10. Panning M, Baumgarte S, Pfefferle S, Meier T, Martens A, Drosten C. Comparative analysis of rabies virus RT-PCR and virus isolation in rabies patient samples. J Clin Microbiol. 2010;48:2960–2. DOI: 10.1128/JCM.00728-10

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Wildlife-associated *Cryptosporidium fayeri* in Human, Australia

To the Editor: Molecular tools are essential for *Cryptosporidium* spp. identification, taxonomy, and epidemiology because of morphologic similarities between species within this genus. Molecular analyses have now identified 22 *Cryptosporidium* spp. and >40 cryptic species (i.e., genotypes) across all vertebrate classes (*1*). The myriad of potential *Cryptosporidium* spp. hosts, in conjunction with the robustness of the infectious stage (oocyst), means diverse *Cryptosporidium* spp. constantly circulate through the environment. This circulation increases the potential for disease from a diversity of contamination sources.

Human cryptosporidiosis is a global problem causing illness in young, elderly, immunocompromised, and immunocompetent persons in both industrialized and developing nations. The 2 most common etiologic agents, responsible for 90% of reported human infections, are *C. hominis* and *C. parvum* (*2*,*3*). Additional species identified as human pathogens are *C. meleagridis*, *C. canis*, *C. felis*, and the *Cryptosporidium* rabbit genotype (*4*). Each of these species was once thought to be specific for turkeys, dogs, cats, and rabbits, respectively. Incidental findings of *C. muris*, *C. andersoni*, *C. suis*, *C. hominis* monkey genotype, *C. parvum* mouse genotype, and *Cryptosporidium* cervine (W4), chipmunk I (W17), skunk, and horse genotypes have also been reported in humans (*4*). The pathogenicity of these zoonotic species and genotypes to humans remains unclear.

In July 2009, a 29-year-old woman who sought care because of prolonged gastrointestinal illness had a fecal test positive for *Cryptosporidium* spp. by the Remel ProSpecT *Giardia*/*Cryptosporidium* microplate assay (Thermo Fisher Scientific, Lenexa, KS, USA). Oocysts were purified from the specimen (*5*) and stained with the *Cryptosporidium* spp.–specific antibody CRY104 labeled with fluorescein isothiocyanate (Biotech Frontiers, North Ryde, Australia) for enumeration. A parasite load of 1.34 × 10^6 oocysts/g feces was determined by using epifluorescence microscopy at 400× magnification.

To identify *Cryptosporidium* spp., DNA was extracted (*5*), and a diagnostic fragment of the small subunit (SSU) rRNA) was amplified (*6*). Clones were screened to identify species and determine the possibility of mixed infection. Plasmids from 50 clones were recovered and digested with the enzyme *SspI* (New England Biolabs, Beverly, MA, USA) (*6*). Two different restriction profiles were visualized. The sequence from each of the restriction types was determined; profile 1 contained *SspI* fragment sizes of 33, 109, 247, and 441 bp; profile 2 had fragments of 33, 254, and 540bp. A BLAST search (www.ncbi.nlm.nih.gov/blast) confirmed the sequences as *C. fayeri* type 1 and type 2. These 2 sequences correspond to known heterogeneity within the SSU rRNA of *C. fayeri* (*7*).

The identification of *C. fayeri* by SSU rRNA was confirmed by the sequence of the actin gene (*8*), showing 99.8% similarity to *C. fayeri* (GenBank accession no. AF112570). Further analysis at the 60-kDa glycoprotein (gp60) locus was used to determine the *Cryptosporidium* subtype family (*5*). The MQ1022 gp60 sequence was 98% similar to *C. fayeri* subtype family IVa (*9*). Analysis of the microsatellite region further characterized isolate MQ1022 to *C. fayeri* subtype IVaA9G4T1R1. The nucleotide sequences generated in this study were submitted to GenBank under accession nos. HQ008932–HQ008934.

Because the patient was imunocompetent, the disease was believed to be self-limiting, and she was lost to follow-up. The patient resided in a national forest on the east coast of New South Wales, Australia, an area where marsupials are abundant. She had frequent contact with partially domesticated marsupials. Notably, *C. fayeri* has been identified in 6 Australian marsupial species. Identification of *C. fayeri* in a human patient is a concern for water catchment authorities in the Sydney region. The main water supply for Sydney, Warragamba Dam, covers 9,050 km^2 and is surrounded by national forest inhabited by diverse and abundant marsupials. A previous study that investigated *Cryptosporidium* spp. in a wild eastern gray kangaroo (*Macropus giganteus*) population reported a prevalence of 6.7% (*10*). Oocyst shedding ranged from 20/g feces to 2.0 × 10^6/g feces (*10*). Subtype IVaA9G4T1R1 identified from the patient in this study has been characterized from eastern gray kangaroos in Warragamba Dam (*9*). Throughout the year, large groups of eastern gray kangaroos graze within riparian zones in the catchment. Such close proximity to the water presents a high possibility that the dam's water is contaminated with oocysts from these animals.

The *Cryptosporidium* genus is diverse, both in species and suitable hosts. The mechanisms of host specificity remain unknown, but the frequency of *Cryptosporidium* spp. crossing the host barrier and becoming

zoonoses is increasing. This increase indicates that *Cryptosporidium* spp. host specificity is not as stringent as previously thought.

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References

1. Fayer R. Taxonomy and species delimitation in *Cryptosporidium*. Exp Parasitol. 2010;124:90–7. DOI: 10.1016/j.exppara.2009.03.005
2. Morgan-Ryan UM, Fall A, Ward LA, Hijjawi N, Sulaiman I, Fayer R, et al. *Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporididae) from *Homo sapiens*. J Eukaryot Microbiol. 2002;49:433–40. DOI: 10.1111/j.1550-7408.2002.tb00224.x
3. Xiao L, Ryan U. Cryptosporidiosis: an update in molecular epidemiology. Curr Opin Infect Dis. 2004;17:483–90. DOI: 10.1097/00001432-200410000-00014
4. Chalmers RM, Davies AP. Minireview: clinical cryptosporidiosis. Exp Parasitol. 2010;124:138–46. DOI: 10.1016/j.exppara.2009.02.003
5. Waldron LS, Ferrari BC, Power ML. Glycoprotein 60 diversity in *C. hominis* and *C. parvum* causing human cryptosporidiosis in NSW Australia. Exp Parasitol. 2009;122:124–7. DOI: 10.1016/j.exppara.2009.02.006
6. Xiao L, Morgan UM, Limor J, Escalante A, Arrowood M, Shulaw W, et al. Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl Environ Microbiol. 1999;65:3386–91.
7. Xiao L, Limor JR, Li L, Morgan U, Thompson RC, Lal AA. Presence of heterogeneous copies of the small subunit rRNA gene in *Cryptosporidium parvum* human and marsupial genotypes and *Cryptosporidium felis*. J Eukaryot Microbiol. 1999;46:44S–5S.
8. Sulaiman IM, Lal AA, Xiao L. Molecular phylogeny and evolutionary relationships of *Cryptosporidium* parasites at the actin locus. J Parasitol. 2002;88:388–94.
9. Power ML, Cheung-Kwok-Sang C, Slade M, Williamson S. *Cryptosporidium fayeri*: diversity within the gp60 locus of isolates from different marsupial hosts. Exp Parasitol. 2009;121:219–23. DOI: 10.1016/j.exppara.2008.10.016
10. Power ML, Sangster NC, Slade MB, Veal DA. Patterns of *Cryptosporidium* oocyst shedding by eastern grey kangaroos inhabiting an Australian watershed. Appl Environ Microbiol. 2005;71:6159–64. DOI: 10.1128/AEM.71.10.6159-6164.2005

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Canine Distemper Epizootic among Red Foxes, Italy, 2009

To the Editor: Canine distemper virus (CDV) is an enveloped, single-stranded, negative RNA virus of the family *Paramyxoviridae*, genus *Morbillivirus* (*1*). The host range for CDV is broad, and infection has been found in several mammalian species of the families Canidae, Mustelidae, Procyonidae, Ursidae, and Viverridae (*2*).

Stelvio National Park (SNP) encompasses 1,333 km^2 of protected land in Italy and covers 2 regions (Lombardia and Trentino Alto Adige); the Lombardia section of the park covers the northern part of Sondrio Province (Valtellina). SNP is surrounded by other parks (Schweitzer National Park, Adamello, and Adamello-Brenta) to form a large protected area (2,500 km^2) in the heart of the Alps mountains. Within SNP, the terrestrial mammals that are susceptible to CDV include red foxes (*Vulpes vulpes*), stoats (*Mustela erminea*), weasels (*Mustela nivalis*), pine martens (*Martes martes*), beech martens (*Martes foina*), badgers (*Meles meles*), and bears (*Ursus arctos*).

In August 2009, three young red foxes were captured in Valtellina (Sondrio), Lombardia, Italy, within the southwestern borders of SNP. The animals showed canine distemper–like signs (e.g., prostration, altered behavior, and conjunctivitis), and CDV infection was confirmed by quantitative reverse transcription–PCR of pooled organs (*3*). In September and October 2009, another 2 young foxes were captured and found to be positive for CDV. From September on, at least 30 foxes with altered behavior were seen near human habitations and facilities in SNP; 10 were captured. In the same period, infected foxes were also reported from Engadina, Switzerland, at the northern and western borders of SNP. In February 2010, two symptomatic foxes were euthanized in Grosotto, 50 km south of where the initial cases were identified. The epizootic appeared to have originated from the eastern regions of Italy (Trentino Alto Adige, and Veneto), where CDV infection had been reported in red foxes and badgers since August 2006 (*4*) (Figure). A large CDV epidemic in foxes in southern Bavaria in 2008 has also been described, thus suggesting spread of the virus throughout the Alps area (*5*).

Reverse transcription–PCR genotyping of the hemagglutinin (H) gene (*6*) identified 15 CDV strains, which were analyzed and characterized as European genotypes. The full-length H gene of the CDV strains was determined (GenBank accession no. HM120874). Sequence analysis of the H gene indicated that the fox CDV