6. Gibbons RV, Kalanarooj S, Jarman RG, Nisalak A, Vaughn DW, Endy TP, et al. Analysis of repeat hospital admissions for dengue to estimate the frequency of third or fourth dengue infections resulting in admissions and dengue hemorrhagic fever, and serotype sequences. Am J Trop Med Hyg. 2007;77:910–3.
7. Capeding RZ, Luna IA, Bomasang E, Lupisan S, Lang J, Forrat R, et al. Live-attenuated, tetravalent dengue vaccine in children, adolescents and adults in a dengue endemic country: randomized controlled phase I trial in the Philippines. Vaccine. 2011;29:3863–72. http://dx.doi.org/10.1016/j.vaccine.2011.03.057
8. Halstead SB, Mahalingam S, Marovich MA, Ubol S, Mosser DM. Intrinsic antibody-dependent enhancement of microbial infection in macrophages: disease regulation by immune complexes. Lancet Infect Dis. 2010;10:712–22. http://dx.doi.org/10.1016/S1473-3099(10)70166-3
9. Kliks SC, Nisalak A, Brandt WE, Wahl L, Burke DS. Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. Am J Trop Med Hyg. 1989;40:444–51.
10. Guy B, Barrere B, Malinowski C, Saville M, Teyssou R, Lang J. From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. Vaccine. 2011;29:7229–41. http://dx.doi.org/10.1016/j.vaccine.2011.06.094

Address for correspondence: Suresh Mahalingam, Institute for Glycomics, Griffith University, Parksland Dr, Gold Coast Campus, Southport, Queensland 4222, Australia; email: s.mahalingam@griffith.edu.au

Novel Norovirus GII.4 Variant, Shanghai, China, 2012

To the Editor: Norovirus (NoV) has been identified as one of the major causal agents of nonbacterial, acute gastroenteritis in humans (*1*). The genetic diversity among NoVs is great, and human strains have been classified into 3 genogroups (GI, GII, and GIV). Despite this diversity, in recent years only a few strains, primarily those of genogroup II, genotype 4 (GII.4), have been responsible for most cases and outbreaks worldwide (*1,2*).

The pattern of epochal evolution of NoV is ongoing, and novel GII.4 variants emerge, which replace previously dominant strains and cause new pandemics. Surveillance systems worldwide showed an increase in NoV activity in late 2012 (*3*). Molecular data shared through NoroNet (www.rivm.nl/en/Topics/Topics/N/NoroNet) suggest that this increase is related to the emergence of a new GII.4 variant, termed Sydney_2012 (*3*). We found that this novel GII.4 variant also emerged in Shanghai, China, and caused increased levels of NoV activity during October–December 2012.

During July 2011–December 2012, fecal specimens from 748 outpatients (≥16 years of age) with acute gastroenteritis who visited 1 of the 2 sentinel hospitals in Shanghai were collected and stored at Shanghai Public Health Clinical Center at −70°C. Molecular detection of GI and GII NoV was performed by using conventional reverse transcription PCR as described (*4*). Full-length viral protein 1 and 639 bp of the 3′ RNA-dependent RNA polymerase gene of 4 randomly selected GII-positive strains were amplified (*5*–*7*). NoV genotypes were classified on the basis of a 280-bp region for GI and a 305-bp region for GII by using the Automated Genotyping Tool (www.rivm.nl/mpf/norovirus/typingtool).

A total of 77 patients showed positive results for GII NoV. An increase in GII NoV activity was observed during October–December in 2012; the detection rate was 46.08% (47 cases in 102 outpatients). The prevalence of GII NoV during the same period in 2011 was low; the detection rate was 6.90% (8 cases in 116 outpatients). Genotyping analysis of the strains detected in these 3 months in 2012 (39 strains were sequenced) showed that except for 1 GII.6 strain and 3 GII.4 2006b strains, the other 35 strains sequenced all belong to the new established cluster of GII.4, termed Sydney_2012. Retrospective analysis indicated that the novel GII.4 variant had already been detected in 2 outpatients during September 2011 in Shanghai.

Phylogenetic analysis of full-length capsid nucleotide sequences for 4 strains randomly selected from the new cluster indicated a novel GII.4 pattern, and new strains clustering separately from previously identified GII.4 pandemic strains (Figure). On the basis of BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) searches, the most closely related NoVs (98%–100% nucleotide identity) were 4 GII.4 viruses recently detected in Australia and Hong Kong. The new GII.4 strains detected in Shanghai also clustered with these strains, a finding that was supported by bootstrap values >70% (Figure). The 3′ end of RNA-dependent RNA polymerase gene sequences also confirmed that the new GII.4 strains were recombinants, with a GII.e polymerase and GII.4 capsid (*3*).

Despite improved control measures to combat NOV, this highly infectious agent continues to cause a large number of epidemics of gastroenteritis globally (approximately every 2 years), and most epidemics have been associated with emergence of a novel GII.4 cluster (*9*). The new cluster reported in the present study was first detected in Australia in March, 2012, followed by detection in France, New Zealand, Japan, the United Kingdom, the United States, and Hong Kong, where increased levels of NoV activity in late 2012 compared with previous seasons were also observed (*3*). This novel GII.4 strain has also emerged in Shanghai, China, and caused increased levels of sporadic cases during October–and December 2012. This new variant has common ancestors, dominant NoV GII.4 variants Osaka_2007 and New

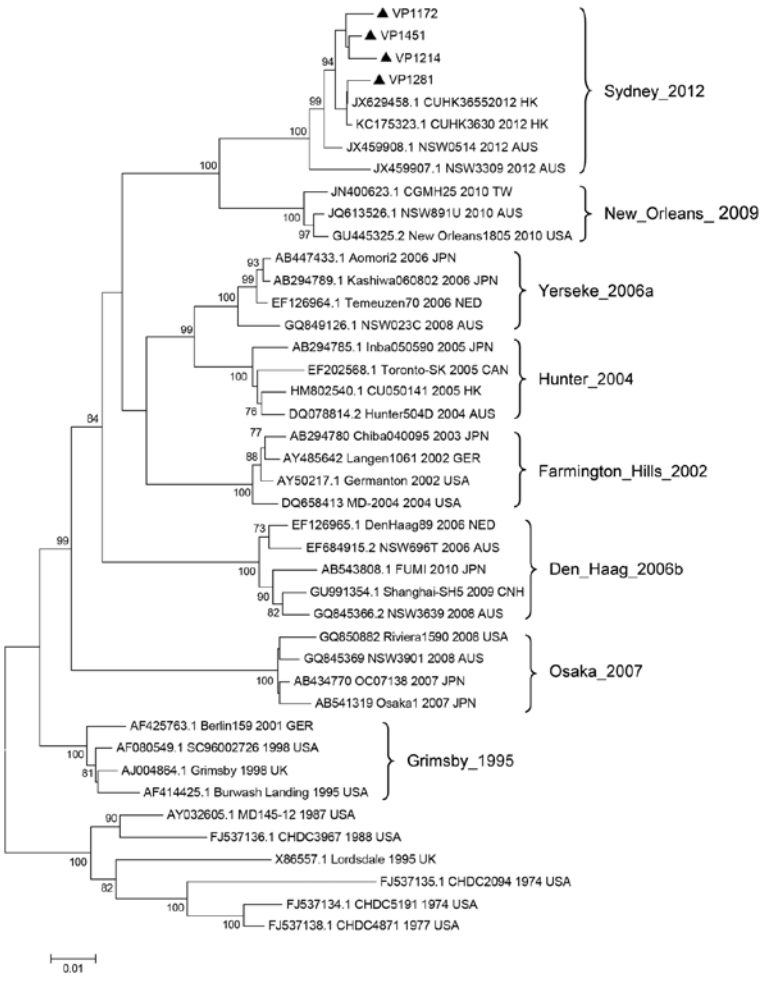

Figure. Phylogenetic tree of norovirus GII.4 capsid nucleotide sequences, Shanghai, China. The dendrogram was constructed by using the neighbor-joining method in MEGA version 5.0 (*8*). Bootstrap resampling (1,000 replications) was used, and bootstrap values ≥70% are shown. Black triangles indicate the 4 representative strains detected in Shanghai (GenBank accession nos. KC456070–KC456073). Reference sequences were obtained from GenBank and are indicated by GenBank accession number, strain name, year, and country of detection. Locations and years on the right indicate previously dominant GII.4 variants. HK, Hong Kong; AUS, Australia; TW, Taiwan; USA, United States; JPN, Japan; NED, the Netherlands; CAN, Canada; GER, Germany; CHN, China; UK, United Kingdom. Scale bar indicates distances between sequence pairs.

Orleans_2009, but is phylogenetically distinct from them. Amino acid changes are present in major epitopes located in the P2 domain, a finding that is consistent with observations from previous epidemics (*3*).

This study was supported by grant (no. 2012ZX10004-211) from the Ministry of Health, People's Republic of China for the National Major Science and Technology Project "Prevention and Treatment of AIDS, Viral Hepatitis, and Other Major Infectious Diseases."

Zhen Shen, Fangxing Qian, Yang Li, Yunwen Hu, Zhenghong Yuan, and Jun Zhang

Author affiliations: Shanghai Public Health Clinical Center, Shanghai, China (Z. Shen, Y. Hu, J. Zhang); Changning District Center Hospital, Shanghai (F. Qian); Shanghai Dongfang Hospital, Shanghai (Y. Li); and Fudan University, Shanghai (Z. Yuan)

DOI: http://dx.doi.org/10.3201/eid1908.130026

References

1. Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis. 2008;14:1224–31. http://dx.doi.org/10.3201/eid1408.071114
2. Kroneman A, Verhoef L, Harris J, Vennema H, Duizer E, van Duynhoven Y, et al. Analysis of integrated virological and epidemiological reports of norovirus outbreaks collected within the foodborne viruses in Europe network from 1 July 2001 to 30 June 2006. J Clin Microbiol. 2008;46:2959–65. http://dx.doi.org/10.1128/JCM.00499-08
3. van Beek J, Ambert-Balay K, Botteldoorn N, Eden JS, Fonager J, Hewitt J, et al. Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. Euro Surveill. 2013;18:8–9.
4. Kojima S, Kageyama T, Fukushi S, Hoshino FB, Shinohara M, Uchida K, et al. Genogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods. 2002;100:107–14. http://dx.doi.org/10.1016/S0166-0934(01)00404-9
5. Yuen LK, Catton MG, Cox BJ, Wright PJ, Marshall JA. Heminested multiplex reverse transcription-PCR for detection and differentiation of Norwalk-like virus genogroups 1 and 2 in fecal samples. J Clin Microbiol. 2001;39:2690–4. http://dx.doi.org/10.1128/JCM.39.7.2690-2694.2001
6. Tu ET, Bull RA, Greening GE, Hewitt J, Lyon MJ, Marshall JA, et al. Epidemics of gastroenteritis during 2006 were associated with the spread of norovirus GII.4 variants 2006a and 2006b. Clin Infect Dis. 2008;46:413–20. http://dx.doi.org/10.1086/525259
7. Bull RA, Hansman GS, Clancy LE, Tanaka MM, Rawlinson WD, White PA. Norovirus recombination in ORF1/ORF2 overlap. Emerg Infect Dis. 2005;11:1079–85. http://dx.doi.org/10.3201/eid1107.041273
8. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9. http://dx.doi.org/10.1093/molbev/msr121
9. Siebenga JJ, Vennema H, Zheng DP, Vinje J, Lee BE, Pang XL, et al. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001–2007. J Infect Dis. 2009;200:802–12. http://dx.doi.org/10.1086/605127

Address for correspondence: Jun Zhang, Department of Clinical Laboratory Medicine, Shanghai Public Health Clinical Center, Fudan University, 2901 Caolang Rd, Jinshan District, Shanghai, China; email: zhangjun@shaphc.org

Human Deaths and Third-Generation Cephalosporin use in Poultry, Europe

Peter Collignon, Frank M. Aarestrup, Rebecca Irwin, and Scott McEwen

Author affiliations: The Canberra Hospital, Garran, Canberra, Australian Capital Territory, Australia (P. Collignon); Australian National University, Woden, Australian Capital Territory, Australia (P. Collignon); EU Reference Laboratory for Antimicrobial Resistance. Copenhagen, Denmark (F.M. Aarestrup); World Health Organization Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens, Copenhagen (F.M. Aarestrup); Public Health Agency of Canada. Guelph, Ontario, Canada (R. Irwin); and Ontario Veterinary College/University of Guelph, Guelph (S. McEwen)

DOI: http://dx.doi.org/10.3201/eid1908.120681

To the Editor: Globally, antimicrobial drug resistance is rapidly rising, with resultant increased illness and death. Of particular concern is *Escherichia coli*, the most common bacterium to cause invasive disease in humans (*1*). In Europe, increasing proportions of bloodstream infections caused by *E. coli* are resistant to third-generation cephalosporins (*1*,*2*).

Resistant *E. coli* can be transmitted to humans from animals. A large proportion of resistant isolates causing human infections are derived from food animals (*3*–*6*). However, lack of data has made it difficult to quantify the proportion of antimicrobial drug resistant *E. coli* infecting persons through food sources and the resultant effects on human health. Recent data from the Netherlands now make such estimates possible (*2*,*6*). The additional illness and death among humans resulting from bloodstream infections caused by third-generation cephalosporin–resistant *E. coli* (G3CREC) has been calculated for Europe (*2*). In the Netherlands, there were 205 G3CREC cases during 2007 (4% of all *E. coli* bloodstream infections) (*2*). Another study in the Netherlands revealed that 56% of the resistance genes in G3CREC in humans were identical to genes derived from *E. coli* isolated from retail chicken samples (*6*). Using the findings of Overdevest et al. (*6*) and de Kraker et al. (*2*), we calculated that, in the Netherlands, infections in humans with G3CREC derived from poultry sources were associated with 21 additional deaths. G3CREC-related illness also resulted in 908 hospital bed-days needed to treat persons with these antimicrobial drug resistant bloodstream infections. If these values were extrapolated to all of Europe (i.e., if 56% of G3CREC were derived from poultry), 1,518 additional deaths and an associated increase of 67,236 days of hospital admissions would be counted as a result of cephalosporin and other antimicrobial drug use in poultry.

To more accurately estimate the associated increased deaths among persons resulting from third-generation cephalosporin use in poultry, detailed data from more countries is essential. Needed data include records of antimicrobial drug use and resistant bacterial strains found in food animals and domestic and imported foods. However, we already know that G3CREC is rapidly rising in many countries, and in Europe, the infection rate is likely to have tripled from 2007 to 2012 (*2*). Globally, billions of chickens receive third-generation cephalosporins in ovo or as day-old chicks to treat *E. coli* infection, a practice that has resulted in large reservoirs of resistant bacteria. In Canada, this practice has been associated with substantial increases in resistance to third-generation cephalosporins in *Salmonella enterica* serovar Heidelberg isolates detected in humans. (*7*). The United States Food and Drug Administration recently prohibited the off-label use of cephalosporins, including prophylactic uses, in major food animal species, including poultry (*8*).

The number of avoidable deaths and the costs of health care potentially caused by third-generation cephalosporin use in food animals is staggering. Considering those factors, the ongoing use of these antimicrobial drugs in mass therapy and prophylaxis should be urgently examined and stopped, particularly in poultry, not only in Europe, but worldwide.

References

1. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2010. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: The Centre; 2011.
2. de Kraker ME, Davey PG, Grundmann H; BURDEN study group. Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. PLoS Med. 2011;8:e1001104. Epub 2011 Oct 11. http://dx.doi.org/10.1371/journal.pmed.1001104
3. Jakobsen L, Spangholm DJ, Pedersen K, Jensen LB, Emborg HD, Agersø Y, et al. Broiler chickens, broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in *Escherichia coli* isolates from community-dwelling humans and UTI patients. Int J Food Microbiol. 2010;142:264–72. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.06.025
4. Vieira AR, Collignon P, Aarestrup FM, McEwen SA, Hendriksen RS, Hald T, et al. Association between antimicrobial resistance in *Escherichia coli* isolates from food animals and blood stream isolates from humans in Europe: an ecological study. Foodborne Pathog Dis. 2011;8:1295–301. http://dx.doi.org/10.1089/fpd.2011.0950
5. Johnson JR, Sannes MR, Croy C, Johnston B, Clabots C, Kuskowski MA, et al. Antimicrobial drug–resistant *Escherichia coli* from humans and poultry