the different regions of Germany. In contrast, within these particular geographic areas, only slight sequence differences were found in longitudinal analysis over several years. This conclusion is supported by the novel human Waldkirchen sequence (H72), which is almost identical to the BF strains from 2004 (*6*,*10*) and the similarity of newly derived human sequences from Munsterland (H208, H303) to the Berkel strain from 1994 (*7*). The molecular characterization of the viral sequences of patient and rodent origin from the outbreak areas demonstrates that PUUV is the causative agent of the current outbreak.

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Chikungunya Virus in *Aedes albopictus*, Italy

To the Editor: Chikungunya virus (CHIKV) infection is a self-limiting illness characterized by fever, headache, weakness, rash, and arthralgia. Some patients show prolonged weakness or arthralgia lasting several months. In 2006, several Indian Ocean states and India experienced outbreaks of CHIKV infection, where the vector was postulated to be *Aedes albopictus* in at least some areas (*1*,*2*).

Starting from mid July 2007, in 2 villages in Ravenna Province in Italy, Castiglione di Ravenna (≈1,700 inhabitants) and Castiglione di Cervia (≈2,000 inhabitants), several residents sought treatment at local hospitals and health centers for high fever and arthralgia, joint and muscular pain, severe headaches, body aches, and in some cases, rash. Since the beginning of August 2007, an increasing number of febrile syndromes associated with arthralgia have been recorded among the residents of the area. By the end of August, the number of sick persons had increased to ≈150 (*3*). At the beginning of September, the disease was confirmed as chikungunya fever by the Superior Institute of Health (*4*).

On August 21 and 22, 2007, an entomologic investigation was carried out in the area. *Ae. albopictus* (215 females and 57 males), *Culex pipiens* (369 females and 15 males), and a few specimens of *Ae. caspius* (5 females) and *Anopheles* spp. (2 females) were collected by using 3 light traps without CO_2 (Centers for Disease Control and Prevention [CDC], Atlanta, GA, USA) and 8 CO_2-baited traps (similar to the CDC light trap) activated once overnight. Collections were obtained by using 2 small-handled aspirators per day of sampling. Collected mosquitoes were divided by species and pooled as described in the Table.

Table. Pooled mosquito samples analyzed for chikungunya virus, Italy

Pool	No. mosquitoes	Sex	Species	Site of collection	PCR results
1	125	F	*Aedes albopictus*	Castiglione Ravenna	Positive
2	90	F	*Ae. albopictus*	Castiglione Cervia	Positive
3	214	F	*Culex pipiens*	Castiglione Ravenna	Negative
4	155	F	*Cx. pipiens*	Castiglione Cervia	Negative
5	5	F	*Ae. caspius*	Castiglione Ravenna and Castiglione Cervia	Negative
6	2	F	*Anopheles* spp.	Castiglione Ravenna and Castiglione Cervia	Negative
7	57	M	*Ae. albopictus*	Castiglione Ravenna and Castiglione Cervia	Negative
8	15	M	*Cx. pipiens*	Castiglione Ravenna and Castiglione Cervia	Negative

Each pool was analyzed for CHIKV or nucleic acid (viral isolation and CHIKV-specific reverse transcription–PCR [RT-PCR]). Total RNA was extracted from supernatant of mosquitoes homogenized in minimal essential medium by using TRIzol LS (Invitrogen, Carlsbad, CA USA), and cDNA was synthesized by using SuperScript II (Invitrogen) and random primers. Two PCR protocols were used on the same samples: a nested RT-PCR (*5*) and a real-time PCR (*6*). Positive results were obtained from samples 1 and 2 (Table) with both PCR protocols. No positive control was available to the authors at the time of the first PCR-positive detection of virus from mosquitoes; therefore, laboratory contamination can be excluded. Moreover, from the same PCR-positive samples of *Ae. albopictus* (Table), viral isolation was achieved.

The 172-bp PCR fragment obtained after the second round of the nested RT-PCR (*5*) was located in the E2 gene between nt positions 9486 and 9660 according to nucleotide sequence of the S27 strain genome (GenBank accession no. AF369024). The sequence of this fragment was obtained by using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and compared with the available CHIKV sequences, including Indian isolates obtained during 2005–2006 (IND-06), the earlier Indian isolates (1963 and 1973), the isolate from Yawat in 2000, Reunion isolates during 2005–2006 (RU), the Senegal strain (1983), and the S27 and Ross isolates (1952). The sequence of the isolate from Italy is clustered into the Central/East African genotype and showed highest nucleotide identity (99.4%–100.0%) with isolates from IND-06 and 100% identity at the amino acid level with isolates from IND-06 and from RU. These results must be considered carefully because the short-sequenced fragment does not enable confirmation of the epidemiologic origin of the isolate from Italy. The sequence of the whole genome of CHIKV isolates from mosquitoes in Italy is an ongoing process; when completed, results will be available in GenBank.

The remainder of insect samples, which included a few male samples of *Ae. albopictus*, generated PCR-negative results with both protocols tested. Male mosquitoes were tested to detect evidence of transovarian transmission, but the small number of mosquitoes tested suggests possible vertical transmission of the virus in the Italian outbreak. Chikungunya fever in Italy has been reported recently by Beltrame et al. (*7*) but those cases involved 9 patients who were infected while traveling in regions where CHIKV was endemic.

In spite of the large diffusion of *Ae. albopictus* in Italy recorded since 1990 and broadly distributed all over the country (*8*), this outbreak of Chikungunya fever is evidence of an active endogenous circulation of the virus and could represent a possible introduction of this disease in Italy. No prediction can be made about spread and persistence of the virus in Italy because the vector is now present in all areas of the country and recent winters have been characterized by mild temperatures. Most likely, because transovarial infection has not been demonstrated for CHIKV, spread of infection will remain limited. However, as this report documents, Italy is at risk for infection with arboviruses, such as dengue virus and West Nile virus, which have serious effects on public health.

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Persistent Human Metapneumovirus Infection in Immunocompromised Child

To the Editor: Respiratory viral infections can be associated with a wide range of clinical manifestations from self-limiting upper respiratory tract diseases to pneumonia (*1*). However, in general, respiratory viral infections are more likely to progress to more severe diseases in immmunocompromised patients. Human metapneumovirus (hMPV) has been reported in most parts of the world as a cause of acute respiratory tract infections in persons of all age groups (*2*). Fatal hMPV infections have been reported in immunocompromised patients, including a 17-month-old girl who had acute lymphoblastic leukemia (*3*) and a 33-year-old woman who had received a hematopoietic stem cell transplant (HSCT) (*4*). In adult HSCT recipients, fatal pneumonia (*5*) and persistent hMPV infection without respiratory symptoms have been described (*6*). In addition, adult lung transplant recipients have been able to clear hMPV infection despite high levels of immunosuppression (*7*). We report a case of persistent hMPV infection in a child with severe combined immunodeficiency disorder (SCID) who shed hMPV during an 11-month period.

The child, a girl who was born in January 2002, received an allogeneic haploidentical stem cell transplant from her father in May 2002 after her diagnosis of SCID. Infection with influenza A virus (H3N2) was diagnosed on April 2005 and progressed to a chronic pneumonitis of the lingula. She received successive courses of anti-influenza agents (amantadine, oseltamivir, and zanamivir) for 1 year during which time several positive influenza cultures were obtained (*8*). Four years after the transplant, she was still lymphopenic (800×10^9/L, mostly T cells) and had chronic graft-versus-host disease, which had been treated with steroids (prednisone 2.5 mg twice a day for many months). She also had a mild chronic cough but did not need supplemental oxygen while she was receiving nebulized zanamivir (10–20 mg twice a day). Her 2 nasopharyngeal aspirate (NPA) specimens from June and July 2006 were negative for influenza virus. However, positive cultures for hMPV were obtained from NPA and bronchoalveolar lavage specimens collected on July 2006. After receiving this result, we performed retrospective and prospective molecular detection studies for hMPV for this patient. HMPV was detected by reverse transcription–PCR for the F and G genes (*9*) in 6 and 7 NPA samples, respectively, collected during an 11-month period from November 4, 2005, through October 4, 2006. These samples were obtained for surveillance of influenza infection in this child with persistent cough.

Amplified hMPV G sequences were aligned by using the Clustal W program (www.molecularevolution.org/cdc/software/clustalw). A phylo genetic tree was constructed with MEGA 3.1 software (www.megasoftware.net) by using the neighbor-joining algorithm with Kimura-2 parameters. Sequence analysis of the hMPV G gene showed that all strains belonged to the B2 genotype, which clustered with hMPV Can98–75 and NL1/94 reference strains (Figure, panel A). Amplified hMPV G gene sequences of the 6 samples collected in 2006 were identical, but they had 96.7% and 92.8% nucleotide and amino acid identities, respectively, with the initial strain from November 2005, which clearly indicates 2 viral strains (Figure, panel B). Similar results were obtained with the F gene (data not shown). Inoculation of the respiratory samples on a panel of 10 cell lines as previously described (*10*) showed that only 2 of 7 NPA samples were positive for hMPV by culture; 2 of the 5 remaining samples were positive for influenza A, which may have masked the cytopathic effects of hMPV on rhesus monkey kidney (LLC-MK2) cells.

Persistent hMPV infection in asymptomatic adult HSCT recipients has been described (*6*). In that study, hMPV was isolated from 2 patients in 2 consecutive samples collected 12–56 days apart. However, virus evolution was not adequately investigated because it was based on sequence analysis of a 150-bp fragment from the highly conserved nucleoprotein gene (*6*). Unlike in previous reports (*6*,*7*), characterization of hMPV strains in our study was performed by sequence