

gastroenteritis. Despite the absence of known pathogens among 6 of 7 BuV-shedding patients, the causative role of BuV in gastroenteritis remains uncertain. Serologic studies will help clarify a possible association between BuVs and diarrhea or other diseases.

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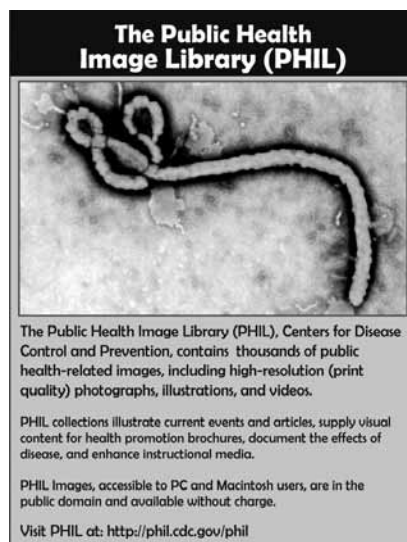
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References

- Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci U S A*. 2005;102:12891–6. <http://dx.doi.org/10.1073/pnas.0504666102>
- Jones MS, Kapoor A, Lukashov VV, Simmonds P, Hecht F, Delwart E. New DNA viruses identified in patients with acute viral infection syndrome. *J Virol*. 2005;79:8230–6. <http://dx.doi.org/10.1128/JVI.79.13.8230-8236.2005>
- Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS Pathog*. 2009;5:e1000391. <http://dx.doi.org/10.1371/journal.ppat.1000391>
- Kapoor A, Slikas E, Simmonds P, Chieochansin T, Naeem A, Shaikat S, et al. A newly identified bocavirus species in human stool. *J Infect Dis*. 2009;199:196–200. <http://dx.doi.org/10.1086/595831>
- Kapoor A, Simmonds P, Slikas E, Li L, Bodhidatta L, Sethabutr O, et al. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *J Infect Dis*. 2010;201:1633–43. <http://dx.doi.org/10.1086/652416>
- Phan TG, Vo NP, Bonkougou IJ, Kapoor A, Barro N, O’Ryan M, et al. Acute diarrhea in West African children: diverse enteric viruses and a novel parvovirus genus. *J Virol*. 2012;86:11024–30. <http://dx.doi.org/10.1128/JVI.01427-12>
- Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ, Qiu J, et al. The family *Parvoviridae*. *Arch Virol*. 2013 Nov 9 [Epub ahead of print]. <http://dx.doi.org/10.1007/s00705-013-1914-1>
- Antikainen J, Kantele A, Pakkanen SH, Laaveri T, Riutta J, Vaara M, et al. A quantitative polymerase chain reaction assay for rapid detection of 9 pathogens directly from stools of travelers with diarrhea. *Clin Gastroenterol Hepatol*. 2013;11:1300,1307.e3.
- Martin ET, Fairchok MP, Kuypers J, Magaret A, Zerr DM, Wald A, et al. Frequent and prolonged shedding of bocavirus in young children attending day-care. *J Infect Dis*. 2010;201:1625–32. <http://dx.doi.org/10.1086/652405>
- Kapusinszky B, Minor P, Delwart E. Nearly constant shedding of diverse enteric viruses by two healthy infants. *J Clin Microbiol*. 2012;50:3427–34. <http://dx.doi.org/10.1128/JCM.01589-12>

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Human Granulocytic Anaplasmosis Acquired in Scotland, 2013

To the Editor: Human granulocytic anaplasmosis is a tick-borne disease caused by *Anaplasma phagocytophilum*, an obligate intracellular gram-negative bacterium that infects granulocytes. The usual clinical signs and symptoms include nonspecific fever, chills, headache, and myalgia. Infection is usually mild or asymptomatic, but severe systemic complications can occur, leading to a need for intensive care and estimated fatality rates of 0.5%–1.0% (1,2).

A. phagocytophilum was first described in 1932 in Scotland as the causative agent of tick-borne fever in sheep (3). Although some clinical cases of human granulocytic anaplasmosis have been reported in Europe, mostly from Slovenia, Sweden, and Poland (4), most cases have occurred in the United States. This difference cannot be explained by the prevalence of the pathogen in ticks or human exposure to the pathogen because the 3% prevalence of *A. phagocytophilum* among *Ixodes ricinus* ticks in Europe seems to be nearly as high as that among ticks in the United States (2). The median seroprevalence rate for *A. phagocytophilum* infection among humans in Europe is 6.2%, reaching up to 21% (2). This incongruence between seroprevalence rate and number of human cases might be associated with underdiagnosis of cases (2), a high rate of asymptomatic disease (5), or cross-reactivities in serologic tests that might lead to overestimation of seroprevalence rate (5).

In August 2013, an immunocompetent 40-year-old man sought treatment for fever ($\approx 39^{\circ}\text{C}$) and other nonspecific symptoms such as malaise, myalgia, and severe headache 3 days after becoming aware of several tick bites received while on a hiking

vacation in Scotland. The man had removed the 3 tick nymphs from his legs immediately after their discovery and stored them in a plastic container; they were later sent to the Consultant Laboratory for Tick-borne Encephalitis in Berlin, Germany, for analysis. When the patient returned to Germany, 5 days after the onset of symptoms and 8 days after tick removal, a blood sample was collected (sample 1) and the bite sites were swabbed with a sterile cotton bud. By that time, the fever was gone, but malaise and other symptoms persisted. The patient began taking doxycycline, and within 2 days all symptoms subsided and the patient recovered completely.

A second blood sample was collected 28 days after tick removal (sample 2). Complete blood counts and chemistry panels were performed for both samples. All values were within the reference range except that for lactate dehydrogenase (248 U/L), which was moderately increased over the baseline value of <245 U/L in sample 1. Values did not differ substantially between the 2 samples.

DNA from whole-blood samples and swabs was extracted (QIAamp DNA Blood Mini Kit; QIAGEN, Hilden, Germany) and tested for *A. phagocytophilum*, *Babesia* spp., *Borrelia* spp., and *Rickettsia* spp. by using commercially available rapidSTRIFE assays for *Anaplasma*, *Babesia*, *Borrelia*, *Rickettsia* (all Analytik Jena AG; Jena, Germany). DNA extracted from blood and swab samples was negative for all tested pathogens.

After the tick specimens were taxonomically identified as *I. ricinus*, DNA/RNA was extracted (blackPREP Tick DNA/RNA Kit; Analytik Jena AG) and tested for the same pathogens. All 3 ticks were negative for *Babesia* spp., *Borrelia* spp., and *Rickettsia* spp., but 2 were positive for *A. phagocytophilum*.

Indirect immunofluorescence assays (Focus Diagnostics, Cypress, CA, USA) performed on the paired

serum samples revealed an increased *A. phagocytophilum*-specific IgM titer, from 20 at 5 days after symptom onset to 80 at 20 days later; the *A. phagocytophilum*-specific IgG titer rose from a high titer of 800 to >3,200 over this period.

The presence and 4-fold increase of *A. phagocytophilum*-specific IgM and IgG in paired serum samples confirmed the diagnosis of human granulocytic anaplasmosis in accordance with Centers for Disease Control and Prevention criteria (6). As described previously for several cases of human granulocytic anaplasmosis, patient blood counts were within reference limits but serum lactate dehydrogenase level was elevated (7). The diagnosis was further corroborated by detection of *A. phagocytophilum* DNA in 2 of the 3 ticks removed from the patient's skin. PCR amplification failed to detect *A. phagocytophilum* DNA in the patient's blood, consistent with previous studies documenting frequent lack of *A. phagocytophilum* DNA detection in whole blood and a substantial drop in PCR positivity after the acute phase of illness (8).

Human granulocytic anaplasmosis is not usually reported in Scotland like it is in the rest of Europe. The case originated from an area with long-established disease occurrence in ruminants, but the literature reports only 1 case of human infection in southwestern Scotland (9), ≈500 km from where this infection was probably acquired. Correct diagnosis would have been difficult had the patient not conserved the ticks and contacted the Consultant Laboratory for Tick-borne Encephalitis immediately after returning to Germany. A large number of human granulocytic anaplasmosis cases might be missed because general practitioners may not be aware of the pathogen's existence or its distribution.

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References

- Bakken JS, Dumler JS. Clinical diagnosis and treatment of human granulocytotropic anaplasmosis. *Ann N Y Acad Sci.* 2006;1078:236–47. <http://dx.doi.org/10.1196/annals.1374.042>
- Dumler JS, Choi KS, Garcia-Garcia JC, Barat NS, Scorpio DG, Garyu JW, et al. Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. *Emerg Infect Dis.* 2005;11:1828–34. <http://dx.doi.org/10.3201/eid1112.050898>
- Woldehiwet Z. The natural history of *Anaplasma phagocytophilum*. *Vet Parasitol.* 2010;167:108–22. <http://dx.doi.org/10.1016/j.vetpar.2009.09.013>
- Blanco JR, Oteo JA. Human granulocytic ehrlichiosis in Europe. *Clin Microbiol Infect.* 2002;8:763–72. <http://dx.doi.org/10.1046/j.1469-0691.2002.00557.x>
- Graf PC, Chretien JP, Ung L, Gaydos JC, Richards AL. Prevalence of seropositivity to spotted fever group rickettsiae and *Anaplasma phagocytophilum* in a large, demographically diverse US sample. *Clin Infect Dis.* 2008;46:70–7. <http://dx.doi.org/10.1086/524018>
- Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance. *MMWR Recomm Rep.* 1997;46(RR-10):1–55.
- Walder G, Fuchs D, Sarcelletti M, Berek K, Falkensammer B, Huber K, et al. Human granulocytic anaplasmosis in Austria: epidemiological, clinical, and laboratory findings in five consecutive patients from Tyrol, Austria. *Int J Med Microbiol.* 2006;296(Suppl 40):297–301. <http://dx.doi.org/10.1016/j.ijmm.2005.12.001>
- Bakken JS, Aguero-Rosenfeld ME, Tilden RL, Wormser GP, Horowitz HW, Raffalli JT, et al. Serial measurements of hematologic counts during the active phase of human granulocytic ehrlichiosis. *Clin Infect Dis.* 2001;32:862–70. <http://dx.doi.org/10.1086/319350>

9. Sumption KJ, Wright DJ, Cutler SJ, Dale BA. Human ehrlichiosis in the UK. *Lancet*. 1995;346:1487–8. [http://dx.doi.org/10.1016/S0140-6736\(95\)92502-3](http://dx.doi.org/10.1016/S0140-6736(95)92502-3)

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Genetic Relatedness of Dolphin Rhabdovirus with Fish Rhabdoviruses

To the Editor: Rhabdoviruses are enveloped, single-stranded, negative-sense RNA viruses that comprise a large and diverse family in the order *Mononegavirales* and infect arthropods, plants, fish, and mammals. There are 9 genera of rhabdoviruses: *Cytorhabdovirus*, *Ephemerovirus*, *Lysavirus*, *Novirhabdovirus*, *Nucleorhabdovirus*, *Perhabdovirus*, *Sigmavirus*, *Tibrovirus*, and *Vesiculovirus*. In addition, a substantial number of plant, vertebrate, and invertebrate rhabdoviruses have not been classified (1). Three genera (*Novirhabdovirus*, *Perhabdovirus*, and *Vesiculovirus*) comprise members that infect fresh water and marine fish (2). Fish rhabdoviruses pose a serious problem for aquaculture because of worldwide outbreaks of disease caused by novirhabdoviruses, perhabdoviruses, and vesiculoviruses (3,4).

In 1992, a rhabdovirus-like virus was isolated from lung and kidney of a white-beaked dolphin (*Lagenorhynchus albirostris*) that had beached along the coast of the Netherlands (5). Although no macroscopic or microscopic lesions were observed at necropsy, negative contrast electron microscopy showed typical rhabdovirus-like, bullet-shaped particles in

Vero cell cultures that showed a focal cytopathic effect (5). After this rhabdovirus-like virus was injected intracerebrally into brains of 1-day-old suckling mice, they died within 5 days (5). We report genetic and phylogenetic characterization of a dolphin rhabdovirus (DRV) and evaluated the seroprevalence of DRV-neutralizing antibodies by using serum samples from various marine mammals collected during a 10-year period (2003–2012).

To characterize DRV, we performed random sequence amplification and deep sequencing with the 454 GS Junior Instrument (Roche, Basel, Switzerland) with DRV-infected Vero cell supernatants as described (6). From this analysis, we determined the complete coding sequence of DRV covered by 42,080 of 49,292 reads (minimum coverage 4 reads, average coverage 872 reads).

Genomic termini of DRV were determined by using a 3' and 5' rapid amplification of cDNA ends PCR and Sanger sequencing of obtained PCR amplicons. The complete genome of DRV (GenBank accession no. KF958252) consists of 11,141 nt and has a typical rhabdovirus gene arrangement of 5 major open reading frames (ORFs) in the order 3'-nucleoprotein (N), phosphoprotein (P), matrix (M) protein, glycoprotein (G), and large (L) protein-5' (Figure, panel A, Appendix, wwwnc.cdc.gov/EID/article/20/6/13-1880-F1.htm). No additional ORFs ≥ 300 nt were detected. Between the major ORFs of DRV, intergenic sequences were present that ranged in size from 34 (P–M) to 83 (G–L) nucleotides. Putative transcription initiation and transcription termination polyadenylation sequences were AAC(A/G/U) and AUGA_n, respectively.

The deduced amino acid sequence of genes of DRV and several other rhabdoviruses were aligned by using MUSCLE in MEGA5 version 5.2 (7). Ambiguous aligned regions

were removed by using the Gblocks program (8). Phylogenetic analysis of the L and G genes was performed by using the neighbor-joining method in MEGA5 (7). This analysis showed that DRV is most closely related to fish rhabdoviruses of the genera *Perhabdovirus* and *Vesiculovirus* and unassigned fish rhabdoviruses with strong bootstrap support (Figure, panels B–D, Appendix).

Deduced amino acid sequences of the 5 major genes had the highest, although weak, homology with those of various fish rhabdoviruses by pairwise identity analyses: N (48%) with hybrid snakehead virus (HSHV), *Monopterus albus* rhabdovirus (MARV), and *Siniperca chautsi rhabdovirus* (SCRV); P (18%–20%) with eel virus European X (EVEX), HSHV, MARV, and SCRV; M (27%–33%) with lake trout rhabdovirus, Swedish sea trout rhabdovirus, and EVEX; G (30%–32%) with perch rhabdovirus, lake trout rhabdovirus, Swedish sea trout rhabdovirus, HSHV, MARV, SCRV, and EVEX; and L (54%–56%) with perch rhabdovirus, HSHV, and EVEX. This close relationship with fish rhabdoviruses is surprising because DRV was isolated from tissues of a mammal and propagated in mammalian cell lines at 37°C, which does not occur with related viruses isolated from fish.

To evaluate whether DRV or related viruses circulate among species of cetaceans, we performed serologic screening by using a virus neutralization assay as described (5). The specificity of this assay was tested by using a panel of rhabdovirus-specific antisera obtained from cetaceans of various species (5). The serum samples had been collected for diagnostic purposes from mainly juvenile cetaceans stranded along the coast of the Netherlands during 2003–2012. These species included 2 Atlantic white-sided dolphins (*Lagenorhynchus acutus*), 79 harbor porpoises (*Phocoena phocoena*), 9 striped dolphins (*Stenella*