

of 54°C, and 45 amplification cycles. PCR blanks containing all reagents except for DNA and extraction blanks were included in every PCR set.

Results of the amplification reactions are listed in the Table. All accompanied extraction and PCR controls remained free of amplification products. All amplicons resulting from suicide PCRs were sequenced. Amplicons resulting from the use of primer pairs YP14F/YP13R and pst-F/pst-R matched the reference sequence to 100% (GenBank accession no. AL109969.1). Amplicons resulting from the use of primer pair PCP-F/PCP-R matched this reference sequence to only 97.78%. This deviation is because of a 2-bp insertion (2 Ts, positions 8531 and 8532, GenBank accession no. AL109969.1) at *Y. pestis* strain CO92 plasmid pPCP1. The sequences obtained from 3 persons' remains showed in the pPCP1 sequence section between nucleotide positions 8528–8532 only 3 Ts instead of 5 Ts described for *Y. pestis* strain CO92 plasmid pPCP1 (GenBank accession no. AL109969.1). The sequences found in this study were deposited in GenBank under accession nos. HQ290521–HQ290523.

To conclude, the successful recovery of several *Y. pestis* plasmid pPCP1 DNA sequences in skeletal finds from the mass burial site excavated in Manching-Pichl suggests that these persons died of plague. Moreover, our findings constitute a molecularly supported confirmation for the presence of *Y. pestis*, the etiologic agent of plague, in late medieval (1250–1500 CE) southern Germany. In future studies, we will attempt to recover chromosomal *Y. pestis* DNA from the mass grave skeletal remains to obtain clues as to the specific *Y. pestis* strain and the microbiology of past plague in Europe.

**Ingrid Wiechmann,  
Michaela Harbeck,  
and Gisela Grupe**

Author affiliations: Ludwig Maximilian University of Munich, Munich, Germany (I. Wiechmann, G. Grupe); and Bavarian State Collection of Anthropology and Palaeoanatomy, Munich (M. Harbeck, G. Grupe)

DOI: 10.3201/eid1611.100598

## References

1. Yersin A. La peste bubonique à Hong Kong. Ann Inst Pasteur (Paris). 1894;8:662–7.
2. Drancourt M, Aboudharam G, Signoli M, Dutour O, Raoult D. Detection of 400-year-old *Yersinia pestis* DNA in human dental pulp: an approach to the diagnosis of ancient septicemia. Proc Natl Acad Sci U S A. 1998;95:12637–40. DOI: 10.1073/pnas.95.21.12637
3. Raoult D, Aboudharam G, Crubezy E, Larrouy G, Ludes B, Drancourt M. Molecular identification by “suicide PCR” of *Yersinia pestis* as the agent of medieval Black Death. Proc Natl Acad Sci U S A. 2000;97:12800–3. DOI: 10.1073/pnas.220225197
4. Gilbert MTP, Cucchi J, White W, Lynnerup N, Titball RW, Cooper A, et al. Absence of *Yersinia pestis*-specific DNA in human teeth from five European excavations of putative plague victims. Microbiology. 2004;150:341–54. DOI: 10.1099/mic.0.26594-0
5. Duncan CJ, Scott S. What caused the Black Death? Postgrad Med J. 2005;81:315–20. DOI: 10.1136/pgmj.2004.024075
6. Garrelt C, Wiechmann I. Detection of *Yersinia pestis* DNA in early and late medieval Bavarian burials. In: Grupe G, Peters J, editors. Decyphering ancient bones; the research potential of bioarchaeological collections. Documenta Archaeobiologiae; Bd.1. Leidorf (Germany): Rahden/Westf.; 2003. p. 247–54.
7. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S, editors. Bioinformatics methods and protocols: methods in molecular biology. Totowa (NJ): Humana Press; 2000. p. 365–86.
8. Drancourt M, Signoli M, Dang LV, Bizot B, Roux V, Tzortzis S, et al. *Yersinia pestis* Orientalis in remains of ancient plague patients. Emerg Infect Dis. 2007;13:332–3. DOI: 10.3201/eid1302.060197
9. Wiechmann I, Grupe G. Detection of *Yersinia pestis* DNA in two early medieval skeletal finds from Aschheim (Upper Bavaria, 6th century A.D.). Am J Phys Anthropol. 2005;126:48–55. DOI: 10.1002/ajpa.10276

Address for correspondence: Ingrid Wiechmann, Ludwig Maximilian University of Munich, Department Biology I, Biodiversity research/Anthropology, Grosshaderner Str. 2, 82152 Planegg-Martinsried, Germany; email: i.wiechmann@lrz.uni-muenchen.de

## Two Clusters of HIV-1 Infection, Rural Idaho, USA, 2008

**To the Editor:** Prevalence of HIV-1 infection in rural areas of the United States has been increasing (1). During 2003–2007, an average of 30 (range 24–42) cases of new HIV-1 infection diagnoses per year among Idaho residents were reported. Of the 152 reported cases during this period, 54 (36%) were related to a person living in a rural area of  $\leq 75,000$  residents and a 60-minute drive from an urban area (2). Of these 54 cases, 19 (35%) were in men who have sex with men (MSM), 5 (9%) were in injection drug users (IDU), and 2 (4%) were in those in both categories.

In March 2008, a cluster of newly identified HIV-1 infections that included 5 cases (cluster A) in a rural southeastern Idaho city (city A) was reported to the Idaho Department of Health and Welfare. Two patients were men and the median age was 26 years (range 18–32 years). One patient was an IDU (Table). Through epidemiologic investigation, 3 additional patients were suspected to be IDUs, but confirmation was not practicable. All reported methamphetamine use. One man and 2 women reported both male and female sex partners.

During September–December of that year, another increase in newly identified HIV-1 infections in southeastern Idaho (cluster B) was reported

to Idaho Department of Health and Welfare. Cluster B included 10 cases, all among men who reported living within a 50-mile radius of city A, with most in a rural city (city B) located <30 miles from city A. The median age of the men in cluster B was 24 years (range 18–37 years). Each case was epidemiologically linked to at least 1 other case in the cluster; each patient reported having had unprotected sex with male partners. Although we suspected transmission of HIV-1 between persons in clusters A and B, whether the clusters were linked epidemiologically remained unclear after an initial investigation.

Although the primary use of HIV-1 sequence data is to assist clinicians in selecting antiretroviral (ARV) therapy, public health practitioners can use HIV-1 sequences from cases and compare those with HIV-1 sequences from others living in the region to explore phylogenetic associations and possible HIV transmission clusters (3). To evaluate links between clusters A and B, HIV-1 *pol* consensus sequence data for 4 of the 5 cases from cluster A and 6 of the 10 cases from cluster B were obtained from 5 commercial laboratories. No case-patients had received ARV. Additionally, we used sequence data from a patient residing in city B who had received an HIV-1 diagnosis in December 2008 but was not epidemiologically linked to either cluster. HIV-1 control sequences from 2 Idaho HIV clinics, including 34 HIV-1-infected persons within a 275-mile radius of city B identified who had not received ARV and who had resistance testing performed during 2005–2008, were used to represent the regional epidemic. Control sequences were aligned with cluster A and B sequences and analyzed as described (4,5).

Ten of the HIV case-patients for whom nucleotide sequence data were obtained were infected with HIV-1, subtype B, and were placed into 2 dis-

tinct phylogenetic-related groupings. Group 1 contained 4 patients from cluster A and 1 patient with no known epidemiologic link to either cluster. Group 2 contained 5 patients from cluster B. The average *pol* genetic distance among virus from members of group 1 was 0.2% (median 0.1%, SD 0.2%) and from members of group 2 was 0.1% (median 0.1%, SD 0.1%). The average distance among the control sequences was 5.1% (median 5.2%, SD 1.2%). The average distance between groups 1 and 2 was 4.8% (median 4.8%, SD 0.2%), which does not demonstrate a linkage between the 2 groups. The 1 case in group 1 that was not initially identified with either cluster had a genetically related HIV-1 sequence to members of cluster A, indicating a potential previously unidentified epidemiologic link. The sequence from 1 case associated with cluster B was not genetically similar to members of either cluster and was more similar to controls. These data do not indicate from whom each patient acquired the infection.

The epidemiologic investigation combined with the molecular analysis

shows transmission of HIV-1 originating from 2 sources occurred within a group of rural MSM in southeastern Idaho and indicates a separate case previously believed to be unrelated to 2 local clusters had genetic similarity to cluster A. Limitations of this investigation include the inability to obtain HIV sequences from all persons identified in clusters A and B and an inability to confirm high-risk behaviors for all identified case-patients.

Previous HIV clusters have demonstrated that infectious persons can spread HIV quickly within a social network and highlighted the importance of timely prevention activities to limit HIV transmission in a community (6,7). Use of phylogenetic analysis of HIV-1 sequences obtained from commercial laboratories showed that clusters A and B were not epidemiologically related and helped target appropriate and specific HIV prevention activities.

#### Acknowledgments

We thank Jeff Doerr, Maggie Mann, and Sherrie Joseph for assistance with the epidemiologic investigation; Shane Ames

Table. Sex and risk factors among patients epidemiologically linked to 2 clusters of HIV-1, southeastern Idaho, USA, 2008\*

Case ID	Sex	Risk factors	Cluster†	Phylogenetic group
1	F	WSMW/SIDU	A	1
2	F	WSMW/SIDU	A	1
3	M	MSMW/IDU	A	1
4‡	M	MSW	A	ND
5	M	MSM	B	2
6‡	M	MSM	B	ND
7‡	M	MSM	B	ND
8‡	M	MSM	B	ND
9	M	MSM	B	2
10§	M	MSM	B	ND
11	M	MSM	B	2
12‡	M	MSM	B	ND
13	M	MSM	B	2
14	F	WSM	A	1
15	M	MSM	B	2
16¶	M	MSM	ND	1

\*ID, identification; WSMW, women who have sex with men and women; SIDU, sex with injection drug users; MSMW, men who have sex with men and women; IDU, injection drug user; MSW, men who have sex with women; ND, not determined; MSM, men who have sex with men; WSM, women who have sex with men.

†Cluster A associated with injection drug use; cluster B associated with MSM.

‡HIV-1 sequence was not available for molecular analysis.

§HIV-1 sequence was similar to controls but not to sequences from either cluster A or B.

¶Case with no epidemiologic link to cluster A or cluster B.

for providing control data; Eoin Coakley, Shannon Utter, and Christopher Lockhart for assistance with acquisition of sequence data; and Alexandra Oster for guidance during this investigation.

The work of L.F. was supported by National Institutes of Health grant 1 U01 AI068632.

**Randall J. Nett,  
Jared L. Bartschi,  
Giovanina M. Ellis,  
David M. Hachey,  
Lisa M. Frenkel,  
J. Clay Roscoe, Kris K. Carter,  
and Christine G. Hahn**

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (R.J. Nett, K.K. Carter); Idaho Department of Health and Welfare, Boise, Idaho, USA (R.J. Nett, J.L. Bartschi, J.C. Roscoe, K.K. Carter, C.G. Hahn); Seattle Children's Hospital Research Institute, Seattle, Washington, USA (G.M. Ellis, L.M. Frenkel); Idaho State University, Pocatello, Idaho, USA (D.M. Hachey); University of Washington, Seattle (L.M. Frenkel); and Family Medicine Residency of Idaho, Boise (J.C. Roscoe)

DOI: 10.3201/eid1611.100857

## References

- Hall HI, Li J, McKenna MT. HIV in predominantly rural areas of the United States. *J Rural Health*. 2005;21:245–53. DOI: 10.1111/j.1748-0361.2005.tb00090.x
- Bowen A, Williams M, Horvath K. Using the Internet to recruit rural MSM for HIV risk assessment: sampling issues. *AIDS Behav*. 2004;8:311–9. DOI: 10.1023/B:AIBE.0000044078.43476.1f
- Robbins KE, Weidle PJ, Brown TM, Saekhou AM, Coles B, Holmberg SD, et al. Molecular analysis in support of an investigation of a cluster of HIV-1-infected women. *AIDS Res Hum Retroviruses*. 2002;18:1157–61. DOI: 10.1089/088922202320567914
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res*. 1997;25:4876–82. DOI: 10.1093/nar/25.24.4876
- Buskin SE, Ellis GM, Pepper GG, Frenkel LM, Pergam SA, Gottlieb GS, et al. Transmission cluster of multiclass highly drug-resistant HIV-1 among 9 men who have sex with men in Seattle/King County, WA, 2005–2007. *J Acquir Immune Defic Syndr*. 2008;49:205–11. DOI: 10.1097/QAI.0b013e318185727e
- Centers for Disease Control and Prevention. Cluster of HIV-positive young women—New York, 1997–1998. *MMWR Morb Mortal Wkly Rep*. 1999;48:413–6.
- Denoon DJ. CDC warns of HIV “clusters” in low-prevalence areas. *AIDS Wkly Plus*. 1999;19:3–4.

Address for correspondence: Randall J. Nett, Montana Department of Public Health and Human Services, 1400 Broadway, Rm C202, Helena, MT 59620, USA; email: gge5@cdc.gov

## Pandemic (H1N1) 2009 and Oseltamivir Resistance in Hematology/Oncology Patients

**To the Editor:** Tramontana et al. (1) recently described characteristics and oseltamivir resistance in hematology and oncology patients infected with pandemic (H1N1) 2009 virus. Such cases merit further study because concurrent medical problems in immunosuppressed patients may obscure and delay diagnosis and management of pandemic (H1N1) 2009 infections. Moreover, severe complications of such infection may be more likely to develop in immunosuppressed patients (2). During the winter of 2009, oseltamivir-resistant pandemic (H1N1) 2009 virus infection was diagnosed for 4 patients at Duke University Medical Center. We describe the clinical features of the infections, the challenges associated

with diagnosis of pandemic (H1N1) 2009 virus infection, and the clinical outcome for the infected patients.

Four immunocompromised patients who received chemotherapy and immunotherapy for solid-organ and hematologic malignancies were hospitalized at our tertiary care medical center during October–November 2009, a period of peak activity of pandemic (H1N1) 2009 in surrounding communities in North Carolina (3). These 4 case-patients experienced symptoms attributable to pandemic (H1N1) 2009 from 0 to 14 days after hospital admission, and the diagnosis of pandemic (H1N1) 2009 was made 0–28 days after symptom onset. Illness, diagnosis, and treatment of the patients are summarized in the Table. One patient reported contact with a family member who had influenza-like illness. Three other patients likely acquired pandemic (H1N1) 2009 in the hospital. An investigation could not conclusively establish whether transmission of pandemic (H1N1) 2009 occurred between case-patients and healthcare workers or visitors (4). All 4 case-patients ultimately died; 2 patients recovered from pandemic (H1N1) 2009 after antiviral drug therapy but died of underlying disease and subsequent bacterial infections. One case-patient did not receive antiviral drugs because the diagnosis was made posthumously.

We learned valuable lessons regarding diagnosis and management of pandemic (H1N1) 2009 in immunocompromised patients. First, pandemic (H1N1) 2009 infection can be difficult to diagnose in immunocompromised hospitalized patients. Such patients do not exhibit consistent symptoms or signs for pandemic (H1N1) 2009. Consistent with Tramontana et al. (1), fever was the most common feature, followed by progressive dyspnea and intermittent cough. None of our patients reported sore throat. Moreover, such nonspecific symptoms may be inadvertently attributed to concurrent medical problems common in immu-