRNA gene of the genotype G6 (camel strain) is distributed together with *E. granulosus* sensu stricto and *E. multilocularis*. The partial rRNA gene of the genotype G6 could be amplified using the present primer set of Ech-LSU/F and Ech-LSU/R; however, it was difficult to differentiate between the genotype G6 and *E. granulosus* sensu stricto because both *Echinococcus* lacked *Ssp* I restriction sites inside the target gene fragment (M. Nakao and N. Xiao, unpublished data). The subsequent sequence analysis of the target gene revealed that a *Bgl* II restriction site is specific to the G6 genotype. In the endemic areas of northern China, an additional cleavage with *Bgl* II is necessary for the PCR-RFLP method to differentiate the genotype G6 from *E. granulosus* sensu stricto.

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