

closest reported autochthonous human cases were in southwestern Hungary (5).

E. multilocularis tapeworms have been reported in foxes in western and central Croatia (6) and likely is in eastern areas, such as Vukovar, because it was found in 17.9% of foxes and 14.3% of golden jackals in the region of Serbia directly across the Danube River from Vukovar (7). Since 2013, rabies vaccination has increased in Croatia, which might give the fox population an opportunity to expand and increase transmission of *E. multilocularis* tapeworms to humans, as noted in Switzerland (8).

Correct diagnosis for this patient took 2.5 years because radiologic findings were inconsistent with cystic echinococcosis and clinicians assumed that was the only type of human echinococcosis in Croatia (9). This case highlights the need for clinicians to include alveolar echinococcosis in differential diagnosis of liver lesions. Imaging provides the first-line approach to such a diagnosis and serology provides strong complementary support. Our case also highlights the usefulness of considering pleural effusion and analyzing archival biopsies to retrospectively diagnose alveolar echinococcosis.

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References

1. Trachsel D, Deplazes P, Mathis A. Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. *Parasitology*. 2007;134:911–20. <https://doi.org/10.1017/S0031182007002235>
2. Stieger C, Hegglin D, Schwarzenbach G, Mathis A, Deplazes P. Spatial and temporal aspects of urban transmission of *Echinococcus multilocularis*. *Parasitology*. 2002;124:631–40. <https://doi.org/10.1017/S0031182002001749>
3. Schweiger A, Grimm F, Tanner I, Müllhaupt B, Bertogg K, Müller N, et al. Serological diagnosis of echinococcosis: the diagnostic potential of native antigens. *Infection*. 2012;40:139–52. <https://doi.org/10.1007/s15010-011-0205-6>
4. Deplazes P, Rinaldi L, Alvarez Rojas CA, Torgerson PR, Harandi MF, Romig T, et al. Global distribution of alveolar and cystic echinococcosis. *Adv Parasitol*. 2017;95:315–493. <https://doi.org/10.1016/bs.apar.2016.11.001>
5. Dezsényi B, Strausz T, Makrai Z, Csomor J, Danka J, Kern P, et al. Autochthonous human alveolar echinococcosis in a Hungarian patient. *Infection*. 2017;45:107–10. <https://doi.org/10.1007/s15010-016-0918-7>
6. Lalošević D, Lalošević V, Simin V, Miljević M, Čabrilo B, Čabrilo OB. Spreading of multilocular echinococcosis in southern Europe: the first record in foxes and jackals in Serbia, Vojvodina Province. *Eur J Wildl Res*. 2016;62:793–6. <https://doi.org/10.1007/s10344-016-1050-9>
7. Morović M. Human hydatidosis in Dalmatia, Croatia. *Epidemiol Infect*. 1997;119:271–6. <https://doi.org/10.1017/S0950268897007760>
8. Schweiger A, Ammann RW, Candinas D, Clavien PA, Eckert J, Gottstein B, et al. Human alveolar echinococcosis after fox population increase, Switzerland. *Emerg Infect Dis*. 2007;13:878–82. <https://doi.org/10.3201/eid1306.061074>
9. Beck R, Mihaljević Ž, Brezak R, Bosnić S, Janković IL, Deplazes P. First detection of *Echinococcus multilocularis* in Croatia. *Parasitol Res*. 2018;117:617–21. <https://doi.org/10.1007/s00436-017-5732-3>

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Two Cases of Newly Characterized *Neisseria* Species, Brazil

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We describe 2 human cases of infection with a new *Neisseria* species (putatively *N. brasiliensis*), 1 of which involved bacteremia. Genomic analyses found that both isolates were distinct strains of the same species, were closely related to *N. iguanae*, and contained a capsule synthesis operon similar to *N. meningitidis*.

Neisseria is a genus containing diverse organisms; most are rarely pathogenic. *N. meningitidis* and *N. gonorrhoeae* are the most clinically relevant species. The polysaccharide capsule is the most critical meningococcal virulence factor, a vaccine target, and the basis for classifying meningococci into serogroups (1). During routine laboratory-based public health surveillance in Brazil, we identified 2 cases of infection caused by a previously uncharacterized species of the *Neisseria* genus.

Clinicians reported 2 cases to the National Reference Laboratory, Adolfo Lutz Institute (IAL), São Paulo, Brazil. Case-patient 1 was a 64-year-old man from Rio Grande do Sul state, Brazil, who, in June 2016, had congestive heart failure with bilateral pulmonary infiltrates and pleural effusion on chest radiograph. Case-patient 2 was a 74-year-old woman with leprosy from Paraná state, Brazil, who, in February 2016, developed a polymicrobially infected ulcer of the left lower extremity. The 2 cases were separated in time and by >400 km and had no known epidemiologic link.

Overnight cultures of blood from case-patient 1 and ulcer exudate from case-patient 2 on brain-heart infusion agar containing 10% chocolate and horse blood at 37°C in the presence of 5% CO₂ both revealed brownish colonies uncharacteristic of *N. meningitidis*. We identified both isolates (N.95-16, from case-patient 1, and N.177-16, from case-patient 2) as gram-negative glucose-fermenting diplococci with positive catalase and oxidase tests. The isolates fermented maltose, lactose, sucrose, and fructose but not mannose; they reduced nitrate and produced a starch-like polysaccharide detected with Gram's iodine but did not produce DNase. Assessment by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy found no species match; the closest matches belonged to the *Neisseria* genus for both isolates.

We performed serogrouping by slide agglutination (2) with polyclonal goat or horse antisera prepared at IAL against the *N. meningitidis* capsule groups (ABCEWXYZ), as described previously (3), and confirmed with real-time PCR (4). Isolate N.95-16 had strong agglutination against serogroup X and nonspecific agglutination against serogroups A, B, C, W, Y, E, and Z antisera; isolate N.177-16 had nonspecific agglutination against A, B, C, W, X, Y, E, and Z antisera. Meningococcal serogroup-specific real-time PCR identified isolate 1 as *N. meningitidis* capsular group X and isolate 2 as capsular group B.

We extracted genomic DNA from overnight cultures and performed library preparation and whole-genome sequencing using a combination of Illumina MiSeq (<https://www.illumina.com>) and Oxford

Nanopore MinION (<https://nanoporetech.com>) technologies. Sequencing reads underwent hybrid assembly using Unicycler (5), which generated a high-quality draft assembly for isolate N.95-16 and a complete genome sequence for isolate N.177-16 (GenBank accession nos. WJXO00000000 and CP046027; PubMLST [<https://pubmlst.org>] identification 94178–94179). We performed species investigation by querying sequencing reads and assemblies against the GenBank and PubMLST reference databases (6). We aligned gene sequences corresponding to 53 conserved ribosomal MLST (rMLST) loci (7) across the *Neisseria* genus and constructed a maximum likelihood phylogenetic tree using RAxML with 1,000 bootstrap replicates (Figure, panel A). We calculated average nucleotide identity (ANI) using OrthoANI (8).

Both isolate genomes were 2.5 Mb and had 49.2% guanine-cytosine (GC) content. ANI was 99.3% between the 2 genomes and <86% relative to all other *Neisseria* species. The closest genome matches were *N. iguanae* and the proposed *N. weixii*, isolated from the intestinal contents of a Tibetan Plateau pika (Figure, panel A) (PubMLST identification 56407–56409; GenBank accession no. CP023429). Both genomes shared identical rMLST profiles (rST 61343); the 4 proposed *N. weixii* genomes shared only 1–2 alleles of 53 rMLST loci with these isolate genomes, and *N. iguanae* shared no rMLST alleles. Both genomes contained an intact capsule gene cluster (*cps*) that was similar in gene organization and sequence identity to *N. meningitidis* (Figure, panel B). The *ctrA-cssA/csxA* promoter region was conserved in both isolates. However, both genomes contained only 1 copy of *galE-rfbCAB* (Region D), compared with 2 copies found in meningococcal reference genomes; the *tex* gene was located >10 kb outside *cps*, upstream from *ctrD* (Figure, panel B). The 2 isolates differed in their sequence of sialic acid biosynthesis genes within region A; isolate N.95-16 contained *csxABC* genes that shared 98% amino acid identity with the meningococcal serogroup X reference strain α388 (1), and isolate N.177-16 contained *cssABC-csb* genes that shared 99% amino acid identity with serogroup B reference strain H44/76 (Figure, panel B). The *cps* differences observed between the isolates were similar to the mosaic recombination pattern associated with meningococcal capsular switching (9). Taken together, the presence of *cps* genes sharing substantial similarity to meningococcal homologs suggests that both isolates have the potential to synthesize meningococcal-like capsules.

In summary, we describe 2 sporadic cases of a new *Neisseria* species (which we propose to name

Neisseria brasiliensis), 1 of which also involved bacteremia. Both genomes contain an intact repertoire of genes for capsule synthesis, a key meningococcal virulence factor. The significance of capsule genes

and potential capsule synthesis in nonmeningococcal *Neisseria* is unknown (10). Continued surveillance is required to establish the pathogenic potential and host range for this apparent new species.

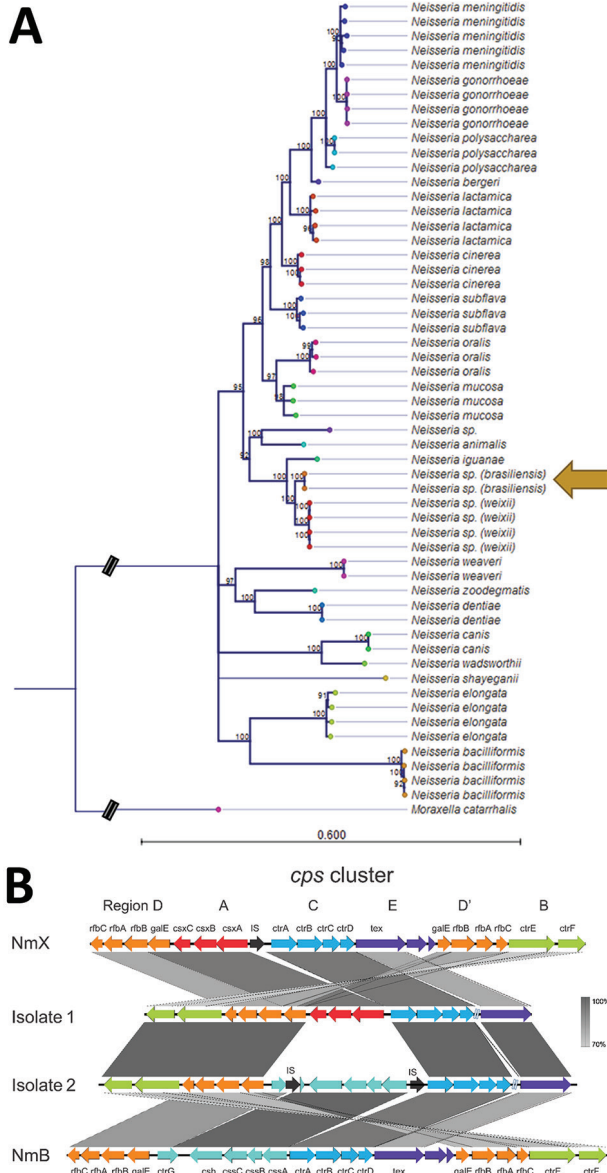


Figure. Analyses of newly characterized *Neisseria* species, Brazil. A) Maximum-likelihood phylogenetic tree of 53 aligned ribosomal multilocus sequence typing genes with 1,000 bootstrap replicates using *Moraxella catarrhalis* as an outgroup. Bootstrap support values <90% are not shown. Scale bar represents number of substitutions per site. B) *cps* sequences in isolates 1 (N.95-16) and 2 (N.177-16) relative to meningococcal reference genomes. Arrows represent genes, which are color coded by previously defined *cps* regions (1). Gray rectangles represent percentage nucleotide identity; darker shading indicates higher identity. The *tex* gene in both isolates is located >10 kb downstream of other *cps* genes. *cps*, capsule gene cluster; IS, insertion sequence; NmB, *N. meningitidis* serogroup B reference genome H44/76; NmX, *N. meningitidis* serogroup X reference sequence α388; IS, *transposase* gene.

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References

- Harrison OB, Claus H, Jiang Y, Bennett JS, Bratcher HB, Jolley KA, et al. Description and nomenclature of *Neisseria meningitidis* capsule locus. *Emerg Infect Dis.* 2013;19:566–73. <https://doi.org/10.3201/eid1904.111799>
- Castillo D, Harcourt B, Hatcher C, Jackson M, Katz L, Mair R, et al. Laboratory methods for the diagnosis of meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*, second edition [cited 2019 Dec10]. [https://www.cdc.gov/meningitis/lab-manual/index.html#/eref>](https://www.cdc.gov/meningitis/lab-manual/index.html#/)
- Alkmin MG, Shimizu SH, Landgraf IM, Gaspari EN, Melles CE. Production and immunochemical characterization of *Neisseria meningitidis* group B antiserum for the diagnosis of purulent meningitis. *Braz J Med Biol Res.* 1994;27:1627–34.
- Mothershed EA, Sacchi CT, Whitney AM, Barnett GA, Ajello GW, Schmink S, et al. Use of real-time PCR to resolve slide agglutination discrepancies in serogroup identification of *Neisseria meningitidis*. *J Clin Microbiol.* 2004;42:320–8. <https://doi.org/10.1128/JCM.42.1.320-328.2004>
- Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLOS Comput Biol.* 2017;13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>
- Bennett JS, Jolley KA, Earle SG, Corton C, Bentley SD, Parkhill J, et al. A genomic approach to bacterial taxonomy: an examination and proposed reclassification of species within the genus *Neisseria*. *Microbiology.* 2012;158:1570–80. <https://doi.org/10.1099/mic.0.056077-0>
- Jolley KA, Bliss CM, Bennett JS, Bratcher HB, Brehony C, Colles FM, et al. Ribosomal multilocus sequence typing: universal characterization of bacteria from domain to strain. *Microbiology.* 2012;158:1005–15. <https://doi.org/10.1099/mic.0.055459-0>
- Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol.* 2016;66:1100–3. <https://doi.org/10.1099/ijsem.0.000760>
- Mustapha MM, Marsh JW, Krauland MG, Fernandez JO, de Lemos APS, Dunning Hotopp JC, et al. Genomic

investigation reveals highly conserved, mosaic, recombination events associated with capsular switching among invasive *Neisseria meningitidis* serogroup W sequence type (ST)-11 strains. *Genome Biol Evol.* 2016;8:2065–75. <https://doi.org/10.1093/gbe/evw122>

10. Clemence MEA, Maiden MCJ, Harrison OB. Characterization of capsule genes in non-pathogenic *Neisseria* species. *Microb Genom.* 2018;4. <https://doi.org/10.1099/mgen.0.000208>

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Hepatitis A Virus Genotype IB Outbreak among Internally Displaced Persons, Syria

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In 2018, a hepatitis A virus outbreak was identified among internally displaced persons in Syria. Sequence analysis based on the viral protein 1/2A junction revealed that the causative virus belonged to genotype IB. A high displacement rate, deteriorated sanitary and health conditions, and poor water quality likely contributed to this outbreak.

Hepatitis A virus (HAV) is the leading cause of acute hepatitis infections worldwide, infecting ≈1.5 million persons annually (1). Symptoms, which are usually mild, include nausea, vomiting, abdominal pain, restlessness, body weakness, myalgia, loss of appetite, and fever. However, HAV may progress into fulminant liver failure, necessitating liver transplant. Generally, HAV is self-limiting (2). HAV (genus *Hepatovirus*, family *Picornaviridae*) is a nonenveloped virus with a single-stranded, positive-sense

RNA linear genome (7.5 kb). The viral protein (VP) 1/2A junction (168 nt) is used to classify HAV into 6 genotypes: I–III (subgenotypes A and B) of human origin and IV–VI of simian origin (3). Genotype IA is the most commonly reported worldwide, whereas genotype IB is predominant in the Middle East (4–6).

On September 9, 2018, the governorate of Aleppo, Syria, informed the World Health Organization office in Syria that internally displaced persons (IDPs; displaced since early 2018) and local host community members in Tal Refaat, Fafin, and surrounding areas in the northwestern and western parts of Aleppo were experiencing a suspected hepatitis outbreak. The affected area included 17 locations in Azaz and Jabal Sem'an districts in western Aleppo (Appendix, <https://wwwnc.cdc.gov/EID/article/26/2/19-0652-App1.pdf>). Outbreak field investigation found sporadic cases of the disease among IDPs starting July 21, 2018; as of November 8, a total of 638 cases of suspected acute hepatitis infection had been reported. Most patients (98.59%) were <15 years of age and the rest 16–54 years of age. A total of 105 patients (16.5%) were admitted into the Fafin hospital; no fatalities were reported. No field investigations were performed in the first half of 2018 because of the crisis that led to weakness in the routine surveillance system.

A total of 48 unidentified serum and plasma samples were collected from 24 IDP children with suspected hepatitis and sent to the laboratory on October 29. The specimens originated from 3 locations in Syria: 13 from Fafin camp in Aleppo, 6 from eastern rural Daraa, and 5 samples from rural Quneitra. Even though the main outbreak was in the Aleppo governorate, Daraa and Quneitra were also experiencing a notable upsurge in reported cases of suspected acute hepatitis infection. For this reason, additional samples were collected from these governorates.

We analyzed the serum specimens by serology (total HAV antibodies and HAV IgM) using the enzyme-linked fluorescent assay VIDAS (bioMérieux Diagnostics, <https://www.biomerieux-diagnostics.com>) and the plasma specimens by real-time reverse transcription PCR (RT-PCR) for the detection of HAV (using the HAVNET protocol) and hepatitis E virus (HEV) (7). Seven samples had insufficient volume to perform both total HAV antibody and HAV IgM tests; thus, only the IgM test was performed. Overall, 19 plasma specimens were positive for HAV and none for HEV by PCR (Table). Eighteen serum specimens had detectable HAV IgM. All the specimens with sufficient volume (n = 17) were positive for total HAV antibodies. Of these, 5 were from past infections, as indicated by the negative HAV PCR and HAV IgM