

shown to be naturally infected with Zaire Ebola virus and Marburg virus. Thus, *R. amplexicaudatus* bats are a possible natural reservoir of REBOV. However, only 16 specimens of *R. amplexicaudatus* bats were available in this study, and it will be necessary to investigate more specimens of this species to detect the REBOV genome or antigens to conclude the bat is a natural reservoir for REBOV.

We have shown that *R. amplexicaudatus* bats are putatively infected with REBOV or closely related viruses in the Philippines. Antibody-positive bats were captured at the sites near the study areas, where REBOV infections in cynomolgus monkeys and swine have been identified. Thus, bats are a possible natural reservoir of REBOV. Further analysis to demonstrate the REBOV genome in bats is necessary to conclude that the bat is a reservoir of REBOV.

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Acute Hepatitis C Outbreak among HIV-infected Men, Madrid, Spain

To the Editor: In the past decade, hepatitis C virus (HCV) has emerged as a sexually transmitted infection (STI) among HIV-infected men who have sex with men (MSM). The epidemic was originally reported in several northern European countries (England, France, Germany, and the Netherlands) (1) and soon after in Australia (2) and the United States (3). Acute HCV acquisition was associated with group sex, unprotected receptive anal intercourse, and according to some studies, concomitant STI (4). Molecular phylogenetic studies suggested evidence of an international transmission network of MSM within northern Europe (1). However, expansion of the HCV epidemic among MSM to Spain (5) or to other

countries of the Mediterranean area had not previously been reported.

We report 4 cases of acute HCV in HIV-infected MSM in Madrid, Spain, 2010. These patients were monitored at a university-affiliated hospital in downtown Madrid, which provides health care to a large MSM community in the Chueca District. Diagnosis of acute HCV was made by using the following criteria of the European AIDS Treatment Network (6): 1) positive HCV RNA; 2) an acute rise in alanine aminotransferase level $>5\times$ the normal upper limit, with documented normal alanine aminotransferase level within 12 months; and 3) negative results for anti-hepatitis A virus immunoglobulin M and anti-hepatitis B core immunoglobulin M (when other causes of acute hepatitis were excluded). An HCV RNA load fluctuation of $>1 \log_{10}$ IU/mL, if present, was considered further evidence of acute HCV infection (7).

All 4 patients were MSM with well-controlled HIV infection who were receiving antiretroviral treatment. During routine medical screening, they were found to have newly elevated liver transaminase levels, and further assessment

confirmed the diagnosis of acute HCV infection (Table). Three patients had received a diagnosis of STI in the previous 6 months, but only 1 patient acknowledged having unprotected anal intercourse. In addition, only 1 patient acknowledged using any recreational drugs (amyl nitrate); all denied using injection drugs (Table). All patients had lived in Madrid for at least 5 years before receiving a diagnosis of acute HCV. No patients reported having sex during international travel, using sex toys, or fisting.

The patients described here lived in the Chueca District of Madrid, the largest MSM community in Spain, which is frequented by MSM traveling from smaller cities in Spain and other countries. Two of the 3 patients were infected with HCV genotype 4, which is unusual in patients from outside the Middle East and Africa (8) yet unexpectedly common in northern European HCV outbreaks (1), which suggests that the patients reported here may have been part of the social network originating in the north. Further sequencing of these isolates is under way to address this issue. The third patient with an identifiable HCV genotype was infected with

HCV genotype 1, the most common genotype among HIV-infected MSM in northern Europe (1). These findings suggest that a larger, undetected outbreak of HCV infection is taking place in Madrid.

Although the patients reported here described fewer risks for sexual acquisition of HCV than patients from northern Europe or the United States, 3 had recent STI, which suggests that they underreported their risks for HCV acquisition. This temporal association between STI and acute HCV in these patients suggests that the pattern of emergence of sexually transmitted HCV among MSM in Spain might be similar to that seen in northern Europe, following regional epidemics of syphilis (starting in 2000) (9,10). We therefore encourage HIV specialists and general practitioners, when investigating an STI, to perform HCV testing on MSM as well as on persons with newly elevated liver aminotransferase levels.

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Table. Description of 4 HIV-infected men with HCV infection, Madrid, Spain, 2010*

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4
Age, y	31	41	39	39
Country of origin	Italy	Ecuador	Spain	Spain
Date of HIV diagnosis	2006 Jun	2000 Jul	1998	2001
Year ART initiated	2008	2000	2006	2006
Prior negative HCV test	2006	None†	None†	2008
Date AHC diagnosed	May 2010	May 2010	May 2010	May 2010
CD4 count, cells/ μ L	562	327	787	750
HIV viral load	Undetectable	Undetectable	Undetectable	Undetectable
Symptoms at diagnosis	No	Mild asthenia only	No	No
ALT/AST levels at AHC diagnosis, U/L	564/331	304/216	222/114	261/125
HCV genotype	4	4	1a	Indeterminate
HCV RNA load, IU/mL (\log_{10} IU/mL)	950,556 (5.98)	629,875 (5.80)	11,827 (4.07)	2,254,258 (6.35)
HCV RNA load fluctuation within 3 mo, \log_{10} IU/mL	4.32	<1.00	1.33	<1.00
Unprotected anal intercourse	No	No	No	Serosorting‡
STI in previous 6 mo	Proctitis	Syphilis	No	Proctitis
Group sex (>2 persons)	Yes	No	No	No
Drug use	Amyl nitrate only	No	No	No

*HCV, hepatitis C virus; ART: antiretroviral therapy; AHC, acute hepatitis C infection; ALT, alanine aminotransferase; AST, aspartate aminotransferase; STI, sexually transmitted infection.

†Both patients had normal transaminase levels in the 4 y before AHC diagnosis.

‡Unprotected sex between seroconcordant partners (HIV positive).

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Saffold Virus Infection in Children, Malaysia, 2009

To the Editor: Since 2007, a new cardiovirus, named Saffold virus (SAFV), has been isolated from human specimens in the United States, Canada, the Netherlands, and People's Republic of China (1–4). Concurrent investigations also showed that SAFV could be detected in feces and respiratory secretions of children in other countries, and genetic analysis showed the circulation of different genetic lineages of SAFVs in various parts of the world. This new virus belongs to the genus *Cardiovirus*, in the family *Picornaviridae* (5). Here we report isolation of a new SAFV in Malaysia, designated SAFV-Penang to reflect the locality of isolation in Malaysia.

A 5-year-old girl was brought to a government outpatient clinic on November 18, 2009, with reported fever and sore throat for 3 days. The fever was described as of high grade with occasional episodes of rigor, accompanied by profuse sweating and myalgia, lethargy, and loss of appetite. The child had nasal blockage, mild runny nose, and dry cough. She vomited twice on day 3 of illness and had abdominal pain but no diarrhea.

There was no history of similar illness affecting other family members, who lived in a semirural area within the state of Penang, Malaysia. Acute pharyngitis/acute influenza-like illness was provisionally diagnosed, and a throat swab specimen was collected in virus transport medium for virus isolation by using established procedures (6).

The throat swab sample was treated with antimicrobial drugs for 1 h before the cells were added to MDCK, Vero, and Hep-2 cells. On the fifth day postinoculation (dpi), a lytic form of cytopathic effect (CPE), similar to the type of CPE from enterovirus infection, was noted in Hep-2 but not in Vero or MDCK cells. The progress of CPE was slow, and full CPE was achieved on 9 dpi. On 8 dpi, a 0.5-mL aliquot containing infected Hep-2 cell suspension was removed and processed for indirect immunofluorescence assay by using a panel of commercial typing monoclonal antibodies for human enteroviruses. The infected Hep-2 cells reacted strongly with broad reactive pan-enterovirus monoclonal antibodies (catalog no. 3360, Chemicon Inc., Temecula, CA, USA) but failed to react with any type-specific monoclonal antibodies (data not shown).

After 3 passages in Hep-2 cells, culture supernatant was subsequently passed into Vero cells. After an additional 3 passages, the virus was fully adapted to grow in Vero cells and was able to induce visible CPE 1 dpi and full CPE by 4 dpi.

Partial genome sequence of the virus was initially obtained by using a random priming and amplification method as described (7). Full-length sequence was then determined by using primers designed according to the partial genome sequences of SAFV-Penang and genome sequences of other SAFV strains available in GenBank (primer sequences are available on request). The viral genome of SAFV-Penang is 8,073 nt