evidence of active disease among these animals. However, pigs in porcine farms not deemed of high-health status are regularly colonized by *S. suis* (*3*). Most cases of *S. suis* disease in humans have been linked to accidental inoculation through skin injuries (*3*). The patient reported that in the days before his hospitalization, a pig died unexpectedly, and he removed it from the pen without using protective equipment such as gloves or safety glasses. However, there was no indication that this animal had died of a *S. suis* infection. The patient also described transient worsening of his bilateral hearing loss during hospitalization. Hearing loss from *S. suis* infection occurs frequently (*9*).

S. suis zoonotic disease has emerged in Asia and occurs frequently in Europe among persons in close contact with pigs (*3*). In contrast, only 8 human *S. suis* cases have been reported in Canada and the United States, which together are the second largest swine producers worldwide (*3*). This lower number of cases may be related to the lower virulence of *S. suis* serotype 2 genotypes circulating in North America (ST25 and ST28) in comparison to serotype 2 genotypes circulating in Europe and Asia (ST1, ST7) (*4*,*6*,*7*). However, *S. suis* infections may be underdiagnosed in North America. Our data and previous reports (*10*) show that the organism is sometimes misidentified as other α-hemolytic streptococci by commercial identification systems. Here, the isolate was initially identified as viridans group *Streptococcus*, and only the use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry at the reference laboratory permitted correct identification. While proper identification was unlikely to have led to a different treatment course in this case, our report underscores the need to increase awareness of *S. suis* as a potential agent of human infections and serves as a reminder to routinely query patients about animal contact, particularly in areas with intensive pig farming operations.

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Moku Virus in Invasive Asian Hornets, Belgium, 2016

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We report the detection of Moku virus in invasive Asian hornets (*Vespa velutina nigrithorax*) in Belgium. This constitutes an unexpected report of this iflavirus outside Hawaii, USA, where it was recently described in social wasps. Although virulence of Moku virus is unknown, its potential spread raises concern for European honeybee populations.

With their work estimated to have a global economic value of €153 billion, insects are critical pollinators of crops in agriculture, with the honeybee (*Apis mellifera*) being by far the major player in this process (*1*). Honeybee populations are decreasing dramatically worldwide, however, threatening food security. Environmental changes, pesticides, pathogens, and parasitic species are all recognized drivers of this decline (*2*). Among these, the Varroa mite (*Varroa destructor*) has been shown to have a critical effect on honeybee populations, both by its direct parasitic effects and through the transmission of pathogenic viruses such as deformed wing virus (*2*).

The Asian yellow-legged hornet (*Vespa velutina nigrithorax*), a natural predator of honeybees, has a native range spanning from India through China and as far as Indonesia (*3*). It is a particularly efficient invader because of its distinctive biology and behavior (*4*,*5*). The hornet was accidentally introduced from China into Europe, with sightings in France in 2004, and has rapidly spread to neighboring countries, including Belgium, since 2011 (*6*). In invaded areas, hornets' feeding sites are primarily apiaries, which present an attractive, abundant, and defenseless prey source (*5*). *V. velutina nigrithorax* hornets not only contribute by hunting to the loss of honeybee colonies but also interact with the honeybees and can act as viral reservoirs, as *V. destructor* mites do, and infect the bees through spillover events (*7*,*8*). To explore the possibility of transmission of viruses from these hornets to honeybees, we performed a viral metagenomic analysis of Asian hornets collected in Belgium in 2016.

We submitted a pool of 5 female and 5 male adult *V. velutina nigrithorax* hornets collected in Belgium in November 2016 to a viral metagenomics analysis by next-generation sequencing (detailed methods in online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/12/17-1080-Techapp1.pdf). A blastx (https://blast.ncbi.nlm.nih.gov/Blast.cgi) alignment to GenBank viral sequences enabled the assignment of

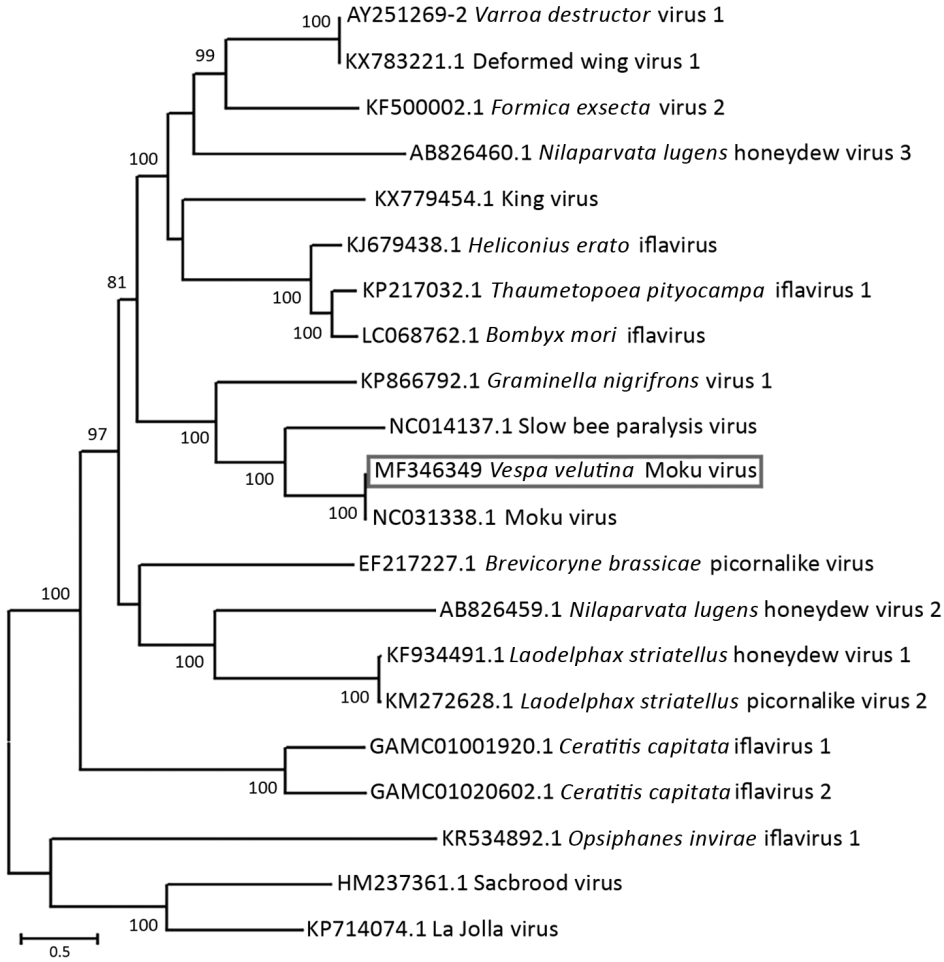

Figure. Evolutionary relationships of Moku virus generated from a pool of 5 female and 5 male Asian hornets (*Vespa velutina*) collected in Belgium in 2016 (box) compared with representative members of the genus *Iflavirus*, based on the maximum-likelihood phylogeny of the polyprotein sequences. The phylogenetic analysis was performed using MEGA6 (*10*) and the LG substitution model, as determined by a model selection analysis. Bootstrap percentages >70% (from 500 resamplings) are indicated at each node. GenBank accession numbers are indicated for each species. Scale bar indicates amino acid substitutions per site.

most viral sequences to phages (not represented) and viruses of the *Partitiviridae* and *Parvoviridae* (*Densovirinae*) families (online Technical Appendix Figure 1); however, a few reads pointed to a member of the *Iflaviridae* family, which contains such notable bee pathogens as deformed wing virus and slow bee paralysis virus (*9*). blastn alignment showed a positive match to Moku virus (*9*). Template-based assembly using Moku virus (GenBank accession no. KU645789) (*9*) permitted a near-full genome reconstruction from 1,215 matching reads out of 4,587,801. We used primer walking PCR and Sanger sequencing to fill the gaps in the genome (online Technical Appendix Figure 2).

The full viral genome sequence we obtained is 10,032 nt in length (GenBank accession no. MF346349) and has a mean nucleotide identity of 96.0% to the Hawaiian Moku virus strain (accession no. KU645789) (*9*), with both viruses showing an open reading frame of the same length (9,153 nt) sharing an amino acid identity of 99.0%. We performed an alignment to the full translated polyprotein amino acid sequence of representative iflaviruses available in GenBank using the Muscle aligner implemented in Geneious version 8.1.8 (Biomatters, Auckland, New Zealand). A maximum-likelihood phylogenetic analysis performed on the full-length polyprotein sequence yielded comparable results to that obtained on a conserved region of the RNA-dependent RNA polymerase (*9*), confirming the high identity of the Moku virus we obtained from the *V. velutina nigrithorax* hornet pools with the Hawaiian isolate of Moku virus, as well as its proximity to slow bee paralysis virus (Figure).

Our results show a large diversity of viruses in invasive Asian hornets collected in Belgium in 2016. Among these, we detected an iflavirus with high identity to the recently described Moku virus found in social wasps (*Vespula pensylvanica*), honeybees, and Varroa mites in Hawaii (*9*). Such a high nucleotide identity unequivocally places both strains in a single species. The potential pathogenicity of Moku virus for honeybees is currently unknown, but its relatively close relationship with the highly virulent slow bee paralysis virus warrants further studies (*9*). There is an urgent need to assess the presence of Moku virus in honeybees and *Varroa* mites in areas of Europe where the Asian hornet has become endemic, such as several regions in France. As highlighted by Mordecai et al. (*9*), the carriage of Moku virus in *V. destructor* mites in Hawaii is of great concern given the role played by this mite in the maintenance and transmission of viruses, including the deformed wing iflavirus, to honeybees. Furthermore, although Moku virus was shown to be highly dominant among viral species infecting *V. pensylvanica* wasps (*9*), suggesting that this species is a likely reservoir of the virus, we could not establish the same relationship for the Asian hornet *V. velutina*, in which *Partitiviridae* were much more abundant. It remains to be determined whether Moku virus is a virus of *Vespulidae* or, more likely given the relatively low number of reads detected, could have been picked up by these hornets from their prey, such as honeybees. Further studies are needed to establish the origin, host range, and transmission route of Moku virus; its virulence; and the risks it may represent for European honeybee populations.

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Angiostrongylus cantonensis DNA in Cerebrospinal Fluid of Persons with Eosinophilic Meningitis, Laos

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Definitive identification of *Angiostrongylus cantonensis* parasites from clinical specimens is difficult. As a result, regional epidemiology and burden are poorly characterized. To ascertain presence of this parasite in patients in Laos with eosinophilic meningitis, we performed quantitative PCRs on 36 cerebrospinal fluid samples; 4 positive samples confirmed the parasite's presence.

Humans are incidental hosts of *Angiostrongylus cantonensis* nematodes; global distribution of these nematodes is being increasingly recognized (*1*). Ingestion of larvae from undercooked infected snails or food contaminated with mollusk secretions can result in the migration of *A. cantonensis* parasites through the human central nervous system (CNS) (*2*). The presence of the parasite and associated inflammation in the CNS can contribute to a meningoencephalitic syndrome, typified by a cerebrospinal fluid (CSF) eosinophilia constituting ≥10% of total CSF leukocyte count. Formal diagnosis of angiostrongyliasis is difficult because the parasite is typically present in low numbers within the CSF (*3*). Serologic methods are limited by cross-reactivity with other helminths (*4*), and antibody-based methods may lack sensitivity, especially during acute illness (*5*).

Host sampling studies have identified *A. cantonensis* parasites in some Mekong region countries but not in Lao People's Democratic Republic (Laos) (*6*). To ascertain the presence of this parasite in patients with eosinophilic meningitis in Laos, we tested samples from a cohort of 1,065 patients suspected of having CNS infection at Mahosot Hospital, Vientiane, Laos, during 2003–2013 by Giemsa staining and identified 54 CSF samples containing ≥10% eosinophils. Of these, 36 samples from 35 patients were available for this study (1 patient underwent lumbar puncture twice) and were tested by conventional and quantitative PCR (cPCR and qPCR). From the same cohort, we also performed qPCR testing on another 50 CSF samples with 1%–9% eosinophils.

DNA was extracted from 200 μL of CSF by using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) and eluted in 30 μL buffer. We ran the extract in parallel with positive samples (*A. cantonensis* DNA from experimentally infected rats, University of Sydney, Sydney, Australia) and negative controls by using published cPCR (*7*) and qPCR (*8*) protocols. We used Platinum PCR SuperMix (Thermo Fisher, Waltham, MA, USA) and performed assays on a Bio-Rad CFX96 (Bio-Rad, Watford, UK).

Among patients with CSF eosinophilia ≥10%, male patients predominated, although sex of patients did not differ significantly among patients with or without CSF eosinophilia (Table). Of the 36 CSF samples that contained ≥10% eosinophils, all were negative by cPCR, but 4 (11.1%) were positive for *A. cantonensis* DNA by qPCR; median quantification cycle was 35.9 (range 34.1–37.4). Sensitivity of qPCR was apparently higher than that of cPCR. The median duration of illness for patients with positive qPCR was 4 (range 0–10) days. Of 3 patients with a positive qPCR, 2 reported that they had eaten raw snails in the previous month.

Results from 2 CSF samples obtained from the same patient and tested by qPCR were discordant; CSF obtained after 7 days of illness was negative for *A. cantonensis* DNA, but a sample obtained on day 13 was positive (quantification cycle 34.1). This finding is consistent with previous observations (*8*), and it is plausible that during the acute stages of infection, insufficient nucleic material is present for detection. Although lumbar puncture is invasive, a high clinical suspicion of angiostrongyliasis in the context of a negative qPCR may therefore warrant a repeated lumbar puncture. All positive samples had CSF eosinophil proportions >40%, and all samples containing a 1%–9% eosinophil proportion tested negative by qPCR, supporting the conventional cutoff of a CSF eosinophilia ≥10% in the diagnosis of CNS angiostrongyliasis.

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Moku Virus in Invasive Asian Hornets, Belgium, 2016

Technical Appendix

Method and Results

A pool of 5 female and 5 male adult hornets collected in Belgium in November 2016 were homogenized and submitted to a viral enrichment step and total nucleic acid extraction, as described previously (*1*,*2*). Libraries for Ion Torrent PGM (Life Technologies, Ghent, Belgium) and Illumina MiSeq (Illumina, San Diego, USA) sequencing were prepared and the run and data analysis were performed as described previously (*1*,*3*) but using the Nextera XT DNA Library Prep Kit for MiSeq sequencing (Illumina, San Diego, USA), according to manufacturer's instructions. We performed viral genome assembly and sequence analysis with Geneious v8.1.8 (Biomatters, Auckland, New Zealand).

We obtained partial genomes or coding sequences of 6 additional viruses by de novo assembly from NGS reads using Geneious v8.1.8. These highly divergent viruses detected by a BLASTX analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were tentatively called *Vespa velutina* partiti-like virus 1 and 2; *Vespa velutina* unclassified RNA virus 1, 2, and 3; and *Vespa velutina* densovirus 1 (Genbank accession nos. MF346350–MF346355). The pathogenic potential of these viruses remains to be determined, but at least the first 2 (*Vespa velutina* partiti-like virus 1 and 2) were relatively abundant in the samples, with 12,621 and 28,126 reads, respectively, matching the partial genomes obtained. Whether these viruses are specific to *Vespa velutina* hornets or have a broader host spectrum, potentially including honeybees, also remains unknown and would deserve further investigations.

We used PCR targeting a 658-bp fragment of the mitochondrial gene cytochrome C oxydase subunit I (COI) (*4*) to determine the origin of *Vespa velutina* hornets found in Belgium. Two Asian hornets collected in Belgium in 2016 (A and B) and 1 collected in France (C) were submitted to the PCR analysis and sequenced using Sanger technology (GenBank accession nos. MF363127–MF363129). All sequences were of the same haplotype (haplotype F) as the

European (GenBank accession no. KX712225) and Jiangsu/Shejiang Chinese (GenBank accession no. JQ780450) isolates (*5*). Although it was obtained on a limited number of insects (N = 3), this result shows that the Asian hornets analyzed in this study share a common origin with the other *Vespa velutina* hornets collected in Europe so far (*5*) and do not belong to a different subset.

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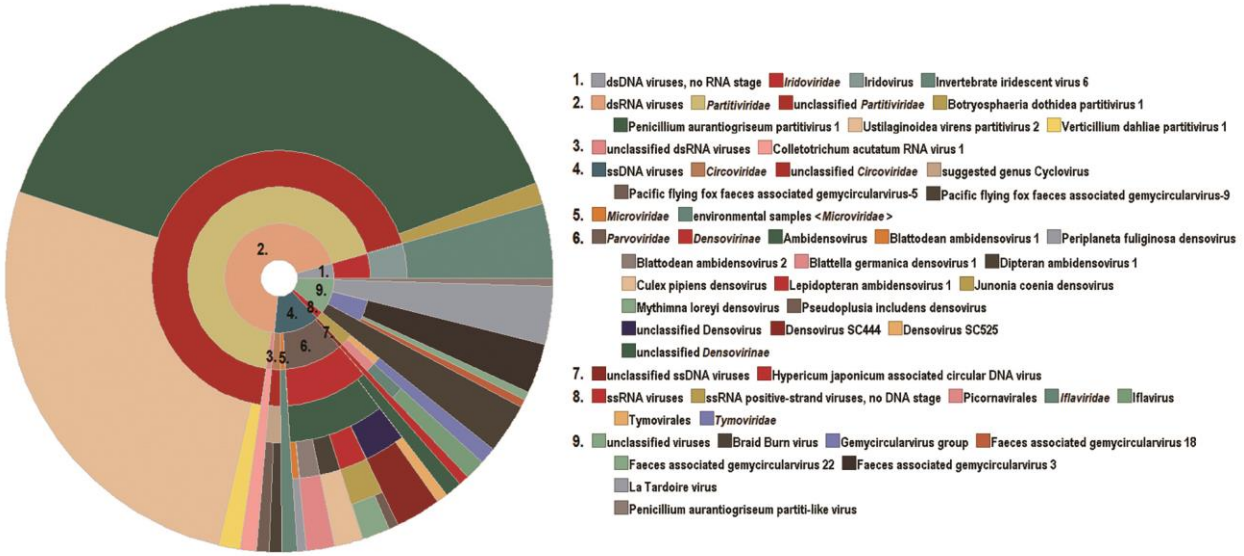
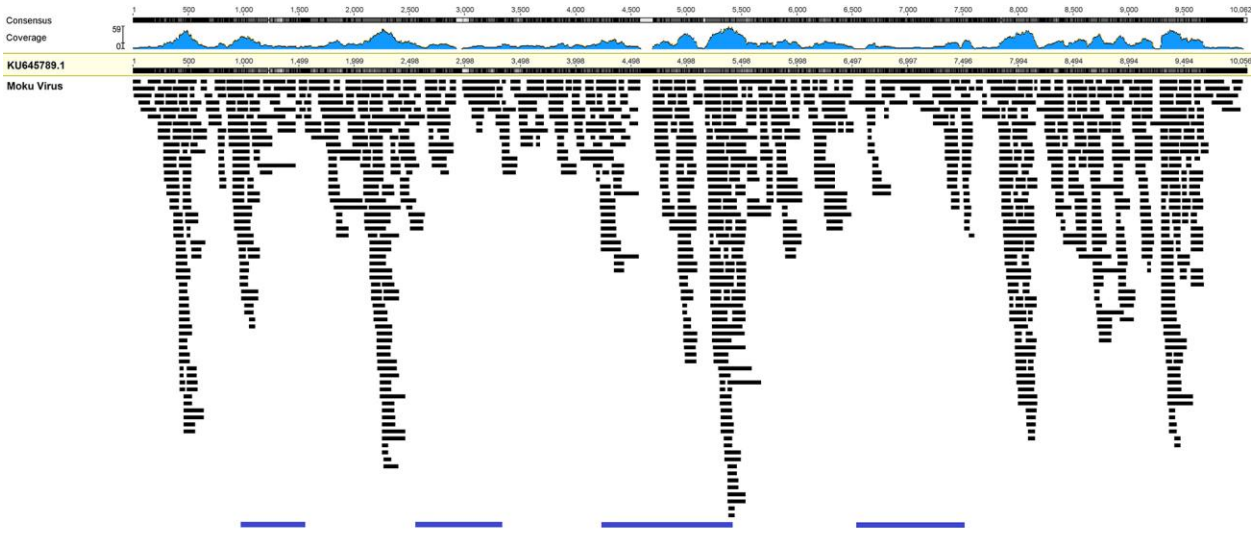


Technical Appendix Figure 1. Radial taxonomic classification tree representing the relative abundance of viral species found in a pool of 5 female and 5 male Asian hornets (*Vespa velutina*) collected in Belgium in 2016, as determined by blastx analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The figure is meant to be read from the center (genome molecular type or viral family) to the periphery (viral species). The surface area for each color is proportional to the relative abundance of reads. Figure drawn using MEGAN6 software (*6*).



Technical Appendix Figure 2. Graphical representation of the mapping of reads obtained by next-generation sequencing from *Vespa velutina nigrithorax* samples collected in Belgium to the Moku virus (GenBank accession no. KU645789) described in *Vespula pensylvanica* in Hawaii (*7*). Gaps or regions of low coverage were filled and confirmed by Sanger sequencing; the corresponding amplicons are shown in blue.