about the patient, who, unfortunately, died due to complications.

Because routine testing did not lead to a diagnosis, a BAL sample and a viral culture were sent to the University Hospital Leuven (Leuven, Belgium). The cell culture was subjected to next-generation sequencing (NGS) with the Novel enrichment technique of ViRomes protocol (5). This protocol includes purification of viral particles, nucleic acid extraction, and random PCR amplification, followed by library preparation and sequencing using NextSeq 500 system NGS technology (Illumina, San Diego, CA, USA) (6). The sequence raws obtained were filtered and trimmed, de novo assembled, and taxonomically classified (7,8). Analysis of the NGS results identified a complete West Nile virus genome (11,060 nt). To confirm this result, a novel RNA extraction and a reverse transcription PCR (Qiagen, Venlo, the Netherlands) with newly developed primers were performed on the BAL sample and cell culture, followed by Sanger sequencing (9). The sequence obtained from the positive sample was identical to the sequence from the complete genome obtained by NGS.

The WNV lineage 2 strain in this study, WNV-2(Belgium2017/Antwerp (GenBank accession number: MH021189), is closely related (99.4%–99.6% nucleotide similarity) to a strain from Hungary and other Balkan countries identified between 2010 and 2015 (Figure), reinforcing the conclusion that this WNV infection was acquired in Hungary. This case illustrates that the combined use of cell cultures and NGS can be a powerful tool for identifying unknown pathogens in clinical specimens when results from routine tests are negative and the patient’s condition is undiagnosed.

About the Author
Elke Wollants currently works as laboratory manager in the Clinical Virology Lab at the Rega Institute, KU Leuven. Her research interests are identifying pathogens not detected in routine diagnostic laboratory tests and next-generation sequencing.

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Address for correspondence: Elke Wollants, Rega Institute, KU Leuven, Laboratory of Clinical Virology, Herestraat 49 box 1040 Leuven 3000, Belgium; email: elke.wollants@kuleuven.be

Disseminated Spiroplasma apis Infection in Patient with Agammaglobulinemia, France

Nicolas Etienne, Laurent Bret, Cécile Le Brun, Hervé Lecuyer, Josquin Moralys, Fanny Lanternaire, Olivier Hermine, Agnés Ferroni, Marc Lecuit, Sabine Pereyre, Laure Beven, Olivier Lortholary

Author affiliations: Paris Descartes University Hospital, Paris, France (N. Etienne, H. Lecuyer, F. Lanternaire, O. Hermine, A. Ferroni, M. Lecuit, O. Lortholary); Centre Hospitalier Régional,
We report a disseminated infection caused by *Spiroplasma apis*, a honeybee pathogen, in a patient in France who had X-linked agammaglobulinemia. Identification was challenging because initial bacterial cultures and direct examination by Gram staining were negative. Unexplained sepsis in patients with agammaglobulinemia warrants specific investigation to identify fastidious bacteria such as *Spiroplasma* spp.

In February 2017, a 40-year-old man in France who was under immunoglobulin replacement therapy for X-linked agammaglobulinemia experienced a migrating nonitchy papular eruption. Skin biopsy revealed nonspecific lymphocytic dermatitis. One month later, he experienced distal interphalangeal arthritis. Histology of a synovial biopsy sample found a nonspecific lymphocytic infiltrate. Bacterial cultures were negative. In April 2017, febrile arthritis of the left ankle and right knee appeared, along with bilateral wrist and finger tenosynovitis (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/12/18-0567-Techapp1.pdf). We initiated ceftriaxone (1 g/d) and performed a left ankle joint aspiration 24 hours later. The patient remained febrile with a pulmonary valve murmur of tricuspid regurgitation and purpuric lesions, tenosynovitis in the left ankle and both wrists, and right knee and interphalangeal polyarthritis. Serum C-reactive protein was 136 mg/dL and IgG serum level 9.4 g/L. Transesophageal echography showed slight tricuspid regurgitation. Arthritis worsened, and we performed a right knee joint aspiration. Four of 6 blood cultures were positive after 55 h of incubation at 37°C (Bactec FX; Becton Dickinson, Franklin Lakes, NJ, USA). Results of direct examination by Gram staining were negative. Unexplained sepsis in patients with agammaglobulinemia warrants specific investigation to identify fastidious bacteria such as *Spiroplasma* spp. and culture) and *Ureaplasma* spp. and *Mycoplasma hominis* (by culture) in urine were negative. We seeded synovial fluid on blood culture, and small colonies developed on horse blood agar. We extracted DNA from the zone of hemolysis using EZ1 DNA tissue kit (QIAGEN, Valencia, CA, USA). We amplified and sequenced the 16S rRNA gene with the primer set 27F/16S1RRB as previously described (1) and performed bacterial identification using phylogenetic analysis on the Bioinformatics Bacterial Identification tool (2). The 491-bp amplification sequence was 100% identical to that of *Spiroplasma apis* strain ATCC 33834 (GenBank accession no. GU993267). We confirmed identification of *S. apis* by specific *S. apis* PCR (3) and culture (on modified SP4 agar) in synovial fluid from left ankle and right knee and in blood culture. Direct examination of synovial fluid and blood culture with dark field microscopy allowed identification of bacteria with a helical morphology and motility evocative of spiroplasmas (Figure). We obtained highly enriched *Spiroplasma* cultures starting from fluid and blood cultures. We retrospectively found specific PCR for *S. apis* positive in synovial biopsy from the distal interphalangeal sample. Antimicrobial drug susceptibility testing showed susceptibility to macrolides, tetracyclines, and fluoroquinolones and resistance to rifampin, cotrimoxazole, and penicillin G. Tetracycline and fluoroquinolone MIC values were similar to those reported for *Ureaplasma* spp.

Patient’s condition gradually improved with 6-week administration of levofloxacin and iterative joint aspirations of the right knee. Because of severe tendinopathy, levofloxacin was stopped after 6 weeks and replaced by azithromycin (500 mg/d) and doxycycline (200 mg 2×/d) for 6 weeks.

Patients with agammaglobulinemia are susceptible to infections, including mycoplasma infections (4). Invasive infections such as endocarditis and septic arthritis have been reported with *M. hominis* and *Ureaplasma urealyticum* in hypogammaglobulinemic patients. Of note, our patient contracted mycoplasma infection despite an appropriate gammaglobulin serum level (IgG 9.4 g/L).

 Spiroplasmas, arthropod-infecting bacteria of the class Mollicutes, are similar to mycoplasmas. Pathogenic species include *S. apis*, detected in hives in southwestern France (5). *S. apis* is associated with the lethal May disease of the European honeybee *Apis mellifera*. Its prevalence in bee colonies in France was not reported but was recently shown to reach high percentages in other countries (6). Only 3 cases of human infection due to *Spiroplasma* spp. have been reported: hepatitis in a lung transplant recipient (7), uveitis in a premature neonate (8), and bacteremia in a patient under ceftriaxone treatment (9). None of these infections correlated with any noticeable insect bite history. In contrast, the patient we report had multiple stings by a flying insect resembling a hornet in August 2016, in the Loiret department of France, leading to cutaneous blebs and transitory rash. This finding raises the possibility that *S. apis* might infect insects other...
than honeybees. The insect stings in this patient are a likely gateway of the reported infection.

In summary, clinicians and microbiologists should be aware of fastidious organisms in atypical infections in immunocompromised patients. Our findings indicate a need for prolonged culture on specific agar on all joint fluids in patients with agammaglobulinemia and targeted molecular methods to identify \textit{S. apis} organisms.

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**About the Author**

Dr. Etienne is an infectious disease clinician and a clinical immunologist in Necker-Pasteur Center for Infectious Diseases, Paris. His research interest focuses on infections in immunocompromised patients.

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Address for correspondence: Olivier Lortholary, Necker-Enfants Malades University Hospital, 149 rue de Sevres 75743, Paris CEDEX, France; email: olivier.lortholary@aphp.fr

**Mycoplasma ovipneumoniae** in Wildlife Species beyond Subfamily Caprinae

Margaret A. Highland, David R. Herndon, Scott C. Bender, Lisa Hansen, Robert F. Gerlach, Kimberlee B. Beckmen

Author affiliations: Washington Animal Disease Diagnostic Laboratory, Pullman, Washington, USA (M.A. Highland); US Department of Agriculture, Pullman (M.A. Highland, D.R. Herndon); Navajo Technical University, Crownpoint, New Mexico, USA (S.C. Bender); Barron Veterinary Clinic, Barron, Wisconsin, USA (L. Hansen); Alaska Department of Environmental Conservation, Anchorage, Alaska, USA (R.F. Gerlach); Alaska Department of Fish and Game, Fairbanks, Alaska (K.B. Beckmen)

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