EMERGING INFECTIOUS DISEASES®

Enteric Viruses

August 2013

EMERGING INFECTIOUS DISEASES®

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On the Cover

Catherine M. Howell (1892–1975)
Oyster Shuckers (1934)
Oil on canvas (101.4 cm × 91.6 cm)
Smithsonian American Art Museum, Transfer from the US Department of the Interior, National Park Service, Washington, DC, USA

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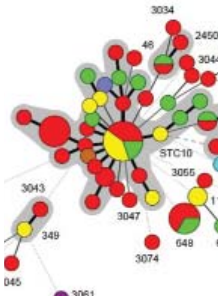

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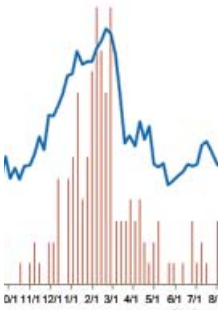

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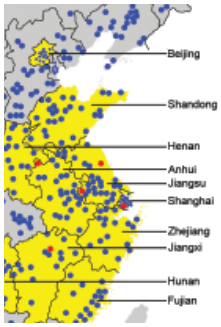

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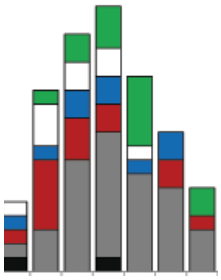

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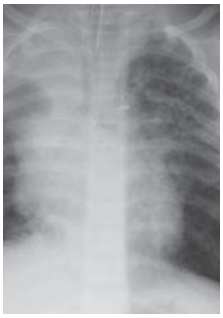

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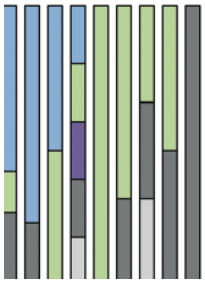

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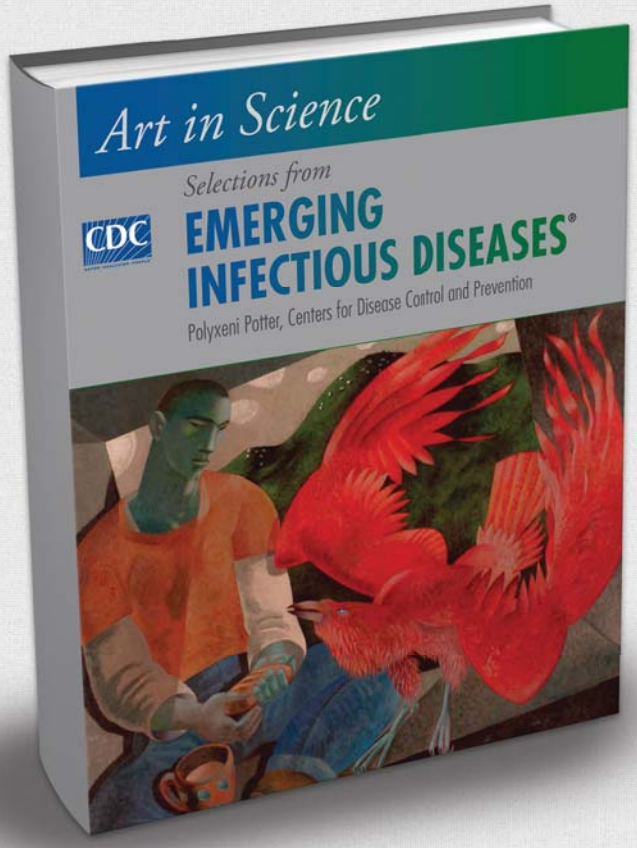

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Rapid Advances in Understanding Viral Gastroenteritis from Domestic Surveillance

Daniel C. Payne and Umesh D. Parashar

"Winter vomiting disease" was the clinical moniker for viral acute gastroenteritis (AGE), including illnesses caused by norovirus and rotavirus, nearly 100 years ago (*1*). This nonspecific diagnosis represented a frequently observed illness, with the symptoms of vomiting and diarrhea that occurred particularly in the colder months. One hundred years later, diagnosing specific AGE pathogens in clinical settings continues to be an elusive task. Clinical treatment options are nonspecific as well—primarily rehydration and supportive therapies—and the identification of the viral pathogens is considered relatively time-consuming and costly.

In truth, viral AGE has likely caused misery, illness, and death among human populations for thousands of years, since people first facilitated disease transmission by congregating in groups. But, it has been only during the lifetime of many current readers (and during the long career spans of several), beginning in the early 1970s, that norovirus, rotavirus, and an expanding collection of other viral AGE pathogens have been discovered. The advent of sensitive laboratory tools to detect and study the genetic evolution of these viruses has uncovered their critical role in the etiology of AGE. The flow of information is now so great that in each year since 2008, >800 scientific papers have been published on this topic as determined by a search of PubMed using the term acute gastroenteritis.

The field of viral gastroenteritis is in the midst of an extraordinary period of rapid development and transition. Vaccines to prevent rotavirus, the leading cause of severe childhood AGE worldwide, are being rolled out globally and have already achieved remarkable success in reducing the burden of this pathogen in many countries, including the United States. In addition, the application of sensitive molecular assays is reaffirming the central etiologic role of noroviruses in both endemic and epidemic AGE, and vaccines against this pathogen are undergoing clinical testing. This issue of Emerging Infectious Diseases highlights recent developments in the field with a collection of timely findings from domestic viral gastroenteritis surveillance, which will further our understanding of disease effects, viral evolution and structure, implications of vaccination, and progress with other preventive measures.

Daniel C. Payne

Dr Payne is an epidemiologist at the National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia. His research interests include viral pathogens, vaccine performance, pediatric diseases, cost-effectiveness, and vaccine safety.

Dr. Parashar leads the Viral Gastroenteritis Epidemiology Team at the Centers for Disease Control and Prevention. His research focuses on prevention and control of gastroenteritis caused by viral pathogens, particularly rotavirus and norovirus, in the United States and globally.

Reference

1. Zahorsky J. Hyperemesis hiemis or the winter vomiting disease. Arch Pediatr. 1929;46:391–5.

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Beyond Discovering the Viral Agents of Acute Gastroenteritis

Roger I. Glass

In the field of enteric microbiology, every major advance in diagnostics has enhanced our understanding of the etiology of gastroenteritis, the role of each pathogen, the different modes of transmission, and control methods that should be considered. Before 1970, >80% of gastroenteritis episodes did not have an etiologic diagnosis; these cases were attributed to weaning, malnutrition, or, most often, idiopathic causes. Then, in 1972, electron microscopists began to examine fecal specimens from patients with acute gastroenteritis, and within a decade, a collection of novel enteric viruses had been discovered: Norwalk virus (noroviruses), rotaviruses, astroviruses, enteric adenoviruses, classic human caliciviruses (sapoviruses), and others. Together, these novel viruses explained most of the severe cases of diarrhea in children and most of the acute outbreaks of disease occurring in the industrialized world.

For rotavirus, research progressed, and simple, sensitive, and inexpensive immunoassays soon displaced electron microscopy as the diagnostic test of choice available in laboratories around the world. On the basis of assay results, rotavirus was determined to be the most common cause of severe diarrhea in children worldwide. By contrast, the other enteric viruses, which are shed in lower quantities, could not be grown in culture and could not initially be detected by simple immunoassays. Consequently, for 2 decades, electron microscopy remained the main diagnostic tool available in a few research laboratories worldwide.

A key breakthrough came around 1990 when the first genetic sequences of these novel viruses were decoded, opening the way for new molecular diagnostics and reverse transcription PCR (RT-PCR). Noroviruses appeared to be prevalent in young children and in adults, including the elderly, and were soon recognized as the most common cause of outbreaks of and hospitalizations for gastroenteritis in industrialized countries.

What we have learned about rotaviruses and noroviruses alone has revolutionized our understanding of the critical role they play in public health and placed the other novel enteric viruses as second-tier agents. However, we are still in the discovery phase with these new viruses, learning about their public health impact worldwide and developing methods for their control. For rotaviruses, the mode of transmission is still in question, but widely introduced vaccines have provided a highly effective measure of disease control. For noroviruses, outbreaks can often be traced to fecally contaminated food or water and person-to-person transmission. The same diagnostics that have enabled our understanding of the high prevalence of disease worldwide have also enabled genetic fingerprinting of strains, a process critical to seeking a common source of exposure and to tracing routes of transmission back to fecally contaminated food, water, or food handlers. Noroviruses were first associated with outbreaks of disease; however, recognition of their key role in the hospitalization of adults with diarrhea indicates that those affected have incomplete immunity against norovirus or that strain diversity is too great to provide adequate cross-protection in persons who have been previously infected with other strains. Noroviruses also demonstrate a unique genetic susceptibility related to secretor histo-blood group antigens, so not all humans are equally susceptible to norovirus infection.

Limitations in our current knowledge of noroviruses and the other novel enteric viruses, excluding rotavirus, can be addressed by research over the next decade. RT-PCR opened the door to detecting these pathogens in research studies, but routine knowledge of the disease they cause remains limited by the lack of widely used, inexpensive, and simple diagnostics tests in the field or at the bedside. This lack of routine tests prevents health care providers from making an etiologic diagnosis and limits our understanding of transmission within hospitals and among the staff and in the community. In developing countries, these pathogens often occur together as mixed infections, making it difficult to assess whether the virus is really contributing to the disease process or merely passing through without replicating or inducing an immune response. RT-PCR and quantitative PCR can detect even a few viral particles, and shedding of these viruses in small numbers can continue for weeks or months, making it difficult to distinguish patients with real

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disease caused by the virus from those who have long since recovered from their infections but are still shedding virus.

This issue of Emerging Infectious Diseases provides readers with an opportunity to become informed about the latest updates in our knowledge of the viral agents of gastroenteritis, the global impact of the disease they cause, and prospects for their treatment and prevention. There is much new today, from the genetic human leukocyte antigen–related predisposition of disease to advances in vaccine development, understanding the diversity and evolution of strains, and testing of new solutions for environmental control, and there is also a robust research agenda ahead. Despite all the attention society has placed on ensuring access to clean food and water, sewage control, and handwashing, gastrointestinal illnesses remain one of the most common afflictions of humankind. We still have a lot to learn.

Dr Glass is director of the Fogarty International Center and associate director for Global Health Research at the National Institutes of Health. His research interests have centered on improving global health, especially by ending preventable childhood deaths from rotavirus infection and other enteric diseases.

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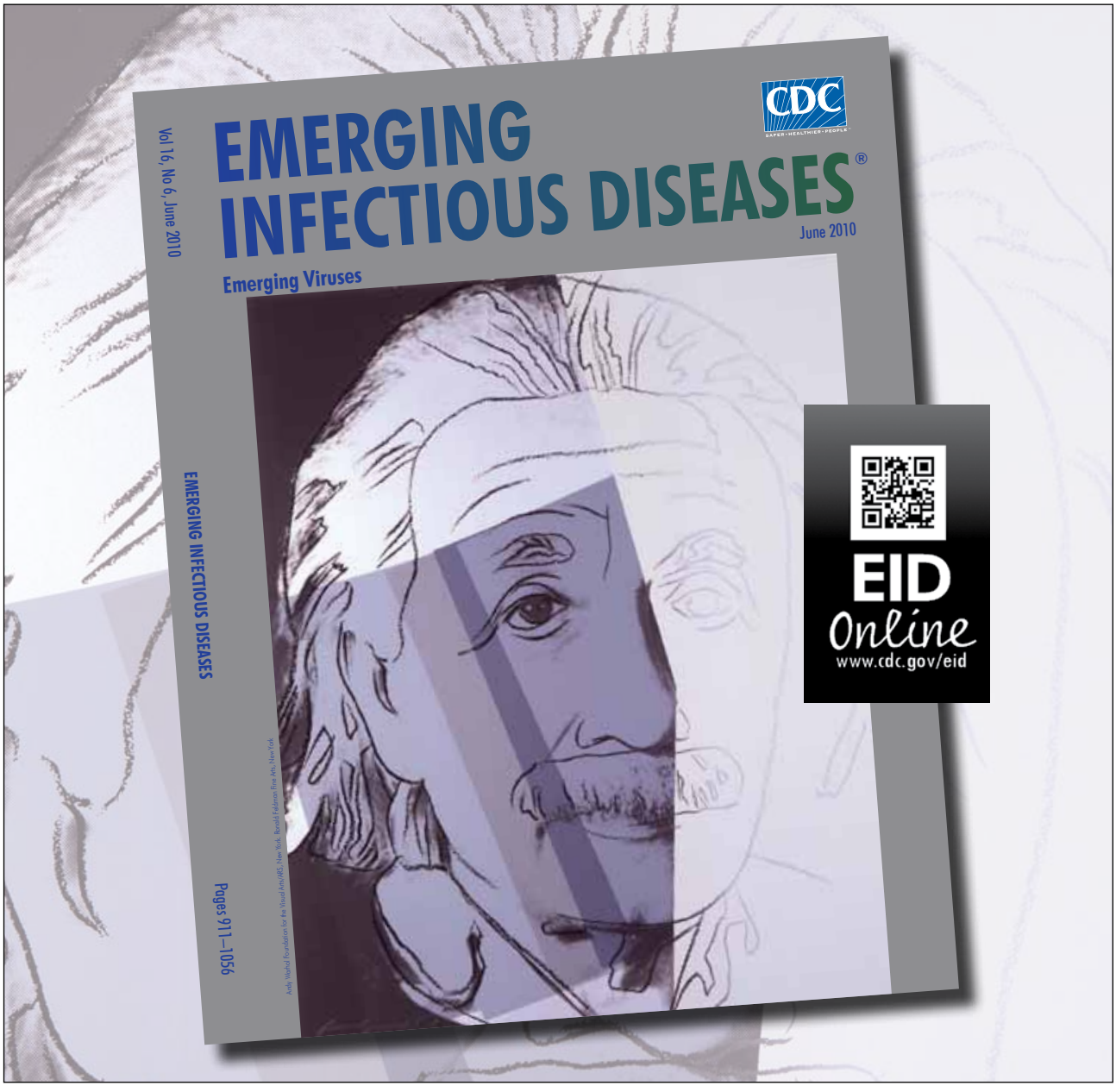

The New Global Health

Kevin M. De Cock, Patricia M. Simone, Veronica Davison, and Laurence Slutsker

Global health reflects the realities of globalization, including worldwide dissemination of infectious and noninfectious public health risks. Global health architecture is complex and better coordination is needed between multiple organizations. Three overlapping themes determine global health action and prioritization: development, security, and public health. These themes play out against a background of demographic change, socioeconomic development, and urbanization. Infectious diseases remain critical factors, but are no longer the major cause of global illness and death. Traditional indicators of public health, such as maternal and infant mortality rates no longer describe the health status of whole societies; this change highlights the need for investment in vital registration and disease-specific reporting. Noncommunicable diseases, injuries, and mental health will require greater attention from the world in the future. The new global health requires broader engagement by health organizations and all countries for the objectives of health equity, access, and coverage as priorities beyond the Millennium Development Goals are set.

> "People are beginning to understand there is nothing in the world so remote that it can't impact you as a person."
>
> –William H. Foege, Director, US Centers for Disease Control, 1977–1983

Health has become an area for diplomatic engagement and a priority subject on the world stage. Funding for global health has reached ≈$30 billion/year, and the United States provides at least one third of this total (*1*). However, too often there is lack of coordination across the inordinately complex architecture of global health. Agencies other than the World Health Organization (WHO), such as the World Bank and the Bill and Melinda Gates Foundation, have become prominent funders that influence policy; new multilateral organizations, such as the United Nations Joint Programme on HIV/AIDS, the Global Alliance for Vaccines and Immunisation, UNITAID, and the Global Fund to Fight AIDS, Tuberculosis and Malaria have sprung up; and civil society groups such as Médecins Sans Frontières implement programs and exert substantial political pressure.

These developments have challenged WHO, which although retaining unique credibility and convening authority, is hampered by funding shortages and donor-imposed earmarks, an inflexible bureaucratic and governance structure, and difficulty prioritizing in the face of unrealistic demands. Many decisions are now made outside the World Health Assembly, the world's senior and most representative forum for global health discussion. Newer global health actors are often seen as swifter and more focused on performance and accountability.

With global emphasis on austerity, there is now more than ever a need for bilateral and multilateral assistance to be coordinated for maximal effect, to avoid duplication and gaps, and to focus on measureable results. The diversity of multilateral agencies working in health distracts from the limited essentials expected from the global sector: estimating fiscal requirements and tracking financing; normative guidance; detecting and coordinating responses to complex emergencies and international health threats; monitoring and communicating health trends; and advocacy. A first requirement, including for bilateral partners, is agreement on what constitutes global health and which agencies are best placed to play particular roles.

This report discusses the evolving nature of global health and its priorities. Progress requires revision of the dichotomous view of a static world of industrialized or developing countries, rich or poor. Today's health disparities are as extreme within countries as between them. A more useful perspective is that global health requires synergistic engagement by all countries in an interdependent world, replacing the model of donors and recipients that characterized earlier international health assistance.

Global Health

The term global health has replaced tropical medicine and international health, disciplines linked to the history of colonialism, the post-independence era of the former European colonies, and the experience of development assistance (*2*,*3*). Global health is multidisciplinary, encompasses many elements besides development, and requires coordination of multiple parties, rather than direction by one organization or discipline. The increased technical and

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political complexity of global health, with many actors, including philanthropic and faith-based organizations, is reflected in its breadth, which covers diverse diseases but deals also with health systems issues and financing.

Global health reflects the realities of globalization, especially the increased movement of persons and goods, and the global dissemination of infectious and noninfectious public health risks. Global health is concerned with protecting the entire global community, not just its poorest segments, against threats to health and with delivering essential and cost-effective public health and clinical services to the world's population. A fundamental tenet is that no country can ensure the health of its population in isolation from the rest of the world, as articulated in the Global Health Strategy of the United States Department of Health and Human Services (*4*). This vision reflects today's health realities but was arrived at through milestones such as the 1993 World Development Report (Investing in Health) (*5*), the 2000 report of the Commission on Macroeconomics and Health (*6*), and the tremendous investment in HIV/AIDS begun earlier this century (*7*).

Development, Security, and Public Health

Three overlapping themes determine global health action: development, security, and public health. These themes provide the humanitarian and political bases for engagement by high-income countries in health matters internationally: for development, to promote health for stability, prosperity, and better international relationships; for security, to protect their populations against internal and external health threats; and for public health, to save lives worldwide and at home. Despite different requirements, organizations and agencies involved must adapt to global trends in socioeconomic development, fertility, population, and urbanization.

Development

Of 214 countries categorized by the World Bank, only 36 (17%) were classified as low-income countries (gross national income per capita in 2011 <$1,025 per year), 26 of which were in Africa (*8*). Economic growth is moving some low-income countries toward middle-income status, and some of the greatest imbalances in wealth may now be within rather than between individual countries. With socioeconomic development, basic health indicators improve but so do countries' abilities to shoulder more of their own health expenditures. Several middle-income countries such as the BRICS (Brazil, Russia, India, China, South Africa), and countries with oil-rich economies are capable of delivering assistance to poorer nations.

A clear correlation exists between countries' gross domestic product and their health indicators, such as mortality rates in children <5 years of age (highest in low-income countries) or life expectancy (highest in high-income countries). Development raises living standards, accompanied by improvement in basic services and drivers of health, such as nutrition and food security; access to potable water and sanitation; maternal and child health interventions, including family planning; and basic education, especially for women. The fundamental responsibility for development agencies, and their greatest contribution to health, is poverty reduction.

Although family planning and maternal and child health remain high on the development agenda, demographic trends are changing rapidly. Since 1980, the world's population has increased by nearly 60%; from ≈7 billion today, global population is projected to reach 9.3 billion by 2050 and 10.1 billion by 2100 (*9*). Decreasing fertility trends in sub-Saharan Africa are now following a similar trajectory as occurred elsewhere, but separated by several decades. By the end of the twenty-first century, the population of Africa will likely have increased by ≈2.6 billion, compared with ≈432 million in Asia. The Democratic Republic of Congo, Ethiopia, and Nigeria will be new demographic giants; it is predicted that in 2100, Nigeria will have a population of 730 million persons (*9*). By 2025 more than half of the world's citizens will live in urban settings, with dozens of megacities characterized by populations >10 million persons, including many in Africa (*9*), all challenged by the need for basic infrastructure and services.

A welcome trend has been renewed attention to reducing avoidable deaths among children. The worldwide reduction in childhood mortality rates means that since the 1980s, deaths among adults have exceeded deaths among children. Recently published estimates of mortality rates among children <5 years of age indicate that there are ≈7.2–7.6 million childhood deaths/year compared with ≈12 million deaths only 2 decades ago (*10–12*). Since 1990, maternal deaths have decreased from ≈526,000 to ≈274,000 (*11*).

Six countries, each with >200,000 deaths annually among children <5 years of age, account for ≈50% of global deaths in children; >50% of deaths in children occur in sub-Saharan Africa. Because of their large populations, India and China contribute substantially to these deaths, as do large countries with poor health indicators, such as Nigeria, the Democratic Republic of Congo, Pakistan, and Ethiopia. Seven countries with >10,000 maternal deaths/year account for >50% of the world's maternal mortality rate. The highest maternal mortality rates are in sub-Saharan Africa, especially western Africa, a finding that is consistent with distribution of adverse rates of child survival. Pakistan and Afghanistan stand out for unfavorable indicators in their region. Further reduction in maternal and child mortality rates globally will require special focus on countries with the greatest absolute numbers of maternal and child deaths.

Health Security

Drawing on earlier United Nations perspectives that characterized poor health as one of several threats to human security and well-being, health security captures the need for collective action and preparedness to reduce vulnerabilities to public health threats that transcend borders (*13*). Earlier optimism predicting the end of infectious diseases was replaced by recognition of the threat to global health from emerging infectious diseases and widespread antimicrobial drug resistance (*14*). The pandemic of HIV/AIDS, repeated outbreaks of Ebola and Marburg virus infections, rapid international dissemination of severe acute respiratory syndrome and pandemic influenza, international spread of several foodborne pathogens, and the intentional transmission of anthrax all convincingly illustrated global vulnerability. Other aspects of globalization negatively affecting health security include the trafficking of drugs and persons and population movement consequent to conflict and instability.

The global framework for health security is embodied in the International Health Regulations that were revised in 2005 and adopted by the World Health Assembly, but whose implementation is lagging behind the 2012 target date (*15*). The diversity of health threats results in involvement of other sectors, such as defense and diplomacy, and linkage with other international agreements, such as those relating to control of chemical, biological, and nuclear weapons.

Surveillance and laboratory capacity through strong national public health institutes are essential components of functioning health systems that provide the basis for health security. Ensuring ability to detect, investigate, diagnose, and rapidly contain public health events of concern wherever they occur requires commitment to global health capacity development in all countries and widespread and supportive public health networks (*16*).

Public Health

The scale-up of programs for HIV/AIDS, malaria, and tuberculosis over the past decade through initiatives such as the Global Fund, the United States President's Emergency Plan for AIDS Relief, and the President's Malaria Initiative led to substantial disease-specific progress. The Global Alliance for Vaccines and Immunisation has positively affected vaccine access. However, these experiences also highlighted the relative neglect of other priority areas and led to criticism that vertical, targeted programs failed to strengthen health systems overall (*17*). As a result, there has been renewed focus on the other health-related Millennium Development Goals (MDGs), especially relating to children's and maternal health (MDGs 4 and 5, respectively). These perceptions contributed to the establishment of the United States government's Global Health Initiative in 2009 (*18*) that addresses all health MDGs and some neglected tropical diseases in a more integrated manner.

The longstanding tension between vertical and horizontal approaches is now better understood, and there is greater emphasis on integration of efforts (*19*). Initiatives to strengthen general health systems have lacked specificity and agreed upon indicators, and they have had more difficulty showing measurable effects than disease-specific interventions that emphasize integration and linkage to other services. Public health agencies have a major role in strengthening specific areas of health systems, such as health information systems and surveillance, laboratory capacity, workforce skills, operational research and evaluation, and capacity for preparedness and program implementation (*20*).

National public health institutes and strong ministries have the core responsibility for defining policies, goals and targets, and assuring technical guidance, supervision, program implementation, evaluation, and accountability (*21*). Although epidemiology remains at the core of such work, the increased complexity of combinations of interventions in public health has highlighted the utility of mathematical modeling for assisting in decision making and policy setting.

Modern public health agencies have to be global in outlook to fulfill their domestic mandates. Because of the credibility emanating from their technical expertise, these agencies play an essential role in health diplomacy and development of public health capacity. Although development agencies concentrate on the needs of the poor, public health agencies potentially interact with all countries to address common challenges. Health systems strengthening, communicable and noncommunicable disease threats, safety and quality of medicines and commodities, and health access and equity are universally challenging to ministries of health, public health institutes and multilateral organizations, which all need to function in a close global network.

Unfinished Business: Infectious Disease Priorities

Recent estimates of the global incidence of disease suggest that communicable diseases account for ≈19% of global deaths (*22*). In Africa, 76% of deaths are still attributable to communicable, maternal, neonatal, or nutritional causes, compared with 25% in the entire world; conditions relevant to MDGs 4, 5, and 6 are responsible for 42% of years of life lost. Focus on infectious diseases remains necessary to prevent their global spread or recrudescence, save lives, enhance economic development, and increase health equity.

Major and persistent infectious disease threats, their global incidence, and some of the global health commitments made to address them are shown in the online Technical Appendix (wwwnc.cdc.gov/EID/article/19/8/13-0121-Techapp1.pdf). The 1993 World Bank report Investing in

Health first highlighted the overwhelming role of HIV/AIDS, tuberculosis, and malaria in Africa (*5*), but only in the past decade have substantially increased investment and effort enabled measurable progress in these major infectious disease challenges. The world needs to maintain momentum to achieve ambitious health targets and implement recent scientific advances while simultaneously coping with economic austerity.

There is increasing pressure to use resources for biomedical interventions with the strongest evidence of efficacy. Efforts toward achieving an AIDS-free generation are centered around HIV treatment scale-up, prevention of mother-to-child transmission (including through immediate and life-long antiretroviral therapy for all HIV-infected pregnant women), medical male circumcision, HIV testing and counseling, and focus on key populations in which HIV infection is concentrated (*23*). The primary current research question in HIV/AIDS is how best to use antiretroviral therapy for individual health and for population-based prevention, and more specifically, whether immediate therapy upon early diagnosis would confer the greatest benefit (*24*,*25*). The commitment to virtual elimination of HIV disease in children (*26*) could usefully link new initiatives to traditional maternal and child health programs delivered through development funding.

Tuberculosis is decreasing in incidence in all regions of the world, although more slowly than expected in some regions (*27*). In the United States, 63% of all tuberculosis cases now occur in foreign-born persons, indicating likely acquisition of the infection outside the United States (*28*). The spread of drug-resistant tuberculosis and extensively drug-resistant tuberculosis (resistant to rifampin, isoniazid, quinolones and injectable antituberculous drugs) highlights global vulnerability and interrelatedness of health systems and challenges health equity. Key scientific advances concern better understanding of the role and use of antiretroviral therapy for persons with tuberculosis co-infected with HIV, new diagnostics with the potential to make case finding more effective, and less strikingly, new drugs.

The tools for combating malaria (insecticide-impregnated bed nets, indoor residual spraying of insecticide, artemisinin-based combination therapies, and intermittent preventive therapy for pregnant women) need further scale-up, but such tools are susceptible to development of resistance on the part of the vector or parasite, and evidence is accumulating that nets may be less durable than assumed (*26*,*29*). Despite the challenges, malaria elimination has risen up the global agenda in recent time.

Poverty-related diseases such as the 17 conditions categorized as neglected tropical diseases have also received increased investment, especially those for which mass drug administration offers a control strategy (*30*,*31*). A concern must be that some major causes of illness and death, such as visceral leishmaniasis and African human trypanosomiasis, remain overshadowed and unaddressed.

Two groups of diseases meriting global health attention are those that are epidemic prone or vaccine preventable, including influenza. The 2009 pandemic of influenza A(H1N1) demonstrated the global nature of the threat, as well as the need to consider strategies for provision of vaccine for all countries (*32*). Dengue and yellow fever are the major mosquito-borne viral infections, and both illustrate the concept of emerging infections promoted by diverse factors, such as urbanization, population growth, inadequate environmental hygiene, and vector resistance to insecticide. In recent years, large outbreaks involving a specific arbovirus, chikungunya virus, have affected the east coast of Africa and islands in the Indian Ocean with importation into Europe.

The second decade of this century has been designated as the decade of vaccines (*33*). The opportunity exists for a notable effect on the 2.5 million deaths of children annually from vaccine-preventable diseases, including through use of new vaccines for prevention of rotavirus and pneumococcal infection, and by strengthening routine services. Vaccination against type A meningococcal meningitis in the Sahel and against hepatitis B virus and human papillomavirus illustrate the unrivaled possibilities in terms of controlling previously deadly epidemics or virus-induced cancers. A major unfinished priority is polio eradication; this goal is particularly threatened by funding shortfalls and ongoing transmission in Pakistan, Afghanistan, and Nigeria, which have seeded infection in other countries in which polio had been eliminated (*34*).

Lack of access to water and sanitation highlights some of the greatest inequities in global health. Approximately 1 billion persons worldwide do not have clean drinking water, and ≈2.5 billion persons have to openly defecate, which is an affront to human dignity (*35*). Large epidemics of waterborne diseases continue to occur, as exemplified by ongoing cholera transmission in Haiti (*36*). It is difficult to explain why investment in separating human drinking water from human feces, the basis of the nineteenth century public health revolution in Europe and North America, has not been a higher political or development priority in resource-poor settings.

Noncommunicable Diseases

The high-level meeting on noncommunicable diseases at the General Assembly of the United Nations in 2011, only the second such meeting devoted to health, emphasized how these diseases now dominate health worldwide (*37*). More than 60% of preventable deaths worldwide are now attributable to noncommunicable diseases (cardiovascular diseases, cancers, diabetes, and chronic respiratory diseases); in low-income and middle-income countries, 48% of such deaths occur in persons <70 years of age, compared with

26% in high-income countries (*38*). The incidence of these conditions is also increasing rapidly in Africa, a region in which urbanization and population growth are most extreme.

The risk factors for noncommunicable diseases are associated with urbanization and altered lifestyles, especially smoking, physical inactivity, air pollution, unhealthy diet, and excessive alcohol use. Hypertension, obesity, and increased cholesterol levels are measurable indicators predicting adverse outcomes, and specific chronic infections, such as those with hepatitis B virus and human papillomavirus, are linked to certain cancers. Injuries and mental health were omitted from the 2011 United Nations agenda despite the increasing incidence of these conditions; each year >5 million deaths worldwide result from injuries and violence (*39*), and ≈1.3 million are caused by road traffic injuries. Mental and behavioral disorders are considered the largest contributor to years lived with disability (*22*).

Global funding for noncommunicable diseases is minimal and coordination is limited, although opportunities exist for integrating approaches to communicable and noncommunicable diseases. Implementation of surveillance to assess incidence and needs along with selected policy interventions to address them will have the greatest immediate effect for the least cost. Examples of such policies include restricting tobacco sales and access, raising tobacco taxes, limiting unsafe use of alcohol, enacting motorcycle helmet and seat belt laws, and reducing salt and trans fats in commercial food products. To encourage countries to take action, WHO is defining population-level targets for noncommunicable diseases and associated risk factors for program implementation (*37*). Experience with HIV/AIDS treatment scale-up (*40*) could provide useful lessons for a standardized approach to management of hypertension and diabetes, thereby enhancing cost-effectiveness; facilitating supervision, monitoring, and evaluation; and ensuring accountability.

Conclusions

Population growth, increased life expectancy of the world's citizens, and decreased age-specific mortality rates in children and young adults, especially those for infectious diseases, have contributed to the altered global health landscape. The New Global Health concerns health in all countries and encompasses poverty alleviation, universal health security, and delivery of appropriate public health and clinical services, including for the increasing prevalence of noncommunicable diseases.

Equity, universal health coverage and access, and fairness in health financing are global aspirations likely to feature prominently in discussions about what comes after the 2015 MDG target date. The unfinished infectious disease agenda will remain a priority, but common approaches will have to address noncommunicable diseases, regulation of commerce in medical technologies and pharmaceuticals, health financing, and systems strengthening. An emerging topic will be surveillance for and mitigation of effects of environmental and climate change.

Surveillance will have to be strengthened globally to track exposure to risk factors for the major causes of disability and death, disease outcomes, and health systems responses. The past and on-going epidemiologic transitions mean that in many countries, the classic health indicators of international health (infant, children under 5, and maternal mortality rates) no longer provide insight into population health. In addition, there is an urgent need for robust vital registration systems and accurate reporting of cause-specific mortality rates across all life stages.

We must not forget the current challenges facing the lowest-income countries, the needs of disenfranchised or displaced populations, societies threatened by conflict and humanitarian emergencies, and the urban and rural poor living conditions in the midst of plenty. Nonetheless, global health practice must adapt to globalization and the rapid evolution in health underway worldwide. For donor countries, this will require clear definition of expectations of development assistance and how this differs from other forms of global health engagement, especially for health security and noncommunicable diseases. How to provide appropriate coordination, the kind of leadership desired, and how to ensure the shared responsibility of funding beyond the traditional donors will all feature prominently. Global interconnectedness requires us to address the health of the planet's entire population, irrespective of national borders. Engagement in global health is not simply a humanitarian concern but a priority for our collective well-being, efficient use of resources, and safeguarding our future.

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Norovirus Disease in the United States

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Although recognized as the leading cause of epidemic acute gastroenteritis across all age groups, norovirus has remained poorly characterized with respect to its endemic disease incidence. Use of different methods, including attributable proportion extrapolation, population-based surveillance, and indirect modeling, in several recent studies has considerably improved norovirus disease incidence estimates for the United States. Norovirus causes an average of 570–800 deaths, 56,000–71,000 hospitalizations, 400,000 emergency department visits, 1.7–1.9 million outpatient visits, and 19–21 million total illnesses per year. Persons ≥65 years of age are at greatest risk for norovirus-associated death, and children <5 years of age have the highest rates of norovirus-associated medical care visits. Endemic norovirus disease occurs year round but exhibits a pronounced winter peak and increases by ≤50% during years in which pandemic strains emerge. These findings support continued development and targeting of appropriate interventions, including vaccines, for norovirus disease.

Recognition of the public health impact of noroviruses has increased in recent years, driven largely by an abundance of reported outbreaks. A systematic literature review identified >900 published reports of laboratory-confirmed norovirus outbreaks during 1993–2011 (*1*). In contrast, studies assessing endemic norovirus disease are limited primarily to etiologic studies of acute gastroenteritis among children seeking medical care (*2*). Such prevalence studies provide valuable insights into the role of norovirus among patients with acute gastroenteritis. However, robust assessment of the norovirus disease burden, which herein refers to the annual number of illnesses and associated outcomes, requires population-based incidence estimates, ideally from national or nationally representative surveillance. However, there are several challenges to generating such estimates for norovirus in the United States, including lack of a widely used, rapid, and sensitive clinical assay; no public health reporting requirement for individual cases; low health care–seeking rates of patients with acute gastroenteritis; and poor sensitivity of norovirus-specific codes in national administrative databases (*3*).

Before 2008, only 1 published report estimated the burden of norovirus disease in the United States (*4*). In that report, as part of a broader effort to estimate the US burden of foodborne disease, Mead et al. generated pathogen-specific estimates of illnesses, hospitalizations, and deaths, and they estimated the fraction of these outcomes caused by foodborne disease transmission. Annual norovirus-associated illnesses (23 million), hospitalizations (50,000), and deaths (310) were based on extrapolation of the norovirus-attributable proportion from a single community-based study in the Netherlands and applied to the US all-cause acute gastroenteritis incidence from the National Hospital Discharge Survey (NHDS) and the first Population Survey of the Foodborne Diseases Active Surveillance Network (FoodNet). Although limited by the absence of direct US data on norovirus prevalence or incidence, this landmark study demonstrated the predominant role of norovirus in causing foodborne disease and became the most widely cited estimate of the US norovirus disease burden for more than a decade.

We review a collection of subsequently published studies that provided population-based incidence rates of norovirus disease in the United States. By comparing the various methods and triangulating the results, we provide summary estimates of the overall US norovirus disease burden, including specific estimates by age groups and disease outcomes. This review facilitates identification of key groups that would benefit from prevention strategies aimed at controlling norovirus and provides the grist for development of appropriate interventions, including vaccines. Such data are particularly timely and relevant given that a candidate norovirus vaccine is approaching a phase 3 efficacy trial and could potentially be licensed within the next 5–7 years (*5*).

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Methods and Findings

Since publication of the original estimates reported by Mead et al. (*4*), seven studies have been published that provide norovirus disease incidence estimates for the United States (Table 1). These studies can be broadly grouped on the basis of methods into the following categories: attributable proportion extrapolation, laboratory-confirmed population-based surveillance, and indirect attribution from regression modeling.

Attributable Proportion Extrapolation

Two studies used the available literature to first estimate the proportion of acute gastroenteritis attributable to norovirus then extrapolated that proportion to all-cause acute gastroenteritis incidence. Patel at al. conducted a systematic literature review of the prevalence of norovirus among persons with acute gastroenteritis in the community, outpatient clinics, emergency departments (ED), and hospitals (*2*). Most of the 31 studies included in the review involved hospitalized children and only 1 of the studies was conducted in the United States, underscoring the limited scope of the available literature. Among hospitalizations or ED visits for acute gastroenteritis in children <5 years of age, a pooled proportion of 12% of cases was attributed to norovirus. This norovirus prevalence was then extrapolated to national estimates of acute gastroenteritis in children <5 years of age from NHDS, the National Ambulatory Medical Care Survey (NAMCS), and the National Hospital Ambulatory Care Survey (NHAMCS) (*12*). The resulting annual estimates of 235,000 outpatient visits, 91,000 ED visits, and 23,000 hospitalizations associated with norovirus in US children <5 years of age suggested that norovirus was second only to rotavirus (before implementation of the national rotavirus vaccine program) as a cause of severe acute gastroenteritis in children.

Building upon the approach taken by Mead et al. (*4*), Scallan et al. reported new estimates of the US burden of foodborne disease (*6*). Although specific data sources had improved over the 12 years separating these 2 reports, the methods for estimating norovirus disease remained largely the same, constrained by the dearth of direct testing data in the United States. On the basis of community studies in the United Kingdom and Australia, and the study in the Netherlands used by Mead et al. (*4*), Scallan et al. estimated that 11% of acute gastroenteritis cases were caused by norovirus (*6*). This attributable proportion was then extrapolated to US rates of all-cause acute gastroenteritis from 3 FoodNet population surveys, hospitalizations from 3 databases (NHDS, the Healthcare Cost and Utilization Project Nationwide Inpatient Sample, and NAMCS/NHAMCS), and deaths from the multiple cause-of-death mortality database in the National Vital Statistics System. The resulting norovirus burden estimates across all ages in the United States were slightly lower than those by reported Mead et al. (*4*) in terms of total illnesses (21 million) but higher with respect to hospitalizations (56,000) and deaths (570). The increased estimate for hospitalizations can be explained in part by the fact that Scallan et al. (*6*) extrapolated the norovirus-attributable proportion to all-cause acute gastroenteritis across all age groups, whereas Mead et al. (*4*) applied

Table 1. Studies estimating incidence of norovirus disease, United States*

Study (reference)	Age group, y	Norovirus-associated outcome	Data source	Data period	Method
Mead et al. (*4*)	All	Deaths, hospitalizations, illnesses	NHDS, FoodNet	1979–1997	Attributable proportion extrapolation
Patel et al. (*2*)	<5	Hospitalizations, ED visits, outpatient visits	NHDS, NAMCS/NHAMCS	1993–2002	Attributable proportion extrapolation
Scallan et al. (*6*)	All	Deaths, hospitalizations, illnesses	NVSS, HCUP-NIS, NHDS, NAMCS/NHAMCS, FoodNet	2000–2006	Attributable proportion extrapolation
Hall et al. (*7*)	All	Outpatient visits, illnesses	HMO passive surveillance, FoodNet	2004–2005	Laboratory-confirmed population-based surveillance
Payne et al. (*8*)	<5	Hospitalizations, ED visits, outpatient visits	NVSN active surveillance, NAMCS/NHAMCS	2008–2010	Laboratory-confirmed population-based surveillance
Hall et al. (*9*)	<5, 5–64, ≥65	Deaths	NVSS	1999–2007	Indirect attribution from regression modeling
Lopman et al. (*10*)	<5, 5–17, 18–64, 65–74, 75–84, ≥85	Hospitalizations	HCUP-NIS	1996–2007	Indirect attribution from regression modeling
Gastañaduy et al. (*11*)	<5, 5–17, 18–64, ≥65	ED visits, outpatient visits	MarketScan	2001–2009	Indirect attribution from regression modeling

*NHDS, National Hospital Discharge Survey; ED, emergency department; NAMCS/NHAMCS, National Ambulatory Medical Care Survey/National Hospital Ambulatory Medical Care Survey; NVSS, National Vital Statistics System; HCUP-NIS, Healthcare Cost Utilization Project Nationwide Inpatient Sample; FoodNet, Foodborne Diseases Active Surveillance Network; HMO, health maintenance organization; NVSN, New Vaccine Surveillance Network.

the norovirus fraction to all-cause acute gastroenteritis only in adults.

Laboratory-confirmed Population-based Surveillance

In recognition of the need to directly assess the incidence of laboratory-confirmed norovirus infections among acute gastroenteritis patients in the United States, 2 surveillance platforms were leveraged to generate this data. In collaboration with FoodNet and a health maintenance organization (HMO) in the state of Georgia, Hall et al. used a passive sampling strategy in a population-based study of acute gastroenteritis incidence among outpatients (*7*). A random sample of fecal specimens submitted for routine clinical diagnostics (i.e., bacterial culture) were aliquoted for subsequent norovirus testing. Because the samples were derived from a known population catchment based on HMO membership, the resulting norovirus prevalence could then be used to calculate incidence. Health care use rates from 3 FoodNet population surveys were used to scale-up the observed prevalence among patients who submitted fecal specimens to outpatient and community incidence. The resulting adjusted outpatient and community incidence rates for norovirus were 64/10,000 population and 650/ 10,000 population, respectively (Table 2). If applied to the US population when the samples were collected (2004), these rates correspond to a national estimate of 19 million illnesses and 1.9 million outpatient visits. This total number of norovirus illnesses was within the uncertainty bounds of the estimate of Scallan et al. (*6*) (90% credibility interval 13–31 million) and provided the first estimate based on direct testing of patients with acute gastroenteritis in the United States. Although this passive sampling approach afforded convenience and required relatively little resources, it is potentially subject to substantial bias for 2 reasons. First, only those fecal samples that had a physician order for bacterial culture were tested. Second, the data may have limited generalizability because the study was conducted in a single, relatively young, privately insured population.

The preferred approach of active surveillance enrollment and laboratory testing of all acute gastroenteritis patients from multiple sites was used in a recent study by Payne et al. from the New Vaccine Surveillance Network (*8*). This network of 3 pediatric hospitals conducted year-round, population-based, active surveillance for hospitalizations, ED visits, and outpatient clinic visits for acute gastroenteritis among children <5 years of age for whom laboratory confirmation of cases was available. Payne et al. reported annual norovirus hospitalization, ED visit, and outpatient visit rates of 7, 141, and 319/10,000 children <5 years of age, respectively, over a 2-year period (*8*). Because the outpatient surveillance in the New Vaccine Surveillance Network used sentinel clinics and was not truly population based, the norovirus outpatient visit rate was based on extrapolation of norovirus prevalence to the all-cause acute gastroenteritis outpatient rates from NAMCS/NHAMCS. Extending these norovirus incidence rates to the ≈20 million US children <5 years of age, Payne et al. estimated 14,000 hospitalizations, 281,000 ED visits, and 627,000 outpatient visits for this age group (*8*). They also reported that the median health care charges for norovirus hospitalizations, ED visits, and outpatient visits were $3,918, $435, and $151, respectively, corresponding to an annual total of $273 million in norovirus-associated treatment costs for US children <5 years of age. Compared with the estimates reported by Patel et al. (*2*) among

Table 2. Population-based rates of norovirus disease–associated outcomes across all age groups by outcome*

Outcome	Study (reference)	Country	Rate/10,000 population (uncertainty bounds)†
Deaths	Scallan et al. (*6*)	United States	0.019 (0.011–0.029)
	Hall et al. (*9*)	United States	0.027 (0.023–0.031)
	Verhoef et al. (*13*)	The Netherlands	0.040 (0.020–0.070)
Hospitalizations	Scallan et al. (*6*)	United States	1.9 (1.1–2.9)
	Lopman et al. (*10*)	United States	2.4 (NR)
	Verhoef et al. (*13*)	The Netherlands	1.2 (0.5–2.0)
Emergency department visits	Gastañaduy et al. (*11*)	United States	13.5 (8–18.9)
Outpatient visits	Hall et al. (*7*)	United States	64.0 (36–120)
	Gastañaduy et al. (*11*)	United States	57.0 (40–74)
	Verhoef et al. (*13*)	The Netherlands	92.0 (50–150)
	Phillips et al. (*14*)	United Kingdom	54.0 (48–60)
	Tam et al. (*15*)	United Kingdom	21.0 (14–30)
	Karsten et al. (*16*)	Germany	63.0 (29–107)
Total illnesses	Scallan et al. (*6*)	United States	698.0 (430–1,028)
	Hall et al. (*7*)	United States	650.0 (370–1,200)
	Verhoef et al. (*13*)	The Netherlands	380.0 (264–544)
	Phillips et al. (*14*)	United Kingdom	450.0 (380–520)
	Tam et al. (*15*)	United Kingdom	470.0 (391–565)
	Thomas et al. (*17*)	Canada	1,040.0 (924–1,163)

*NR, not reported.
†Uncertainty bounds represent 95% CIs for all studies, except for Scallan at al. (*6*), Hall et al. (*7*), and Thomas et al. (*17*), who used 90% credible intervals.

children <5 years of age, Payne et al. estimated ≈55% fewer hospitalizations but ≈2 times as many ED and outpatient visits (Figure 1). Moreover, Payne et al. reported that norovirus had become the leading cause of medically attended acute gastroenteritis in children during the post-rotavirus vaccine era.

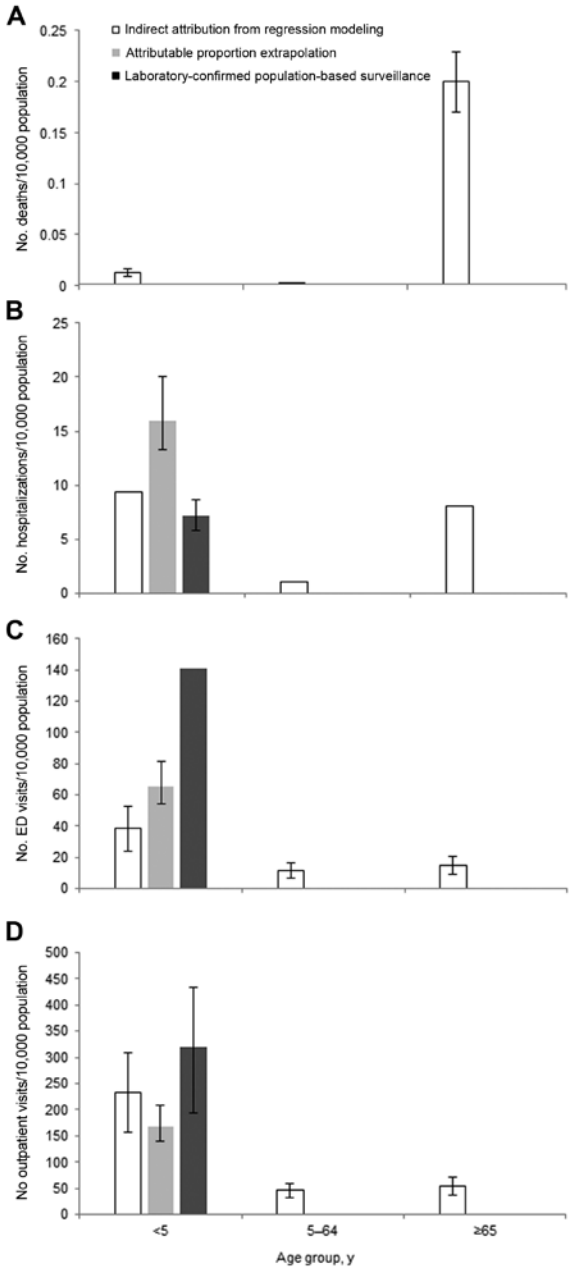

Figure 1. Rates of A) norovirus-associated deaths. B) hospitalizations, C) emergency department (ED) visits, and D) outpatient visits by age group, United States. Data were derived from studies using indirect attribution from regression modeling (*9*–*11*), attributable proportion extrapolation (*2*), and laboratory-confirmed population-based surveillance (*8*). Error bars indicate 95% CIs if reported.

Indirect Attribution from Regression Modeling

Population-based databases that use International Classification of Disease (ICD) coding are often used to estimate trends of specific syndromes or pathogens. Those databases that are national or nationally representative can be particularly helpful in overcoming the generalizability limitations of studies performed in specific populations that may not be broadly representative. However, ICD coding for specific pathogens is typically used only when there is laboratory confirmation (*18*). Given the limited availability of direct testing for norovirus among sporadic acute gastroenteritis cases, norovirus-specific coding in these databases is insensitive and unreliable. For example, Payne et al. retrospectively retrieved ICD–9-CM discharge diagnosis codes for 278 medically attended laboratory-confirmed norovirus cases identified by active surveillance and found that none had been assigned the norovirus ICD-9-CM code (008.63) (*8*).

To overcome this issue and still use these robust sources of data, we conducted a series of modeling studies to indirectly estimate the proportion of cause-unspecified acute gastroenteritis (which represents most acute gastroenteritis–coded events) likely caused by norovirus. In brief, time-series regression models used monthly counts of acute gastroenteritis attributed to specified causes other than norovirus to estimate the number of cause-unspecified acute gastroenteritis cases likely attributable to those causes. Events attributed to these other causes and to background nonseasonal causes were subtracted from the total cause–unspecified acute gastroenteritis, and the remaining unattributed events (i.e., model residuals) were then analyzed to generate norovirus estimates. Models were developed for specific age-groups to the extent this was possible for each specific outcome.

Applying this method to national mortality data from National Vital Statistics System, Hall et al. estimated that norovirus is associated with an average of 797 deaths/year (*9*). Most (90%) of these norovirus-associated deaths and the highest mortality rate (0.20 deaths/10,000 population) occurred among persons ≥65 years of age (Figure 1, panel A). Using Healthcare Cost and Utilization Project Nationwide Inpatient Sample data, Lopman et al. estimated an average of 71,000 norovirus-associated hospitalizations each year, resulting in $493 million in health care charges (*10*). Norovirus-associated hospitalization rates exhibit a U-shaped curve (Figure 1, panel B); the highest rates occur among persons <5 years of age (9.4 hospitalizations/10,000 population) and ≥65 years of age (8.1 hospitalizations/10,000 population). To estimate rates of norovirus-associated ambulatory visits, Gastañaduy et al. applied this same method to MarketScan insurance claims databases and reported norovirus associated with 13.5 ED visits and 57.2 outpatient visits/10,000 population across all age groups (*11*). In

contrast to rates of norovirus-associated mortality, rates of ambulatory visits associated with norovirus are highest in children <5 years of age (Figure 1, panels C, D). When Gastañaduy et al. extrapolated these rates to the US population, they estimated a national incidence of 399,000 ED visits and 1.7 million outpatient visits/year, corresponding to $284 million in health care charges.

Although this indirect modeling method has the potential for biases that might overestimate (e.g., assuming all residual seasonality in acute gastroenteritis is caused by norovirus) and underestimate (e.g., assuming none of the background nonseasonal incidence is associated with norovirus) norovirus incidence, it yielded temporal trends highly consistent with what is known about norovirus that help to ensure the validity of this method. These trends included a pronounced winter peak, with 63%–73% of all norovirus-associated events occurring during October–March and increases by ≤50% during years pandemic strains of norovirus emerged (i.e., 2002–2003 and 2006–2007) (Figure 2). These patterns have been well described through US surveillance of norovirus outbreaks (*19*–*21*) but had not been previously described among cases of sporadic norovirus illness. In addition, estimated rates of norovirus-associated outcomes from these models were generally consistent with those generated from population-based testing and attributable proportion extrapolation. For example, the all-ages outpatient rate modeled by Gastañaduy et al. (*11*) (57 outpatient visits/10,000 population) was within the uncertainty bounds of the estimate of Hall et al. (*7*) (90% credible interval 36–120 outpatient visits/10,000 population) from direct testing in the Georgia HMO population (Table 2). Likewise, the modeled hospitalization rate in children <5 years of age reported by Lopman et al. (*10*) (9.4 hospitalizations/10,000 population) was between the estimates obtained through direct testing by Payne et al. (*8*) (7.2 hospitalizations/10,000 population) and attributable proportion extrapolation by Patel et al. (2) (16 hospitalizations/10,000 population) (Figure 1, panel B).

Discussion and Conclusions

Over the past 5 years, substantial improvements have been made in our understanding of the burden of norovirus disease in the United States, which now represents the leading contributor to acute gastroenteritis across all age groups. By summarizing findings from studies using different methods and published over the past 5 years, we conclude that norovirus causes on average 570–800 deaths, 56,000–71,000 hospitalizations, 400,000 ED visits, 1.7–1.9 million outpatient visits, and 19–21 million total illnesses each year in the United States (Figure 3). On the basis of

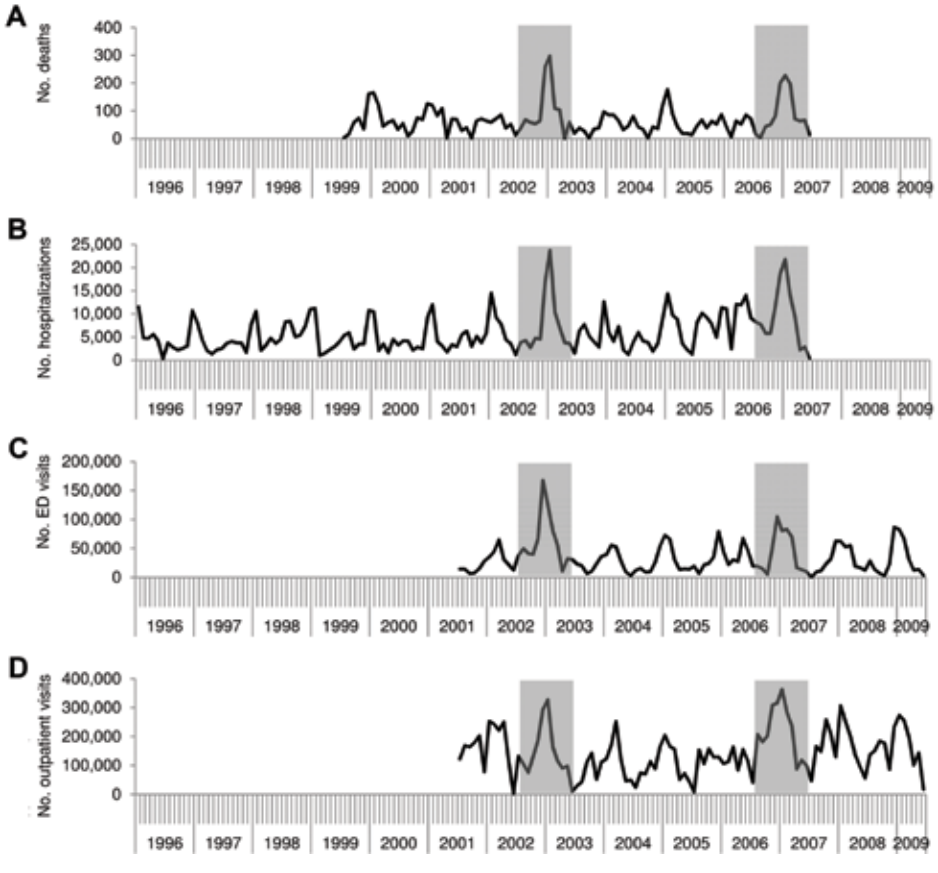

Figure 2. Number of A) norovirus-associated deaths, B) hospitalizations, C) emergency department (ED) visits, and D) outpatient visits across all age groups, by month and year, United States. Data were derived from studies using indirect attribution from regression modeling (*9*–*11*). Shaded areas indicate years of pandemic strain emergence (2002–2003 and 2006–2007).

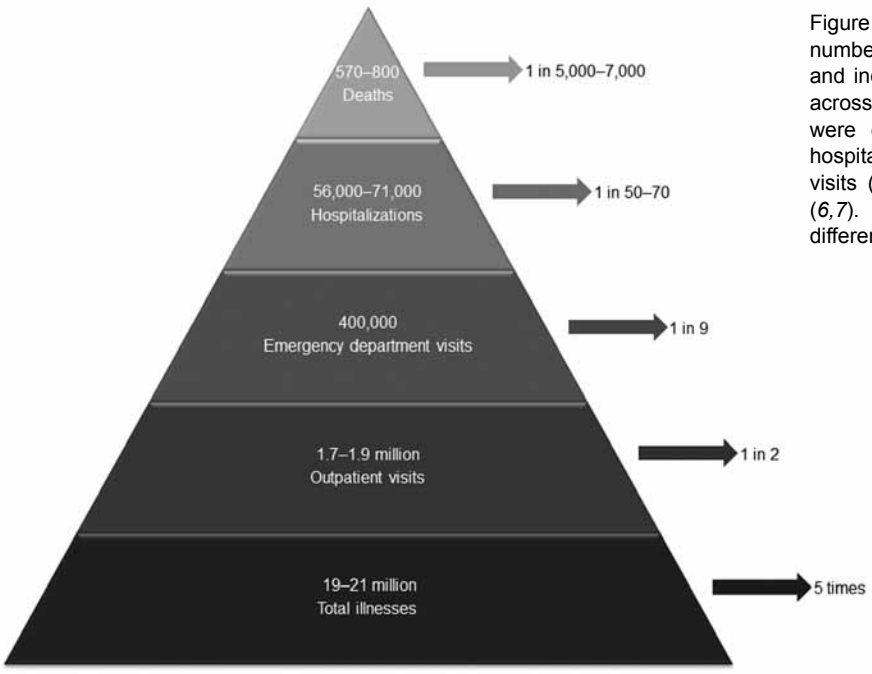

Figure 3. Estimates of annual burden (annual number of illnesses and associated outcomes) and individual lifetime risks for norovirus disease across all age groups, United States. Data were derived from estimates of deaths (*6,9*), hospitalizations (*6,10*), emergency department visits (*13*), outpatient visits (*7,11*), and illnesses (*6,7*). Ranges represent point estimates from different studies, not uncertainty bounds.

these rates of disease and a life expectancy of 79 years, a US resident would experience 5 episodes of norovirus gastroenteritis in his or her lifetime and an average lifetime risk for norovirus-associated outpatient visit, ED visit, hospitalization, and death of 1 in 2, 1 in 9, 1 in 50–70, and 1 in 5,000–7,000, respectively. Through age-group specific analyses, we identified that older Americans ≥65 years of age have the greatest risk for norovirus-associated deaths, and children <5 years of age have the highest rates of norovirus-associated medical care visits. In addition, we consistently observed across the reviewed studies increases in norovirus disease during the winter months and during years in which pandemic strains emerged.

Although the estimates summarized herein were developed by using distinct methods, each with their own strengths and limitations, the broad agreement among them is reassuring and provides a clearer picture of the norovirus disease burden in the United States. Population-based surveillance for laboratory-confirmed norovirus disease provides the most direct assessment of disease incidence, but depending on the study population, might have limited generalizability. Indirect attribution from regression modeling makes use of the most nationally representative data available but relies on temporality of acute gastroenteritis to ascribe etiology, as opposed to diagnostic testing. Attributable proportion extrapolation is somewhat of a hybrid between these 2 methods, being limited primarily by the comparability of the 2 populations involved in the extrapolation. Aside from differences in methods, the variation between estimates from the different studies might be partly caused by different time periods from which they were derived, given major year-to-year fluctuations in norovirus disease driven by the emergence of new strains.

Comparison of US norovirus incidence estimates with the few similar such estimates available from other industrialized countries showed general consistency in magnitude, especially when one considers that the uncertainty surrounding these estimates often exceeds 50% (Table 2). For example, a recent study in the Netherlands (*13*) reported a slightly higher norovirus-associated mortality rate (0.40 deaths/10,000 population) than the 2 recent US estimates (0.027 and 0.019 deaths/10,000 population) but a lower hospitalization rate (1.2 vs. 2.4 and 1.9 hospitalizations/10,000 population, respectively) (*6,9,10*). Rates of outpatient norovirus incidence from 2 studies in the United Kingdom (21 and 54 outpatient visits/10,000 population) (*14,15*) and 1 study in Germany (63 outpatient visits/10,000 population) (*16*) were consistent with 2 recent US estimates of 57 and 64 outpatient visits/10,000 population (*7,11*). Estimates of community norovirus incidence determined on the basis of 2 large-scale prospective cohort studies in the United Kingdom (470 and 450 illnesses/10,000 population) (*14,15*) and 1 study in the Netherlands (380 illnesses/10,000 population) (*13*) were all lower than the 2 recent US estimates (650 and 700 illnesses/10,000 population) (*6,7*). In contrast, a recent estimate in Canada (*17*) (1,040 illnesses/10,000 population) (*17*) was higher than estimates in the United States. However, the uncertainty bounds for the US estimates overlaps with those surrounding estimates for the United Kingdom, the Netherlands, and Canada (Table 2).

Although differences in health care delivery systems and payment structures confound direct comparisons of health care visits and associated costs between countries, the substantial burden of norovirus disease is clearly not unique to the United States.

Great strides have been made in characterizing the incidence of norovirus disease in the United States; however, additional work is needed to fill some key gaps. Age-specific rates of norovirus disease, ideally from direct laboratory testing among population-based community cohorts, would help identify groups most often infected and thus those likely serving as primary human reservoirs for transmission. The causal role of norovirus and common concurrent conditions in norovirus-associated deaths also requires further clarification to help protect the most vulnerable populations. In addition, stable surveillance platforms that enable systematic and ongoing assessment of endemic norovirus disease are needed to characterize long-term trends, annual fluctuations, and effects of emergent norovirus strains.

As progress continues in the arena of norovirus vaccine development (*5*), such endemic norovirus disease data will be critical to guide formulation and quantify potential effects of vaccine. The burden of norovirus disease in the United States justifies continued efforts toward developing potential norovirus vaccines and identification of specific groups for such interventions. Our review suggests that for a vaccine to have maximal impact, it would need to demonstrate safety and effectiveness in young children and the elderly, groups at the highest risk for severe norovirus disease. Other groups at risk for epidemic disease might also include health care workers, travelers, and military personnel. Data from our review can inform cost-effectiveness and modeling studies to define an investment case and public health strategy for controlling norovirus disease in anticipation of completion of vaccine development and licensure.

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Dr Hall is an epidemiologist at the Centers for Disease Control and Prevention, Atlanta, GA. His research interests focus on all aspects of the epidemiology of noroviruses and other agents of viral gastroenteritis.

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Extended-Spectrum β-Lactamase–producing *Enterobacteriaceae* among Travelers from the Netherlands

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Distinguish the rate of acquisition of multidrug-resistant *Enterobacteriaceae* (MDR-E) after foreign travel
- Analyze geographic areas associated with the highest risk for acquisition of MDR-E
- Evaluate other risk factors for the acquisition of MDR-E during travel
- Assess characteristics of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in the current study.

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A prospective cohort study was performed among travelers from the Netherlands to investigate the acquisition of carbapenemase-producing *Enterobacteriaceae* (CP-E) and extended-spectrum β-lactamase–producing *Enterobacteriaceae* (ESBL-E) and associated risk factors. Questionnaires were administered and rectal swabs were collected and tested before and after return. Of 370 travelers, 32 (8.6%) were colonized with ESBL-E before travel, 113 (30.5%) acquired an ESBL-E during travel, and 26 were still colonized 6 months after return. No CP-E were found. Independent risk factors for ESBL-E acquisition were travel to South and East Asia. Multilocus sequence

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typing showed extensive genetic diversity among *Escherichia coli*. Predominant ESBLs were CTX-M enzymes. The acquisition rate, 30.5%, of ESBL-E in travelers from the Netherlands to all destinations studied was high. Active surveillance for ESBL-E and CP-E and contact isolation precautions may be recommended at admission to medical facilities for patients who traveled to Asia during the previous 6 months.

The effect of international travel on the spread of multidrug-resistant *Enterobacteriaceae* (MDR-E) became more evident during 2007–2010. Data obtained during that time from prospective studies among returning travelers from Australia, Canada, Sweden, and the United States (New York, New York) revealed high rates of extended-spectrum β-lactamase producing *Enterobacteriaceae* (ESBL-E) carriage, varying from 18% to 25% after foreign travel (*1*–*4*). Two of these studies also reported a pretravel ESBL-E carriage rate of 7.8%.

The identification of carbapenemase-producing *Enterobacteriaceae* (CP-E) produced another set of challenges. Carbapenemases, such as *Klebsiella pneumoniae* carbapenemases (KPC), New Delhi metallo-β-lactamase (NDM), OXA-48, VIM and IMP, are plasmid-encoded enzymes, which have emerged worldwide. The rate of acquisition of CP-E during foreign travel is unknown; no surveillance system to date tracks these rates, and such rates are included sporadically in case reports, such as the situation recently reviewed by Van der Bij and Pitout (*5*). In the Netherlands, CP-E were found for the first time in 2010 (*6*).

No data were available on the pre-and post-travel carriage rates among travelers from the Netherlands. Our objective was to investigate whether these travelers are at risk of MDR-E (ESBL-E and/or CP-E) by use of a prospective cohort study design. Because detailed microbiological data of the isolates and epidemiologic data are crucial for assessing the real public health impacts of these organisms, we also investigated the persistence of intestinal colonization and possible spread to household contacts 6 months after the travelers returned.

Materials and Methods

Study Design

A prospective cohort study was conducted at the travel clinic at the Leiden University Medical Center and at the Hollands Midden Municipal Health Services in Leiden, the Netherlands. During March–September 2011, all adults who made an appointment for travel advice and had the intention to travel to areas outside Europe, North America, and Australia were invited to participate in the study. Travelers <18 years of age and those who traveled >3 months were excluded. Only 1 person in a couple or travel group was included.

Participants were asked to complete an electronic questionnaire and to deliver a rectal swab sample immediately before and immediately after travel. Questionnaires were used to collect demographic data, previous medical history, and travel information. Travelers who acquired MDR-E after foreign travel were asked to fill out a third questionnaire and deliver a third rectal swab 6 months after return.

If travelers were positive for MDR-E 6 months after return, their household contacts were also requested to submit a rectal swab and questionnaire. Household contacts were defined as persons who shared the same household with a participant on a regular basis. MDR-E–positive participants were asked to deliver a fourth rectal swab at the same time. The study was approved by the Leiden University Medical Center medical ethics committee.

Bacterial Isolates

Rectal swab samples were collected with Stuart Agar Gel Medium Transport Swabs (Copan Diagnostics, Corona, CA). The swabs were inoculated in trypticase soy broth supplemented with cefotaxime 0.25 mg/L and vancomycin 8 mg/L (MP products, Groningen, the Netherlands) and incubated for 24 hours at 37°C. After overnight incubation, the trypticase soy broth samples were subcultured on chromogenic ESBL screening agar (ESBL-ID; bioMérieux, Marcy-l'Étoile, France) and sheep blood agar as a growth control. All gram-negative rods growing on the ESBL-ID were identified by using MaldiTof-MS with BioTyper software version 3.0 (Bruker Daltonics, Breman, Germany), and antimicrobial drug susceptibility testing was performed by using the VITEK2 system (BioMérieux). All isolates underwent ESBL confirmatory disk testing by disk diffusion for ceftazidime and cefotaxime or cefepime (in cefoxitin-resistant isolates), with and without clavulanic acid, as recommended by Clinical and Laboratory Standards Institute guidelines (www.clsi.org).

MICs for meropenem and ertapenem were determined by using Etests (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. MICs were interpreted by using EUCAST criteria (www.eucast.org/clinical_breakpoints/).

Molecular Characterization of β-Lactamases

Molecular characterization of the β-lactamase genes in ESBL-E was performed by using Check-MDR CT103 version 1.1 (Check-Points B.V., Wageningen, the Netherlands) to test microarrays. The principals of the microarray system and interpretation software have been described (*7*). Concisely, the system combines ligation-mediated amplification with the detection of amplified products on

a microarray to detect the various carbapenemase genes: OXA-48, NDM-1, IMP, VIM, and KPC; CTX-M groups: CTX-M group 1, 2, 9 or combined 8/25; and the most prevalent ESBL-associated single-nucleotide polymorphisms in TEM and SHV-variants. Furthermore, the 6 plasmid-mediated AmpC β-lactamases can be identified (www.lahey.org/studies).

Molecular Typing of *Escherichia coli* Isolates

Multilocus sequence typing (MLST) was performed on all *E. coli* isolates by using 7 housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA*, and *recA*) to determine the corresponding sequence type (ST) and to designate the sequence type complex (STC) by using the MLST Databases at the Environmental Research Institute, University College Cork website (http://mlst.ucc.ie/mlst/dbs/Ecoli).

Data Analysis

A logistic regression model was used to determine risk factors for the acquisition of ESBL-E/CP-E after foreign travel for a total of 338 participants. Associations between acquiring an ESBL-E/CP-E after travel and different variables are calculated as odds ratios and p-values. Participants who were positive for ESBL-E/CP-E before travel were analyzed separately. Database processing and statistical analyses (univariate and multivariate analysis) were performed by using the SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). MLST analysis was performed by using BioNumerics software v.6.6 (Applied Maths, St-Martens-Lathem, Belgium).

Results

Study Population and Travel Characteristics

In total, 521 travelers were invited to participate in the study; 370 travelers completed 2 questionnaires and sent in 2 rectal swabs and were included in the analysis (Figure 1). The median age of the study population was 33 years (range 19–82), and 234 (63.2%) were women. The median length of stay abroad was 21 days (range 6–90 days). The most common reason for travel was vacation (n = 277).

Of the 370 participants, 113 (30.5%) whose pretravel swab samples were negative acquired MDR-E during foreign travel. Of these 113 participants, 19 (16.8%) still carried MDR-E 6 months after return. In 32 of the 370 participants (8.6%), MDR-E was identified before travel. Twenty (62.5%) of these 32 participants returned with MDR-E, 7 (35.0%) of whom were still colonized after 6 months. No MDR-E was found before or after travel in 225 (60.8%) participants.

Travel-associated Risk Factors for ESBL Acquisition in Returning Travelers

For the analysis of travel-associated risk factors, data for 338 participating returning travelers with negative pretravel rectal swab sample test results were used (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/19/8/13-0257-Techapp1.pdf). In total, 65 countries were visited; these are subdivided in 10 subcontinents. The most common destinations were Indonesia (n = 62), Thailand (n = 30), Malaysia (n = 27), Cambodia (n = 21),

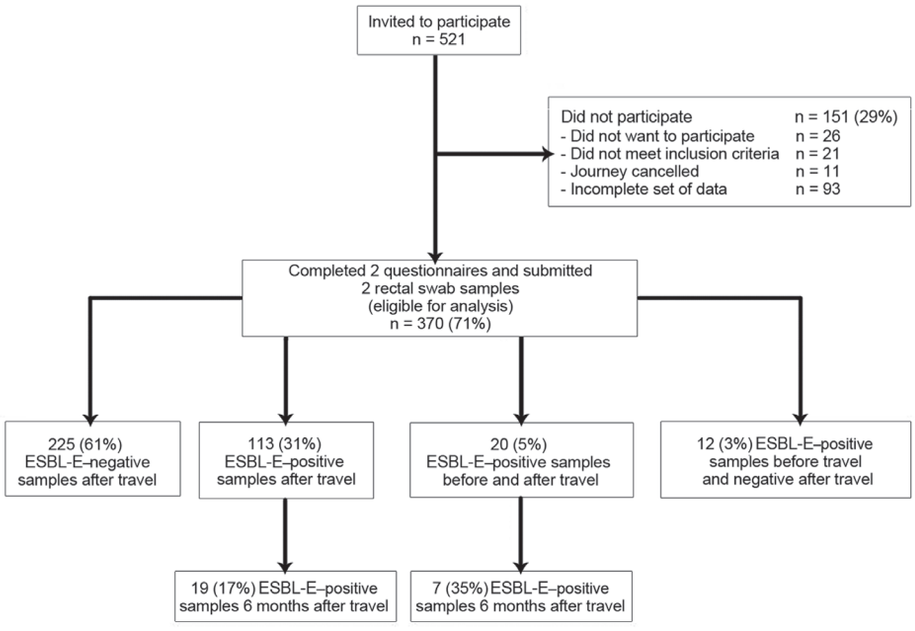

Figure 1. Participant colonization by *Enterobacteriaceae* species immediately before, immediately after, and 6 months after travel. ESBL-E: extended-spectrum β-lactamase producing *Enterobacteriaceae*.

People's Republic of China (n = 39), Kenya (n = 30), Tanzania (n = 24), Surinam (n = 20), and South Africa (n = 19).

The highest ESBL-E acquisition rates were identified among participants who visited countries in Asia: 73% in South Asia and 67% in East Asia. Univariate and multivariate analyses showed that the travel destinations South and East Asia were significant risk factors for the acquisition of ESBL-E (p<0.001). Participants traveling to Asia (all subcontinents) were more likely to return with ESBL-E colonization after a self-arranged trip (odds ratio 1.7; p = 0.07) or if they stayed in hostels/lodges (odds ratio 1.9; p = 0.08), although this finding was not statistically significant. There were no other risk factors for the acquisition of ESBL-E after foreign travel. The incidence proportions of ESBL-E after foreign travel are listed in Table 1.

Microbiological Results and Molecular Characterization

A total of 133 participants were colonized with MDR-E after travel. This group consisted of 113 travelers who had initially negative pretravel swab samples. In addition, 20 participants who had positive pretravel samples also returned colonized with MDR-E. The ESBL-E of these 133 post-travel swab samples consisted of 146 *E. coli*, 10 *K. pneumoniae*, and 2 *Enterobacter cloacae* isolates.

No carbapenemase-producing MDR-E were found among the pre-and post-travel isolates. Molecular characterization of the post-travel isolates demonstrated that CTX-M group 1 ESBL (n = 110) predominated (CTX-M-1–like, n = 4; CTX-M-3–like, n = 1; CTX-M-15–like, n = 85; CTX-M-32–like, n = 20), followed by CTX-M group 9 ESBL (n = 42), CTX-M group 2 (n = 2), and CTX-M group8/25 (n = 1). One *E. coli* isolate carried an SHV-ESBL (238S+240K). In addition, some isolates coproduced plasmid-mediated AmpC β-lactamase, ACT/MIR (n = 1) or CMY-2 (n = 2).

Thirty-four ESBL-E were isolated from pretravel rectal swab samples from 32 participants: 29 (85.3%) samples were positive for *E. coli,* 4 for *K. pneumoniae* (11.8%), and 1 for *Citrobacter freundii* (2.9%). The CTX-M group 1 ESBL (n = 22) comprised (CTX-M–1 like, n = 4; CTX-M-15–like, n = 16; CTX-M-32–like, n = 2); the remaining ESBL isolates belonged to CTX-M group 9 ESBL (n = 8) and CTX-M group 2 (n = 1). Two *E. coli* isolates carried an SHV-ESBL (238S+240K).

Coresistance to other classes of antimicrobial drugs was common in pre- and post-travel isolates; 67% displayed resistance to trimethoprim/sulfamethoxazole, 36% to ciprofloxacin, 37% to tobramycin, 35% to gentamicin, and 29% to nitrofurantoin. All isolates were susceptible to colistin and carbapenems.

MLST of ESBL-producing *E. coli* Isolates

MLST of 146 *E. coli* isolates from the post-travel samples identified 86 different STs; 31 new STs were found. The most prevalent STs were: ST38 (12%; n = 17), ST10 (7%; n = 10), and ST131 (4%; n = 9). The distribution of the CTX-M groups and types and STs is displayed in Figure 2. There was no association between ST and ESBL type, nor were STs associated with specific travel destinations. Pretravel isolates showed a similar diversity of STs, of which 3 were ST131.

Prolonged Carriage and Household Contacts

Of the 133 participants whose samples were positive for ESBL-E after return, 127 (95.4%) completed the follow-up survey and provided samples after 6 months. ESBL-E was isolated from 26 (20.4%) samples (Table 2). None of these participants reported the use of antimicrobial drugs or were hospitalized during the previous 6 months; none were health care workers, and none reported contact with farm animals. Diarrhea was reported by 7 participants.

Of 113 participants who had initially negative pretravel samples and positive samples immediately after return, 19 (16.8%) were still colonized after 6 months.

Table 1. Incidence proportions and incidence rates for extended-spectrum β-lactamase producing *Enterobacteriaceae* colonization in 338 travelers from the Netherlands*

Destination	No. travelers	No. (%) travelers with ESBL-E after return	Incidence proportion, % (SE)	Person-days, all travelers	Mean duration of travel, all travelers, d	ESBL incidence rate/100 pdt (SE)
Southeast Asia	110	37 (34)	34 (4.5)	2,980	27	1.24 (0.20)
East Asia	33	22 (67)	67 (8.3)	776	24	2.83 (0.60)
South Asia	25	18 (72)	72 (9.2)	599	24	3.01 (0.70)
Central Asia	3	1 (30)	33 (33.3)	94	31	1.06 (1.06)
North Africa	10	4 (40)	40 (16.3)	112	11.2	3.57 (1.76)†
Central Africa	56	17 (30)	30 (6.2)	1,637	29	1.04 (0.25)
Southern Africa	26	3 (12)	12 (6.6)	631	25	0.48 (0.27)
Middle East	15	2 (13)	13 (9.1)	222	14.8	0.90 (0.64)
Central America and the Caribbean	28	7 (25)	25 (8.3)	544	19	1.29 (0.48)
South America	32	2 (6)	6 (4.4)	922	29	0.22 (0.15)
Total	338	113 (33)	33 (2.6)	8,536	25	1.32 (0.12)

*ESBL-E, extended-spectrum β-lactamase–producing *Enterobacteriaceae*; SE, standard error; pdt, person-days of travel.
†The ESBL incidence rate/100 pdt is represented by 4 travelers returning from North Africa who carried ESBL-E: 3 of them had traveled for 7 days and 1 had a 25-day stay abroad, which accounts for the high SE.

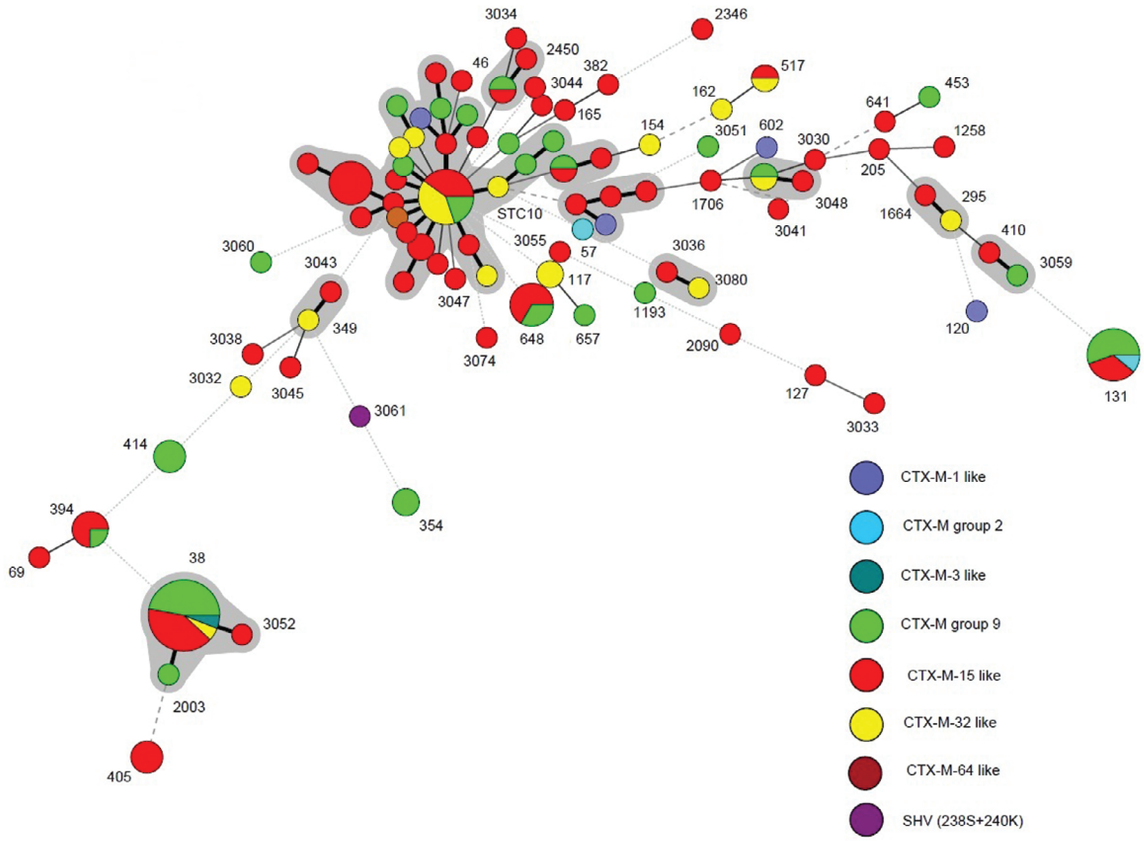

Figure 2. Multilocus sequence typing of *Escherichia coli* (n = 146) from the post-travel isolates of 133 travelers from the Netherlands. The numbers indicate the most prevalent sequence types (STs). Gray shadow indicates that >1 ST belongs to the same complex. The following sequences belong to STC10: ST4,10, 34, 43, 44, 48, 167, 193, 215, 218, 227, and 617. Thick connecting lines indicate single-locus variants; thin connecting lines indicate variants with 2–3 loci differences; dashed connecting lines indicate variants with 4 loci differences; dotted connecting lines indicate 5–7 loci differences.

Of these, 7 participants had samples that were positive for *E. coli* with the same ST 6 months after return. Nine participants were positive for *E. coli* and had a different ST 6 months after return; 3 were positive for a different species 6 months after return. Eleven household contacts of 4 MDR-E–positive participants agreed to cooperate and submitted a rectal swab sample. ESBL-producing *E. coli* was isolated from 2 (18.1%) household contacts, each from different households. The first household contact carried a different ESBL-producing *E. coli* than the associated traveler before and after the trip. Both isolates carried a CTX-M group 9 enzyme. The second household contact was positive for SHV-ESBL-producing *E. coli* ST2599. The associated traveler's samples were positive for *E. coli* ST617 and ST38 immediately after the trip, *K. pneumoniae* 6 months after return, and the fourth rectal swab sample was positive for a CTX-M-15–like *E. coli* ST3363.

Of 20 participants whose samples were positive before and after return, 7 (35.0%) participants were still colonized 6 months after return. Of these 7 participants, 5 carried a similar strain: 2 carried a CTX-M group 9–producing *E. coli* with an identical ST as before the trip, 2 carried a similar ST but with a different CTX-M group enzyme as before the trip, and 1 participant carried a CTX-M group 1–producing *K. pneumoniae* during the study period; 2 participants returned with *E. coli* with a different ST. No household contacts were included in this subgroup of travelers.

Discussion

The results of this study show a high ESBL-E carriage rate of 30.5% among healthy participating travelers from the Netherlands after return. This finding is worrisome, because this ESBL-E carriage rate is higher compared with those in recent studies that identified international travel as an independent risk factor for ESBL-E colonization (*1–4*).

Table 2. Microbiological and molecular characteristics of rectal swab samples collected from travelers from the Netherlands immediately pre- and post-travel and 6 mo after return*

	Pretravel sample			Immediate post-travel samples						Post-travel sample 6 mo after return†		
				Isolate 1			Isolate 2					
ID	Species	CTX-M group	ST	Species	CTX-M group	ST	Species	CTX-M group	ST	Species	CTX-M group	ST
25	Neg	NA	NA	*E. coli*	9	131	Neg	NA	NA	*E. coli*	9	131
45	Neg	NA	NA	*E. coli*	1	405	*E. coli*	9	38	*E. coli*	1	405
56	Neg	NA	NA	*E. coli*	1	3036	*E. coli*	1	517	*E. coli*	1	3267
60	Neg	NA	NA	*E. coli*	1	648	*K.p.*	1	ND	*E. coli*	1	648
61	Neg	NA	NA	*E. coli*	1	648	*E. coli*	9	227	*E. coli*	1	131
62	Neg	NA	NA	*E. coli*	9	3037	Neg	NA	NA	*E. coli*	9	501
80	Neg	NA	NA	*E. coli*	1	131	Neg	NA	NA	*E. coli*	9	1177
86	Neg	NA	NA	*E. coli*	1	93	*E. coli*	1	2090	*E. cloacae*	9	ND
137	Neg	NA	NA	*E. coli*	1	155	*E. coli*	1	617	*E. coli*	9	131
204	Neg	NA	NA	*E. coli*	1	38	Neg	NA	NA	*K.p.*	1	ND
211	Neg	NA	NA	*E. coli*	1	3044	Neg	NA	NA	*K.p.*	1	ND
222	Neg	NA	NA	*E. coli*	9	2003	Neg	NA	NA	*E. coli*	9	2003
238	Neg	NA	NA	*E. coli*	9	414	Neg	NA	NA	*E. coli*	9	10
251	Neg	NA	NA	*E. coli*	1	34	Neg	NA	NA	*E. coli*	1	450
309	Neg	NA	NA	*E. coli*	1	3045	Neg	NA	NA	*E. coli*	1	3045
373	Neg	NA	NA	*E. coli*	1	38	Neg	NA	NA	*E. coli*	1	3266
387	Neg	NA	NA	*E. coli*	1	131	Neg	NA	NA	*E. coli*	1	131
454	Neg	NA	NA	*E. coli*	9	10	Neg	NA	NA	*E. coli*	9	10
474	Neg	NA	NA	*E. coli*	1	154	Neg	NA	NA	*E. coli*	1	131
12	*E. coli*	9	38	*E. coli*	1	3074	Neg	NA	NA	*E. coli*	1	38
105	*E. coli*	1	191	*E. coli*	1	120	*E. coli*	1	38	*E. coli*	1	120
255	*E. coli*	9	131	*E. coli*	1	617	Neg	NA	NA	*E. coli*	9	131
269	*K.p.*	1	ND	*K.p.*	1	ND	Neg	NA	NA	*K.p.*	1	ND
283	*E. coli*	9	131	*E. coli*	1	46	Neg	NA	NA	*E. coli*	9	131
505	*E. coli*	1	1163	*E. coli*	1	69	Neg	NA	NA	*E. coli*	9	3268
512	*E. coli*	9	657	*E. coli*	9	657	Neg	NA	NA	*E. coli*	1	657

*ID, participant identification number; CTX-M, extended-spectrum β-lactamase enzyme; ST, sequence type; Neg, no species were isolated from sample; *E.*, *Escherichia*; *K.p.*, *Klebsiella pneumoniae*; NA, not applicable; ND, no sequence type data available.
†None of the participants with a positive rectal swab sample after 6 months reported antimicrobial drug use during the 6 months after return.

It is striking that none of the potential travel-associated risk factors investigated in this study, other than traveling to South and East Asia, were found to contribute to this high ESBL-E carriage rate. Additional risk factors were not revealed by including in the univariate analysis the 13 participants who had a positive pretravel sample and acquired an ESBL-producing *E. coli* during travel with a different ST than before the trip.

Tangden et al. associated gastroenteritis during travel with the risk for ESBL-E acquisition among travelers from Sweden (*3*). That association was not found in this study, which may reflect less fecal–oral contamination while traveling. Baaten et al. reported that diseases transmitted by the fecal–oral route among travelers to nonindustrialized countries have declined because of improved hygiene standards at the destination as measured by the human developmental index, sanitation index, and the water source index (*8*). The sanitation index levels, which represent the proportion of the population that has access to sanitation, were the lowest for sub-Saharan Africa and the Indian subcontinent. On the basis of these indices, we would expect the incidence of ESBL-E acquisition to be similar among travelers in countries in Asia and Africa. Nonetheless, participating travelers to Asia had the highest post-travel colonization rates. Travelers to Asia most likely differ in their eating habits compared with travelers to African countries, since the former are more likely to eat in individual establishments outside of hotels or from street vendors. Thus, the high incidence rate found for returning travelers from Asia in this study may result from the increased risk for foodborne exposure.

No CP-E were found despite the fact that countries were visited where CP-E are prevalent in hospitals and in the environment (*5*). Other known risk areas besides India for the acquisition of CP-E, such as the United States, Greece, Italy, and the Balkan region were not included in this study, because these travelers do not visit the Travel Clinic of the Leiden University Medical Center. Many citizens from the Netherlands have relatives in North African countries or Turkey whom they visit frequently. OXA-48–producing bacteria are endemic to these countries (*9*). These travelers do not consult travel clinics and may well return carrying OXA-48–producing isolates unnoticed.

Peirano et. al. (*2*) reported that the prevalence of ST131, a uropathogenic *E. coli* notorious for its worldwide expansion and spread of CTX-M-15, was similar among travelers and non-travelers from the Calgary region. The most prevalent ESBL among the travelers participating in this study was the CTX-M-15–like enzyme. However, this enzyme was found in a plethora

of different STs of *E. coli*. Participants in the Leiden area not only showed a great heterogeneity of STs but also harbored different CTX-M types after travel and 6 months after return. The majority of the *E. coli* strains identified in the participants in this study were of STs that clustered around ST10 and belonged to ST complex 10 (STC10). STC10 strains essentially belong to the nonvirulent, commensal phylogenetic group A (*10*). In a recent study based in France, isolates belonging to STC10 were found to be the most prevalent among fecal samples from healthy carriers of nalidixic acid–resistant (but ESBL-negative) *E. coli* (*11*). It is also the most prevalent STC in the MLST database. Data from this study show that transmissible genetic elements containing resistance genes are exchanged with naive *E. coli* strains of the human intestinal microbiota during foreign travel combined with foodborne exposure.

Although 26 participants had positive results for ESBL-E 6 months after travel, they were not all positive for the same enterobacterial strain that was identified immediately after travel. In 8 participating travelers colonized with *E. coli*, an ESBL of the same CTX-M group was identified in the immediate post-travel sample as after 6 months, but *E. coli* with a different ST was detected. In 11 travelers, the strain persisted during the study period. It is possible that more strain types were present in the rectal samples where colony morphology of different strains was not discriminative. However, it is also possible that the transfer of ESBL genes between strains within a host is a frequent occurrence. Or, the acquisition of a new ESBL-E occurs at the expense of the resident strain.

Interhousehold transmission of ESBL-E has been demonstrated in the community setting (*12,13*). Clonally related strains could be found for 66% of the isolates from infected community patients and their corresponding household contacts (*13*). Because of the limited data on household contacts in the present study, the transmission dynamics of ESBL-E in households after foreign travel remain to be discovered.

The high pretravel ESBL-E carriage rate among our study participants (8.6%) was an unexpected finding. Two recent studies on the ESBL-E carriage rate in the community have been conducted in the Amsterdam area. In the first study, 10.1% of the fecal samples from outpatients with gastrointestinal discomfort being assessed by their general practitioners yielded ESBL-E, predominantly CTX-M-15–producing *E. coli* (*14*). In a second study, investigating the prevalence of ESBL-E carriage in the general community, a carriage rate of 8.5% was found (E.A. Reuland et al., unpub. data). Although no data on travel history were given, the investigators pointed out that foreign travel might be responsible for at least part of ESBL-E carriage rates among outpatients from the Netherlands. This finding is supported by data from our study: 50% of participants who had a positive pretravel sample had traveled during the previous 12 months. This high percentage of carriers identified in this study before travel points toward ongoing importation of ESBL-E. Other potential reservoirs for ESBL-E are poultry and other retail meats, which have been found to be contaminated with ESBL-producing *E. coli* strains harboring the genes on identical plasmids as found in human isolates (*15,16*).

International travel is growing and the number of intercontinental flights has increased during the past decade. The findings in this study support the role of international travel on the ESBL-E acquisition and carriage rates among travelers from the Netherlands, especially to South and East Asia. The high pre- and post–travel carriage rates among persons traveling from the Netherlands indicate that the consequences of increased foreign travel are already manifest in this country. The lack of apparent travel-associated risk factors, the spread of CTX-M enzymes through a highly diverse population of *E. coli,* the association of ESBL production with multidrug resistance, and the possible role of other sources make containing the spread difficult. These factors also complicate the implementation of other strategies, such as pretravel advice, and imply that all travelers to Asia should be considered for carriage of ESBL-E. Although CP-E were not found in this study, CP-E have been introduced into the Netherlands by returning travelers (*6,17–19*), and introduction by asymptomatic travelers to the Netherlands from countries where CP-E are endemic may largely go unnoticed. There is no reason to assume that, after CP-E are introduced, their spread will be less dynamic than that of ESBL-E. This inference has serious implications for the implementation of screening methods and effective infection control strategies. On the basis of the results of this study, we recommend active surveillance of CP-E and ESBL-E and at least temporary contact isolation precautions for patients being admitted to hospitals after travel to Asia during the previous 6 months.

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Emergency Department Visit Data for Rapid Detection and Monitoring of Norovirus Activity, United States

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Noroviruses are the leading cause of gastroenteritis in the United States, but timely measures of disease are lacking. BioSense, a national-level electronic surveillance system, assigns data on chief complaints (patient symptoms) collected during emergency department (ED) visits to 78 subsyndromes in near real-time. In a series of linear regression models, BioSense visits mapped by chief complaints of diarrhea and nausea/vomiting subsyndromes as a monthly proportion of all visits correlated strongly with reported norovirus outbreaks from 6 states during 2007–2010. Higher correlations were seen for diarrhea (R = 0.828–0.926) than for nausea/vomiting (R = 0.729–0.866) across multiple age groups. Diarrhea ED visit proportions exhibited winter seasonality attributable to norovirus; rotavirus contributed substantially for children <5 years of age. Diarrhea ED visit data estimated the onset, peak, and end of norovirus season within 4 weeks of observed dates and could be reliable, timely indicators of norovirus activity.

Noroviruses are the most common cause of epidemic and sporadic gastroenteritis worldwide (*1*–*4*). In the United States, norovirus gastroenteritis causes an estimated 21 million cases of illness and ≈800 deaths annually (*5*,*6*), resulting in an estimated 1.7 million physician's office visits, 400,000 emergency department (ED) visits, and 71,000 hospitalizations each year. The estimated annual cost for norovirus-related health care in the United States is $777 million (*7*,*8*).

Timely monitoring of norovirus activity has remained elusive in part because of the scarcity of diagnostic testing for patients with suspected disease. Approximately 90% of persons with acute viral gastroenteritis do not seek medical attention; of those who do, only 6% submit stool specimens for diagnostic testing, in part because testing is often not deemed necessary for self-limited illness (*2*). Furthermore, no rapid and sensitive clinical assay is widely available in the United States, and definitive diagnosis requires PCR, which is used primarily in public health laboratories; therefore, few nonoutbreak cases are laboratory confirmed (*9*). As a result, existing US norovirus surveillance depends on outbreak investigations that can be subject to substantial delays in reporting. This process of notifying local/state health departments of disease, subsequently investigating the outbreak, testing specimens, and voluntarily reporting to the national surveillance system can vary widely in duration (days to months), which makes timely and uniform monitoring on a national level challenging (*9*,*10*).

The timing and magnitude of norovirus seasonal activity varies from year to year (*11*,*12*). Timely monitoring could rapidly identify the season onset and elevated levels of activity, which could potentially improve prevention and control efforts by public health and infection control personnel in health care settings, help with planning for increased health care utilization in facilities, and alert the public with timely prevention messages. Syndromic surveillance data based on ED visits related to gastroenteritis might be robust and timely surrogate measures of norovirus activity, given its characteristic wintertime seasonality.

BioSense is a timely, national-level electronic health surveillance system that receives and processes healthcare encounter data to conduct syndromic surveillance and is maintained by the Centers for Disease Control and Prevention. A subset of the data that BioSense receives is ED visit data: codes from the International Classification of Diseases, 9th Revision, Clinical Modification (www.cdc.gov/nchs/icd/icd9cm.htm), and chief complaint (i.e., patient-reported symptoms) information entered in text format. These data are then mapped in near real-time to 15 syndromes and 78 subsyndromes, including those related to gastroenteritis

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symptoms (*11*,*12*). For this study, we assessed whether BioSense chief complaint–based ED visit data could be a reliable indicator of norovirus activity in the United States by determining the degree of correlation of these data with reported norovirus outbreaks.

Methods

Data Sources

BioSense Chief Complaint Data

ED chief complaint–based data used in analyses were collected and processed daily in the BioSense 1.0 platform from >600 participating nonfederal hospitals in 26 states during January 2005–June 2011. In 2008, the median time from patient visit to chief complaint data receipt was 4 hours (*13*). Daily counts of ED visits that mapped to either of 2 BioSense subsyndromes, "Diarrhea" or "Nausea and Vomiting" (hereafter as diarrhea and nausea/vomiting), on the basis of chief complaint text were aggregated into weekly counts by state and age group. For example, any visit with a chief complaint text field featuring keywords such as "nausea" or "vomiting," as well as associated abbreviations and misspellings, was mapped to the nausea/vomiting subsyndrome. Diarrhea and nausea/vomiting visits were not necessarily mutually exclusive, because a given chief complaint text substring can be assigned to ≥1 subsyndrome by the BioSense system (*13*). Weekly counts of all-cause ED visits by state and age group were also compiled so that the proportion of ED visits mapped to each subsyndrome could be calculated.

Norovirus Outbreak Surveillance Data

Monthly norovirus outbreak counts were based on reports of suspected and confirmed norovirus outbreaks elicited from 30 states during January 2007–April 2010 (*12*); these monthly data were used as the main comparison for the BioSense data. For a separate analysis, weekly norovirus outbreak surveillance data were obtained from the National Outbreak Reporting System (NORS), a national-level, Internet-based reporting platform established in 2009 (*10*). These weekly data were based on suspected and confirmed norovirus outbreaks as reported by 47 state and territory health departments during January 2009–December 2011.

Rotavirus Laboratory Surveillance Data

To control for the possible contribution of rotavirus, another diarrheal pathogen with distinct winter seasonality, we obtained data reported to the National Respiratory and Enteric Virus Surveillance System by participating laboratories in 24 states during January 2006–June 2011. These laboratories report data aggregated on a weekly basis that includes the total number of rotavirus tests performed and the number of tests that were positive for rotavirus.

Data Analyses

Statistical Methods

Linear regression models were fitted to assess the degree of correlation between syndromic surveillance ED visit data from the BioSense system and norovirus outbreak activity following an approach that has been described (*14*). In a series of linear regression models, BioSense ED visits mapped by chief complaint to diarrhea and nausea/vomiting (as a monthly proportion of all ED visits) were regressed on monthly reported norovirus outbreaks from January 2007–April 2010. We restricted analysis to the 6 states (Georgia, Missouri, Ohio, Pennsylvania, Tennessee, and Wyoming) that had uninterrupted data during that time for 1) BioSense, 2) monthly norovirus outbreaks, and 3) rotavirus antigen tests (≥120 tests/year). For these analyses, weekly BioSense and rotavirus test data were aggregated by month. Linear regression models were fitted separately for 5 age groups (0–4, 5–17, 18–64, ≥65 years, and all ages). To avoid attributing secular trends in syndromic data to norovirus, a sequential variable for month of study was included in the models. In addition, a term for laboratory-reported test data for rotavirus, a major cause of gastroenteritis in those <5 years of age, was also included in the model initially. For the sake of parsimony, the rotavirus term was subsequently removed from models in which its coefficient was not significant or positive in preliminary analyses.

The models can be expressed with the following formulas:

$$pCC_{x,y} = \alpha + (\beta_1 \times Noro_y) + (\beta_2 \times Rota_y) + (\beta_3 \times Time_y)$$

for models in which the rotavirus term was significant and positive, and

$$pCC_{x,y} = \alpha + (\beta_1 \times Noro_y) + (\beta_2 \times Time_y)$$

for all other models, where pCC is the proportion of ED visits mapped by chief complaint to the subsyndrome of interest, Noro is the count of reported norovirus outbreaks, Rota is the proportion of positive rotavirus antigen tests, in age group x and year-month y, the intercept α represents the background proportion of ED visits mapped by chief complaint to the subsyndrome of interest, and Time is the sequential variable for month of study included to account for secular trends. These models assumed that the relationships between BioSense data and 1) norovirus outbreak and 2) rotavirus test data were constant over time. The Pearson correlation coefficient (R) and the coefficient of determination (R^2) were calculated for each model across the 5 age

groups to determine the strength and direction of the relationship between ED visits mapped by chief complaint to diarrhea or nausea/vomiting and norovirus outbreak data.

The proportions of diarrhea-related visits predicted to be attributable to norovirus, rotavirus, background, or secular trends for each month were estimated by multiplying the monthly values for each predictor with its corresponding coefficient in the regression model. The proportions of diarrhea visits attributable to background and secular trends were combined to establish a nonseasonal baseline proportion of diarrhea visits with other etiologies. To determine whether the relationship between BioSense ED visit data and monthly norovirus outbreaks was robust at the state level, linear regression models for the all-ages group were fitted separately for each of the 6 states.

Microsoft Excel (Microsoft, Redmond, WA, USA) and SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA) software were used to perform all analyses. Assumptions of linearity, common variance, and normality were checked by visual inspection of residual plots for all models.

Estimating Norovirus Seasonal Time Markers

To determine if BioSense ED visit data could be used to predict norovirus season markers (i.e., onset, peak, and end), we compared weekly diarrhea visits mapped by chief complaint as a proportion of all visits (for all ages) with weekly norovirus outbreak data from NORS for the 2009–2011 seasons on a national level. All states/territories that contributed data to BioSense (n = 26) and/or NORS (n = 46) during those seasons were included in this analysis. Outbreak data from NORS were used because they are weekly, which allowed for detection of seasonal time markers at the week level for these 2 seasons. First, NORS data were used to identify the onset, peak, and end weeks of the 2009–2010 norovirus season (weeks ending July 11, 2009–July 3, 2010). Season onset was defined as the week by which at least 10% of the year's cumulative total number of outbreaks had occurred, and season end was defined as the week by which at least 90% of the year's cumulative total number of outbreaks had occurred. The peak was defined as the week with the highest number of outbreaks. Using these definitions, we then used the observed onset, peak, and end week dates to formulate rules by which corresponding diarrhea visit data could be used to estimate the season's onset, peak, and end. These rules were then applied to 2010–2011 data (weeks ending July 10, 2010–July 2, 2011) to test their performance in estimating the time markers for that season.

Results

Correlation

During January 2007–April 2010, the 6 states analyzed reported 1,048 norovirus outbreaks and 32,455 rotavirus antigen tests (4,197 [13%] with positive results). During the same period, BioSense received data from the 6 states for 20,205,284 total ED visits, of which 277,433 (1.4%) were diarrhea and 1,165,414 (5.8%) were nausea/vomiting visits. More than half (56%) of all ED visits were by patients in the 18–64-year age group. For each month, the proportion of ED diarrhea visits was higher for children <5 years of age (0.022–0.054) than for persons in any other age group (0.008–0.025). Over the 40-month period, a seasonal pattern was observed in the proportion of diarrhea visits for each age group that mirrored the seasonal variation observed in the reported norovirus outbreaks, with peaks in the winter months (Figure 1). The proportion of positive rotavirus test results peaked 1–2 months after the peaks of norovirus outbreaks.

The monthly proportion of visits for diarrhea or nausea/vomiting had strong linear relationships with norovirus outbreaks for each age group ($p<0.001$), with stronger

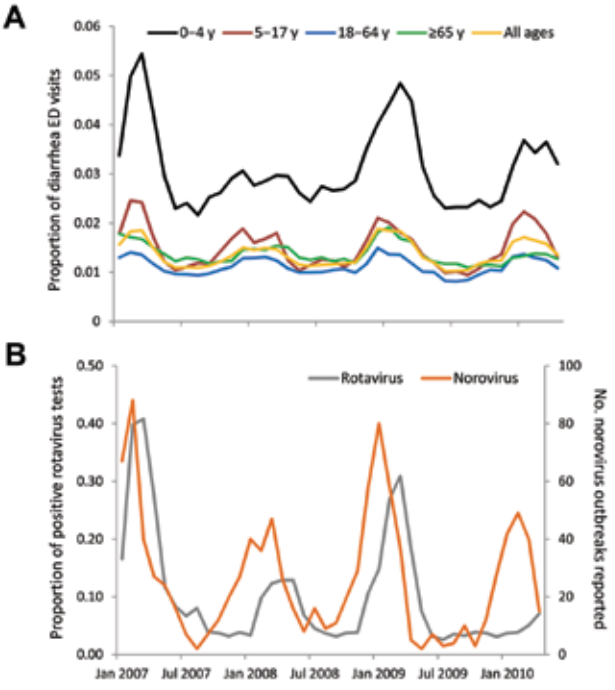

Figure 1. Proportion of BioSense emergency department (ED) visits for diarrhea subsyndrome (A) and norovirus and rotavirus surveillance data (B), United States, January 2007–April 2010. The proportion of ED visits mapped by chief complaint to diarrhea subsyndrome in the 6 states analyzed (Georgia, Missouri, Ohio, Pennsylvania, Tennessee, and Wyoming) and reported norovirus outbreak data displayed seasonal peaks in the winter months. This seasonal pattern was observed for all 5 age groups (0–4, 5–17, 18–64, ≥65 years, and all ages); a higher proportion of ED visits for diarrhea was seen among children <5 years of age. Rotavirus activity, as measured by the proportion of positive antigen tests, also showed winter seasonality, with peaks that lagged behind those of norovirus.

Table 1. Linear regression model estimates of the association between norovirus outbreaks and BioSense emergency department visit data, by age group, United States, January 2007–April 2010*

Subsyndrome and age group, y	Norovirus,† $\beta_1, \times 10^{-4}$ (95% CI)	p value‡	*R*
Diarrhea			
0–4§	1.72 (1.15–2.29)	<0.0001	0.926
5–17¶	1.59 (1.23–1.95)	<0.0001	0.828
18–64¶	0.70 (0.56–0.83)	<0.0001	0.864
≥65§¶	0.71 (0.55–0.86)	<0.0001	0.917
All ages§	0.94 (0.74–1.14)	<0.0001	0.910
Nausea/vomiting			
0–4§	7.01 (4.93–9.09)	<0.0001	0.866
5–17	5.73 (4.24–7.23)	<0.0001	0.796
18–64¶	1.60 (1.14–2.06)	<0.0001	0.758
≥65	0.61 (0.31–0.91)	0.0002	0.729
All ages	2.78 (2.15–3.40)	<0.0001	0.832

*Emergency department chief complaint–based subsyndrome visits as a monthly proportion of all visits in 6 states (GA, MO, OH, PA, TN, WY) regressed on norovirus and rotavirus surveillance data and time variable. Intercept for each model p<0.0001.
†Suspected and confirmed norovirus outbreaks.
‡By *t* test.
§Included term for proportion of rotavirus tests positive in the model, as it is significant and positive (p<0.05). β_2 for 0–4-y age group diarrhea model = 0.0539 (95% CI 0.0403–0.0674); β_2 for 0–4-y age group nausea/vomiting model = 0.0745 (95% CI 0.0252–0.1237); β_2 for ≥65-y age group diarrhea model = 0.0048 (95% CI 0.0011–0.0084); β_2 for all-ages age group diarrhea model = 0.0065 (95% CI 0.0018–0.0112).
¶Time variable not significant in model (p≥0.05).

correlations for diarrhea visits (R = 0.828–0.926) than for nausea/vomiting visits (R = 0.729–0.866) (Table 1). The proportion of positive rotavirus test results was significant and positive in the diarrhea and nausea/vomiting models for the 0–4-year age group, as were diarrhea models for the ≥65-year and all-ages groups (p<0.05). The time variable was kept in the models to give conservative estimates of the contribution of norovirus outbreaks, even though it was not significant for all models. Although a few models showed possible evidence of skew at extreme values, we kept our linear models for the sake of parsimony and used diarrhea models for all subsequent analyses because diarrhea had higher correlation across all age groups. The best-fit models were for diarrhea in the 0–4-year, ≥65-year, and all-ages groups (R^2 = 0.829–0.857). Overall, the models indicate that the seasonal variation by month in the proportion of diarrhea visits as reported by BioSense can be attributed to reported norovirus outbreak activity, with rotavirus also playing a role in the 0–4-year, ≥65-year, and all-ages groups.

Predicted Contribution of Norovirus/Rotavirus to BioSense Activity

The models predicted a linear baseline proportion of diarrhea visits that occur year-round, above which seasonal variation that is observed can largely be attributed to predicted values of norovirus (for all ages) or norovirus and rotavirus (for the 0–4-year age group) (Figure 2). For patients of all ages, norovirus was estimated to account for 17.5% of the predicted proportion of BioSense diarrhea visits over the 40-month span (rotavirus accounted for 4.7%), or 23.9% when restricting analysis to months that typically encompass the norovirus season (November–April). For children <5 years of age, we excluded data before August 2007 to capture the contribution of norovirus and rotavirus after the introduction of rotavirus vaccination. Norovirus accounted for 13.6% of the predicted proportion of diarrhea visits among children in this age group and rotavirus accounted for 13.7%.

State-level Robustness

When considering the relationship between BioSense diarrhea visit data for all ages and reported norovirus outbreaks at the state level, correlation was generally higher in states with a greater number of recorded ED visits/month. R≥0.60 was observed for all states with ≥5,000 BioSense ED visits/month (Figure 3; Table 2).

Estimating Season Markers

By applying our definitions of norovirus seasonal time markers to the 2009–2010 season by using weekly NORS outbreak data, we observed the following season markers (by week/year): onset, 47/2009; peak, 8/2010; and end, 19/2010. On the basis of visual inspection of the all-ages weekly national BioSense data for diarrhea visits for the 2009–2010 season, we determined the following rules to define the onset, peak, and end: 1) norovirus season begins when the proportion of diarrhea ED visits is ≥0.0125 (1.25%) for 2 consecutive weeks, 2) season peak occurs when the proportion of diarrhea ED visits is ≥0.0170 (1.70%) for 2 consecutive weeks, and 3) season ends when the proportion of diarrhea ED visits is ≤0.0125 (1.25%) for 2 consecutive weeks. Applying these rules to the 2009–2010 season yielded estimates for each season marker within 2 weeks of the observed dates (Figure 4). The rules were then tested on NORS data from the 2010–2011 season, yielding estimates within 4 weeks of the following observed marker dates: onset, 41/2010; peak, 52/2010; and end, 15/2011. Notably, the proposed rules correctly predicted the earlier observed onset, peak,

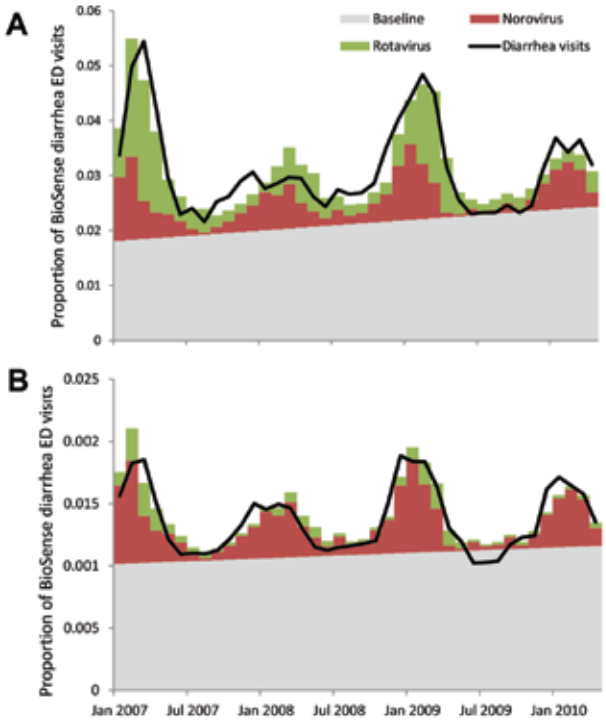

Figure 2. Model attribution of the proportion of BioSense emergency department (ED) visits mapped by chief complaint to diarrhea subsyndrome compared with estimates of norovirus and rotavirus infections, United States, January 2007–April 2010. A) Patients 0–4 years of age; B) patients of all ages. Predicted norovirus largely accounted for the observed seasonal variations in the proportion of diarrhea visits in the all-ages group (17.5% of predicted total, January 2007–April 2010), with rotavirus making a smaller contribution (4.7%); norovirus (13.6%, August 2007–April 2010) and predicted rotavirus (13.6%) equally accounted for the seasonality in the 0–4-year age group. All other etiologies captured by the background and secular increase (baseline) did not contribute to the observed winter seasonality.

and end dates for the 2010–2011 season when compared with the previous season.

Discussion

We found that BioSense syndromic data based on patient–reported symptoms (chief complaints) from ED visits correlated strongly with norovirus outbreak activity. These findings demonstrate that temporal signals in community syndromic surveillance time series can accurately reflect trends in norovirus activity across several regions and age groups. Specifically, the monthly proportion of BioSense ED diarrhea visits exhibited winter seasonality that mirrored that seen in reported norovirus outbreaks over 3 seasons, with the best-fit models accounting for >82% of the variation observed in the chief complaint–based syndromic data. Nausea/vomiting models did not fit as well as diarrhea models, which may be because the definition for the nausea/vomiting subsyndrome is less specific, as suggested by the fact that nausea/vomiting visits greatly outnumbered diarrhea visits. Our diarrhea models predict that much of the observed seasonal variation can be attributed to norovirus, with rotavirus also contributing substantially to seasonality in children <5 years of age. Nationally, this system has the potential to identify key attributes of the norovirus season (i.e., onset, peak, and end) in near real-time. The robustness of this relationship depends on the number of ED visits captured by the syndromic surveillance system; states with ≥5,000 recorded ED visits per month may be able to reliably use BioSense to accurately monitor community norovirus activity at the state level. Overall, our results suggest that BioSense chief complaint–based ED diarrhea visits can be a useful and timely indicator of norovirus disease in the United States.

The proportion of ED visits mapping to diarrhea exhibited a seasonality that can largely be explained by norovirus, which accounted for 17.5% of the overall ED activity for diarrhea. Although our estimates are based on proportions of diarrhea visits rather than exact counts, useful comparisons can still be made to norovirus prevalence reported for previous studies in the ED setting, including 18% for gastroenteritis patients of all ages (*8*) and 26% for adults (*15*). For children <5 years of age, our models suggest that norovirus (13.6%) and rotavirus (13.7%) caused similar proportions of diarrhea ED visits contributing to seasonal variation after rotavirus vaccination was introduced in the 2007–2008 season (*16*). This level of activity is lower than

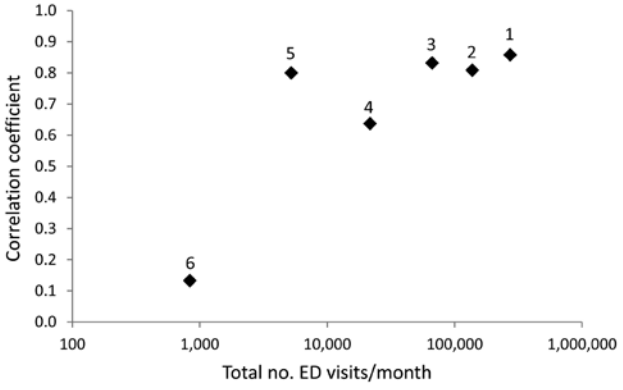

Figure 3. Correlation between the proportion of BioSense emergency department (ED) visits mapped by chief complaint to diarrhea subsyndrome and norovirus outbreaks as a function of total BioSense ED visits per month using state-specific data for the 6 states analyzed, United States, January 2007–April 2010. Correlation coefficients for each state are plotted by corresponding total ED visits/month on a logarithmic scale. Models tended to perform better in states with greater total ED visits. Higher correlation ($R \geq 0.60$) was observed for states with ≥5,000 BioSense ED visits/month. State number labels on data points correspond to those in Table 2.

Table 2. Parameters of linear regression models of the association between norovirus outbreaks and BioSense emergency department diarrhea subsyndrome visit data, by state, United States, January 2007–April 2010*

State no.	Total no. emergency department visits/mo	Norovirus,† $\beta_1, \times 10^{-4}$ (95% CI)	p value‡	*R*
1	273,218	2.86 (1.72 to 4.00)	<0.0001	0.858
2§	137,584	6.91 (4.60 to 9.22)	<0.0001	0.809
3¶	66,597	3.79 (2.74 to 4.83)	<0.0001	0.832
4¶	21,684	7.06 (0.73 to 13.40)	0.0298	0.637
5¶	5,214	2.18 (1.57 to 2. 78)	<0.0001	0.800
6§¶	836	0.53 (–8.77 to 9.84)	0.9082	0.133

*Emergency department chief complaint–based visits for diarrhea subsyndrome as a monthly proportion of all visits regressed on norovirus surveillance data, rotavirus antigen test data, and time variable. Intercept for each model p<0.0001.
†Suspected and confirmed norovirus outbreaks.
‡By *t* test.
§Time variable not significant in model (p≥0.05).
¶Proportion of rotavirus tests positive variable not significant in model (p≥0.05).

the norovirus prevalence (23%) reported in a recent US study through laboratory testing of children <5 years of age with gastroenteritis over 2 seasons from 2008–2010 (*17*) but more closely approximates the pooled proportion (12%, 95% CI 10%–15%) of children <5 years of age with severe diarrhea reported by a systematic review of 19 studies from low- to high-income countries (*18*). This winter seasonality is a well-described attribute of norovirus outbreaks (*19*,*20*) that has been used to estimate rates of norovirus-associated ED and ambulatory care visits, hospitalizations, and deaths (*6*–*8*,*21*–*23*). In addition, new variants of norovirus emerge every 3–5 years and are sometimes associated with surges in incidence (*11*), a pattern that was detected by a local ED-based syndromic surveillance system in Boston, Massachusetts, USA, during the winter of 2006–2007 (*24*). Our study expands on these observations by correlating ED-based syndromic signals with disease outcomes in a simple model.

The need for timely estimates of norovirus activity has led to previous efforts to develop surrogate measures of disease from other types of syndromic data. In the United Kingdom, an early warning system for norovirus activity based on the subjects of calls to a national telemedicine hotline gave up to 4 weeks advance warning of season onset (*25*). More recently, Internet search trends for terms related to gastroenteritis symptoms have been examined as timely surrogates for norovirus activity (*26*,*27*); in the United States, high correlation with outbreaks was demonstrated at national and regional levels (*26*). However, a limitation of Internet search data is their potential to be affected by media and social interest rather than disease incidence. Here we have shown ED visit data containing BioSense diarrhea subsyndrome chief complaints also have good potential for estimating norovirus season onset, as well as its peak and end, and have the ad-

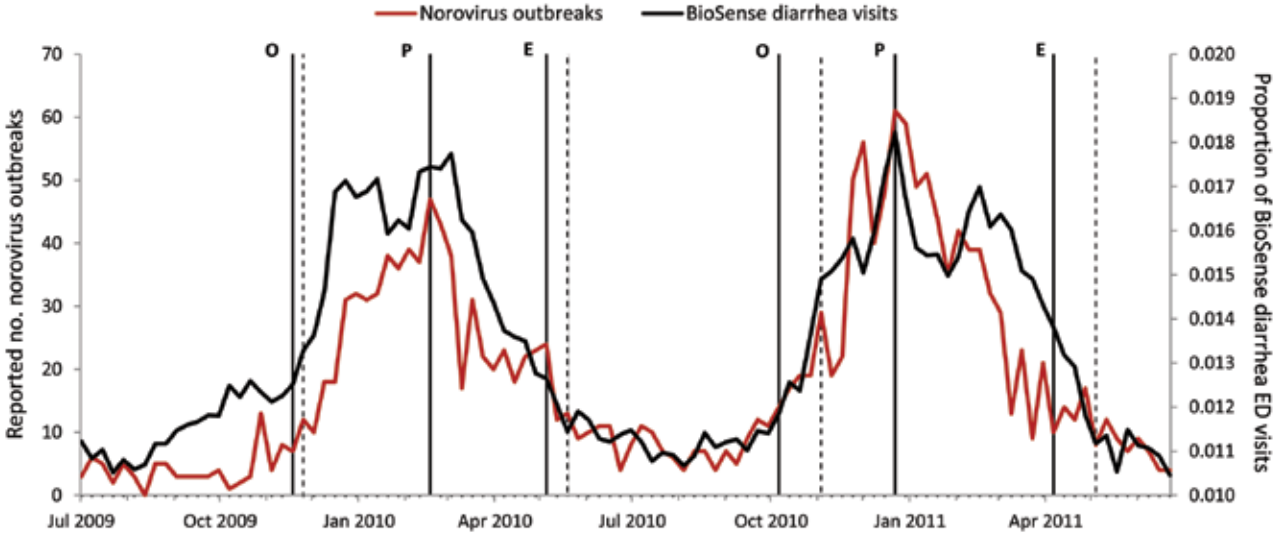

Figure 4. Estimation of norovirus season time markers using BioSense data on emergency department (ED) visits mapped by chief complaint to diarrhea subsyndrome, United States, 2009–2011. Observed season time markers (solid vertical lines) as defined by norovirus outbreak data are labeled as follows: season onset (O), season peak (P), and season end (E). Applying these rules yielded estimates for each season marker (dotted vertical lines) within 2 weeks of observed dates for the 2009–2010 season and within 4 weeks of observed dates for the 2010–2011 season.

vantage of being more closely linked to clinical outcome than are Internet search trends.

Certain limitations should be considered when interpreting our findings. First, chief complaint data, like all syndromic data, lack specificity in etiology. Our model acknowledges that other infectious and noninfectious illnesses can result in ED visits mapped to diarrhea by including a background term and only attributes the seasonal fraction to norovirus (and rotavirus in certain age groups). Although other pathogens that exhibit seasonality, such as astrovirus and sapovirus, were not accounted for in our model, these pathogens are detected at substantially lower rates than norovirus (*2*,*3*,*15*).

A second limitation is that each facility's catchment area and the duration of its participation during the study period were not known, so population-based rates could not be calculated. We accounted for facilities coming in and out of the system by using as our primary outcome the proportion of diarrhea visits. However, this measure may be subject to bias if the denominator (total all-cause ED visits) fluctuates for reasons independent of norovirus activity. For example, an increase in total ED visits because of respiratory illnesses would lower the proportion of diarrhea visits, even if the number of diarrhea visits remained unchanged. However, none of these considerations appear to diminish the value of using BioSense data as a timely indicator of norovirus activity.

Finally, our analyses could not account for any local variations in model performance resulting from any other factor beyond the volume of visits. The strength of correlation did vary from state to state, but the overall high degree of correlation suggests that this is not a fundamental limitation. Indeed, expanding the scope of our analyses with national-level data to develop rules for predicting season onset, peak, and end yielded promising results that can be used as a starting point for further refinement and prospective validation in the future.

The impact of norovirus in the United States is becoming increasingly clear, but traditional surveillance has not been sufficiently timely in identifying aberrant activity. Although syndromic surveillance lacks specificity, the strength of correlation with reported norovirus outbreaks we observed highlights the value of these data for rapid detection of norovirus activity. On a practical level, early detection that the norovirus season has started can serve as warning to infection control practitioners and the general public and might also help to detect the emergence of novel norovirus strains with pandemic potential. In this way, the proposed surveillance method applied within BioSense and described here can serve as a useful adjunct to existing surveillance systems. The emergence of the GII.4 Sydney norovirus strain during the 2012–2013 season (*28*), in particular, serves as a reminder of the need for timely surveillance tools to assess the timing and magnitude of norovirus activity.

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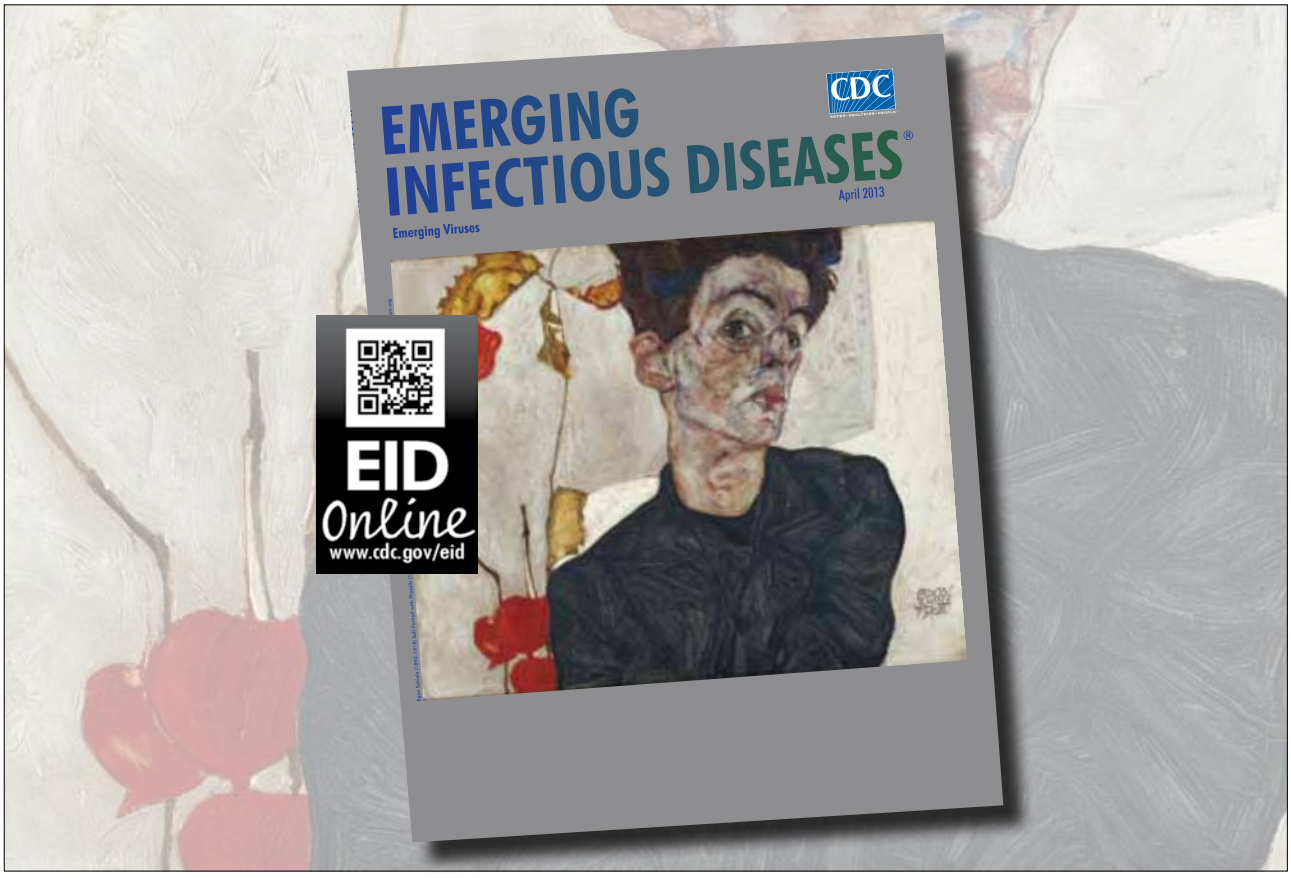

Aichi Virus in Sewage and Surface Water, the Netherlands

Willemijn J. Lodder, Saskia A. Rutjes, Katsuhisa Takumi, and Ana Maria de Roda Husman

Detection of Aichi virus in humans was initially reported in Japan in 1989. To establish a timeline for the prevalence of Aichi virus infection among humans in the Netherlands, we conducted molecular analysis of archival water samples from 1987–2000 and 2009–2012. Aichi virus RNA was detected in 100% (8/8) of sewage samples and 100% (7/7) of surface water samples collected during 1987–2000 and 100% (8/8) of sewage samples and 71% (5/7) of surface water samples collected during 2009–2012. Several genotype A and B Aichi virus lineages were observed over the 25-year period studied, but the time course of viral genetic diversity showed recent expansion of the genotype B population over genotype A. Our results show that Aichi virus has been circulating among the human population in the Netherlands since before its initial detection in humans was reported and that genotype B now predominates in this country.

Gastroenteritis is a common waterborne disease in humans of all ages worldwide. Children and the elderly are most severely affected, especially in low-income countries (*1*). A number of viral etiologic agents, such as picornaviruses, caliciviruses, rotaviruses, human adenoviruses, and astroviruses, have been identified in the past few decades. However, a diagnostic gap remains in samples for which no causative agent is determined. It has been suggested that other picornaviruses may be involved (*2*).

Aichi viruses (species *Aichivirus*, genus *Kobuvirus*, family *Picornaviridae*) are small, nonenveloped viruses with a single-stranded, positive-sense RNA genome. Aichi virus in humans was reported in 1989 in Japan from a sample collected during an oyster-associated gastroenteritis outbreak (*3*); the complete nucleotide sequence of an Aichi virus was described in 1998 (*4*). Clinical signs and symptoms of Aichi virus infection include diarrhea, abdominal pain, nausea, vomiting, and fever, reflecting gastroenteritis (*3*,*5*). Aichi virus has been found at low incidence in patients with gastroenteritis in several regions around the world, including South America (*6*), Asia (*7*,*8*), Europe (*6*,*9*–*12*), and Africa (*13*). Serologic studies indicate that up to 90% of the human population has been exposed to Aichi viruses by the age of 40 years (*14*). However, the epidemiology of gastroenteritis caused by Aichi virus is, to a large extent, unknown.

Aichi viruses have mainly been detected by PCR targeting the 3CD junction of the virus genome (*15*). The 3CD junction region has been described as conserved, and the viral protein (VP) 1 region is more genetically diverse (*4*,*6*,*9*,*16*). VP1 sequence typing is standard for the classification of picornaviruses (*17*), but analysis of the 3CD region has been used to divide Aichi viruses into 3 genotypes: A, B, and C (*9*,*15*).

Aichi viruses excreted with human feces contaminate surface waters directly or after discharge of treated or untreated sewage (*18*). Humans could be exposed to these viruses in surface waters used for the production of drinking water (after insufficient treatment) or for recreational purposes and after consumption of raw shellfish cultivated in contaminated surface waters. One indication that Aichi viruses may be transmitted by the fecal–oral route is the detection of these viruses in sewage samples in Tunisia (*19*), in surface waters in Venezuela (*20*), and in sewage and river waters in Japan (*21*). Some of these studies demonstrated a high Aichi virus prevalence in water samples. Viruses in sewage are thought to reflect the viruses circulating in the human population, originating from asymptomatic and symptomatic persons (*22*). Hence, environmental surveillance studies are extremely useful to determine the circulation of viruses in the human population (*22*,*23*) and to obtain sequence information of the circulating strains.

To establish a timeline for the emergence of Aichi viruses among the human population in the Netherlands, archival sewage and surface waters sampled over a >25-year

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period were subjected to molecular analysis targeting VP1 and the 3C region of the Aichi virus genome. The detected viruses were typed by sequence analysis to determine genetic variability. These environmental Aichi virus strains were subsequently compared with strains previously isolated from clinical materials and environmental samples worldwide. The possible emergence of the Aichi virus infections in humans was inferred by analyzing the population dynamics of these Aichi viruses.

Material and Methods

Samples

Depending on water type and the pressure during the membrane filtration, different volumes of water were concentrated by using a conventional filter adsorption-elution method. The resulting eluates were further concentrated by using an ultrafiltration method as described (*24*). The remaining samples were stored at –70°C or –30°C.

Fifteen archival concentrates from these samples, originating from 1987–2000, were randomly selected for analysis. Of these samples, 8 were raw sewage samples and 7 were surface water samples. The time of storage of the concentrates did not influence the results, as a previous study also found (*25*).

Fifteen additional archival samples, originating from 2009–2012, also were selected. Of these, 8 samples originated from raw sewage and 7 samples from surface waters (*18*,*26*); of the sewage samples, 4 were tested directly, without sample concentration.

An Aichi virus–positive control, a culture supernatant of the Japanese isolate A846/88, was kindly provided by Erwin Duizer (Laboratory for Infectious Diseases and Perinatal Screening, National Institute for Public Health and the Environment [RIVM], Bilthoven, the Netherlands).

RNA Extraction

Genomic material was isolated from 12.5-µL and 125-µL volumes of the water concentrates, corresponding to 26 mL–2,000 mL of the original surface water and 0.5 mL–176 mL of original sewage, depending on the concentration factor obtained by filtration. The NucliSENS miniMAG Nucleic Acid Isolation Kit (bioMérieux, Zaltbommel, the Netherlands) was used as described (*24*).

For 4 raw sewage samples collected in 2010 and 2011, RNA was extracted directly from 1 mL and 5 mL of sewage. Nucleic acids were eluted from the silica in 100-µL elution buffer containing RNase inhibitor (Promega, Leiden, the Netherlands), and the eluate was further purified and concentrated by using the RNeasy MinElute Cleanup Kit (QIAGEN, Hilden, Germany). The extracted RNA was used directly in the reverse transcription reaction or stored at –70°C until use.

cDNA Synthesis

cDNA was synthesized by using random hexamers. In brief, for each water concentrate, 5 µL of undiluted RNA and a sample of 10× diluted RNA were tested; in addition, a sample of 100× diluted RNA from each 125-µL water concentrate was tested. These volumes corresponded to 170 µL–140 mL of surface water and 3.4 µL–12 mL of sewage, depending on the extraction volume and the dilution factor. The RNA was added to 1.5 µg of random hexamers (Roche, Almere, the Netherlands) and the mixtures were heated at 70°C for 5 min and then chilled on ice for 5 min. Subsequently, 1X First Strand Buffer (Invitrogen, Leek, the Netherlands), 0.5 mmol/L dNTP (Roche), 2.5 mmol/L DTT (Roche), 0.2 U RNase inhibitor (Promega), and 5 U Superscript II (Invitrogen) were added at room temperature, resulting in a final volume of 20 µL. The mixture was incubated in a thermal incubator at 42°C for 60 min, heated at 95°C for 5 min, and then chilled on ice for 5 min. The synthesized cDNA was used directly in a PCR reaction or stored at –70°C until use.

Nested PCR

VP1

For Aichi virus detection and typing, cDNA samples were amplified by a nested PCR using primers developed in this study to target the VP3 and VP1 regions (Table 1). In brief, an aliquot of 5 µL of synthesized cDNA was added to 45 µL of the first-round PCR reaction mixture containing 1X PCR reaction buffer with $MgCl_2$ (Roche), 0.2 mmol/L dNTP (Roche), 1 µmol/L each primer (F1 and R1), and 2.5 U of Taq DNA Polymerase (Roche). The PCR protocol was as follows: a denaturation and activation step at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s. A 1-µL volume from the first-round PCR was used as a template for the second-round PCR mixture, containing 1X PCR reaction buffer with $MgCl_2$ (Roche), 0.3 mmol/L dNTP (Roche), 0.1 µmol/L each primer (primer combinations F2/R2 and F3/R2 were used), and 2.5 U of FastStart Taq DNA Polymerase (Roche). The PCR protocol was as follows: a denaturation and activation step at 95°C for 5 min, followed by 40 cycles of 95°C for 20 s, 60°C for 30 s, and 72°C for 40 s. The second-round PCR products were separated on 2% agarose gels and visualized by ultraviolet illumination after staining with SYBR Gold Nucleic Acid Gel Stain (Molecular Probes, Leiden, the Netherlands) to identify positive samples. DNA fragments of 530 bp were amplified for primer pair F2/R2 and fragments of 264 bp for primer pair F3/R2. Positive second-round PCR products were purified by using a QIAquick PCR Purification Kit (QIAGEN), according to the manufacturer's instructions. All purified PCR products were stored at –20°C until further use.

Table 1. Oligonucleotide sequences of the VP1 and VP3 primers developed and used for study of Aichi virus in sewage and surface water, the Netherlands*

Primer	Sequence, 5′→3′†	Nucleotide location‡	PCR
AiV-VP3-F1	CACACCGCCCCTGCGTCRGCCCTCGT	2912–2937	First-round
AiV-VP1-F2	CTCGATGCRCCMCAAGACACCGG	3023–3045	Nested
AiV-VP1-F3	GTGCTTCACRTACATCGCYGCGG	3289–3311	Nested
AiV-VP1-R2	CCTGACCAGTCCTCCCAWCCGAAGTA	3552–3527	Nested
AiV-VP1-R1	GAGAGCTGGAAGTCRAAGGG	3651–3632	First-round

*VP, viral protein; AiV, Aichi virus; F, forward; R, reverse.
†R indicates A or G; M indicates A or C; Y indicates C or T; W indicates A or T.
‡Compared with Aichi virus reference strain (GenBank accession no. AB010145).

3C Region

For Aichi virus detection and typing, cDNA samples were amplified by a nested PCR using primers targeting the 3C region as described (*6*). PCR mixtures and protocol were as described for the VP1 PCR and amplified by using the same cycling parameters, except that the annealing step in the second-round PCR was performed at 55°C. For the first-round PCR, DNA fragments of 313 bp were amplified; for the second-round PCR, fragments of 180 bp. Positive PCR products were purified and stored at -20°C until further use.

Cloning and Sequencing

The purified PCR products were cloned into a pCRII-TOPO Vector (Invitrogen), according to the manufacturer's instructions; the construct was subsequently transformed into JM109 competent cells. Approximately 5–7 clones were randomly selected per purified PCR product and were checked by using M13 primers supplied by the manufacturer (Invitrogen). Up to 6 positive clones from each sample were randomly selected and subjected to sequence analysis of both strands with M13 primers by using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA).

Phylogenetic Analysis

The obtained 3C and VP1 sequences were edited with BioNumerics software version 6.6 (Applied Maths, Kortrijk, Belgium) and compared with all available Aichi virus sequences from GenBank. Multiple DNA sequences from each genotype were aligned by using MAFFT version 6.847b (*27*). We estimated phylogenies of the dated samples using a Bayesian Markov chain Monte Carlo method implemented in the Bayesian evolutionary analysis by sampling trees (BEAST version 1.7.4 [*28*]) and estimated coalescent effective population sizes using skyline plots (*29*). Skyline plots represent a nonparametric flexible method based on coalescent theory; the method was used to reconstruct changes in population sizes through time. The Hasegawa-Kishino-Yano model of DNA evolution with a uniform mutation rate across branches (strict clock) was used with default priors. Simulations were run for 30 million updates after discarding burn-in. The resulting tree was summarized by using TreeAnnotator version 1.7.4 (*28*), and the maximum-clade credibility tree was visualized and edited with FigTree software version 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

We found Aichi viruses were present in sewage and surface water samples originating from both sampling periods, 1987–2000 and 2009–2012 (15 samples from each period). Aichi virus RNA was detected in 93% (28/30) and 83% (25/30) of water samples by testing that targeted the 3C and VP1 regions, respectively. Aichi virus RNA was detected by both detection methods in all 16 sewage samples from both sampling periods and in 12 (86%) of 14 and 9 (64%) of 14 surface water samples for the 3C region and the VP1 region, respectively (Table 2).

The 2 primer pairs, F2/R2 and F3/R2, used in the VP1 second-round PCR showed similar results. Because of the larger product of the F2/R2 primer pair (530 bp), PCR products obtained with this primer pair were further used for cloning and sequence analysis. Viral population dynamics was estimated over time by using Bayesian coalescent analysis of the VP1 nucleotide sequence alignment of the isolates from the Netherlands and GenBank reference strains. The phylogenetic tree in Figure 1 shows that the 151 sequences obtained from the strains isolated from the Netherlands were in several different clusters and clustered between the limited available GenBank sequences obtained from other countries. To explore the possible expansion of Aichi virus lineages in the Netherlands in the sampled period, the genetic variability of the genotype A and B Aichi viruses was analyzed separately (Figure 2, panel A; Figure 3, panel A). The phylogeny of genotype A Aichi viruses showed predominantly strains from samples taken early in the sample period; only 1 genotype A was found in a location sampled in 2011 (Figure 1; Figure 2, panel A; Table 2). Genotype B showed 2 distinct clusters that resulted in a ladder-like structure suggesting a continual replacement of lineages through time (Figure 1; Figure 3, panel A). Translation into amino acid sequences of VP1 showed a high similarity (<4% difference) between the genotype A strains and genotype B strains, but 2 separate clusters of genotype A and B were seen (data not shown).

Table 2. Sample characteristics and summarized results per genomic region of sewage and surface water samples collected during 1987–2000 and 2009–2012 and tested for Aichi virus, the Netherlands*

Sample no.	Sampling month	Sample type	Genotype†	
			3C	VP1
1987-49	July	Sewage	B	B
1987-56	August	Surface water	B	B
1987-75	September	Surface water	B	B
1989-33	April	Sewage	A, B	B
1991-29	April	Sewage	B	A, B
1994-10	February	Surface water	A	A
1995-44	July	Surface water	B	ND
1997-27	May	Surface water	A	ND
1997-31	June	Sewage	A	A
1997-39	June	Sewage	A	B
1998-20	February	Surface water	A, B	A
1998-56	May	Sewage	‡	A
1998-62	May	Sewage	A, B	A
1999-46	April	Sewage	CK	B
2000-12	February	Surface water	A	ND
2009-011	January	Sewage	B	B
2009-074	May	Surface water	B	B
2009-075	May	Surface water	B	B
2009-064	April	Surface water	B	B
2010-007	January	Surface water	B	B
2010-033	March	Surface water	B	B
2010-210	September	Sewage	B	B
2010-216	October	Sewage	B	B
2011-024	February	Sewage	CK	A
2011-129	May	Sewage	B	B
2011-221	June	Sewage	B	B
2011-579	September	Sewage	A	B
2011-331	August	Sewage	B, CK	B
2012-063	April	Surface water	ND	ND
2012-195	June	Surface water	ND	ND

*VP, viral protein; ND, not detected; CK, canine kobuvirus.
†Result per genomic region.
‡Not clustering with the known genotypes A, B, or C.

A Bayesian skyline plot model was used to reconstruct a time course of viral genetic diversity (*30*). Although the dataset is limited (36 clones from 7 samples), a constant diversity was seen in genotype A Aichi virus strains detected until the 1990s, followed by an apparent drop in genotype A detection (Figure 2, panel B). A constant diversity was also seen in genotype B strains (Figure 3, panel B). Overall, genotype B Aichi viruses have been more prevalent in the Netherlands in the past decade than have genotype A Aichi viruses.

After cloning of the 3C positive PCR products, phylogenetic analysis of the cloned sequences of the 3C region showed several different clusters within the known genotypes A and B (Figure 4). The phylogenetic tree in Figure 4 shows that the 127 sequences obtained from the environmental strains in this study were more divergent than the 3C sequences of Aichi viruses obtained from GenBank. No obvious differences were seen in the number of strains found in sewage or surface water. In the samples originating from 1987–2000, 3C sequences clustered with the known genotypes A and B and with canine kobuvirus strains. In contrast, for the samples originating from 2009–2011, sequences clustered only with genotype B and canine kobuvirus strains. The genotype B sequences obtained from both sampling periods showed 2 distinct clusters; the sequences of the first sampling period disappeared after 2009. Nevertheless, the amino acid sequences of the genotype A and B strains were very similar and did not show distinct clusters (data not shown). The phylogeny of the 3C sequences of genotype A and B Aichi viruses showed a ladder-like structure suggesting a continual replacement of lineages over time (Figure 4).

The sequence obtained from a sewage sample collected in 1998 (sewage/NL/1998-56) differed by up to ≈20% from the available Aichi virus sequences in GenBank (genotypes A, B, and C) (Figure 4). This high nucleotide divergence could suggest that this strain belongs to a new genotype of Aichi virus. Three sewage samples contained sequences that were highly similar (95%–96%) to recently discovered canine kobuviruses (GenBank accession nos. JN088541 and JN387133; Figure 4).

Discussion

Reuter et al. (*14*) showed that the seroprevalence of Aichi virus in the human population worldwide is high. Up to 95% of persons 30–40 years of age have antibodies against Aichi virus, indicating a high rate of exposure to the viruses. By contrast, Aichi viruses are found at low

incidence in clinical materials (*7–11*,*13*,*31*). More data are needed to gain better insight into the epidemiology and pathogenesis of Aichi viruses. Environmental surveillance of enteric viruses may give information on the possible circulation of Aichi viruses in the human population in the Netherlands, as well as their evolutionary dynamics. Therefore, we tested different water samples collected in the Netherlands over a period of 26 years (1987–2012) and found a high prevalence of Aichi viruses in samples from sewage and surface waters. We dected Aichi virus RNA in water samples from the Netherlands originating from 1987, which precedes description of Aichi virus in the literature, in fecal samples from patients affected in a 1989 oyster-related gastroenteritis outbreak in Japan (*3*).

Three previous studies have described the detection of Aichi viruses in environmental samples and found different prevalence levels. In Venezuela, 5 of 10 tested surface water samples contained Aichi virus RNA (*20*), but in Tunisia, only 15 of 250 (6%) tested raw and treated sewage samples contained Aichi virus RNA (*19*). Much higher prevalence was found in samples from Japan: raw sewage, 100% (12/12); treated sewage, 92% (11/12); and surface water, 60% (36/60) (*21*). Our study also found a high prevalence of Aichi viruses in water samples: 100% (14/14) of sewage and 85% (12/14) of surface water samples. Two surface water samples from 2012 tested negative for the presence of Aichi virus RNA, but this may have been explained by the origins: a large lake and a storage reservoir for the production of drinking water. These sources are differentfrom the other waters tested, which included large, relatively polluted rivers. To resolve possible transmission routes, Aichi viruses could be quantified in source waters for drinking water production and recreational waters by cell culture methods followed by quantitative microbial risk assessment to estimate public health risks from such exposures (*32*).

Several studies have compared the available Aichi virus sequences of the 3CD and VP1 regions and described the 3CD junction region as conserved and the VP1 region as more variable (*4*,*6*,*9*,*16*). Lukashev et al. (*33*) showed that the VP1 genome region, coding for structural proteins that express the antigenicity of the virus, is particularly suitable for distinguishing subtypes of Aichi viruses, whereas the sequence data of the more conserved 3CD junction region did not seem to provide sufficient sequence diversity for subtyping. The 3CD region, however, may be useful for the detection of a wider range of Aichi virus genotypes. The primers targeting the VP1 region used in this study proved to be useful for the detection of Aichivirus genotypes A and B. Sequence comparison with the limited sequence information of the other genotypes showed that our primers may have detected the Aichi virus genotype C less sensitively. In addition, the canine kobuviruses might

Figure 1. Maximum-clade credibility tree showing the phylogenetic relationships between Aichi virus isolates from the Netherlands and other locations, based on a multiple alignment of nucleotide sequences (481-nt) of the viral protein (VP) 1 region. The rooted tree was generated by the Bayesian Markov chain Monte Carlo method in BEAST (*28*), using CK as an outgroup, visualized in FigTree (http://tree.bio.ed.ac.uk/software/figtree/), and plotted on a temporal *y*-axis scale in units of 1,000 years. Aichi virus strains from the Netherlands isolated from sewage (red) and surface waters (blue) are indicated; reference strains (black) are shown with GenBank accession numbers. Clusters of sequences of the same sample are represented by triangles (a collapsed branch), and the number of isolates in each triangle is shown in parentheses. Aichi virus genotypes are shown on the right (A, B, and C). CK, canine kobuviruses.

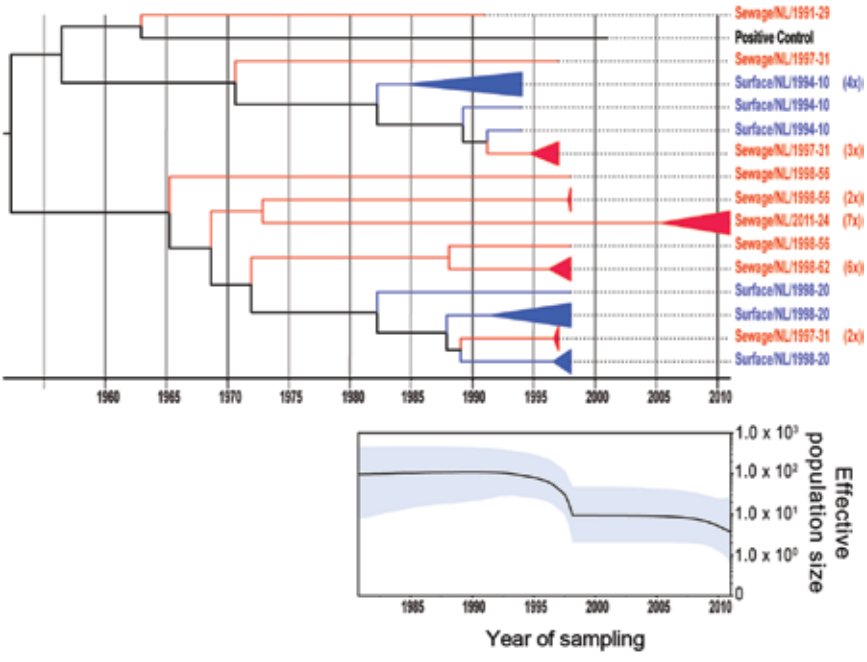

Figure 2. Phylogenetic relationships and genetic diversity over time for 37 sequences of Aichi virus genotype A strains collected in the Netherlands. A) Maximum-clade credibility tree was generated by the Bayesian Markov chain Monte Carlo method in BEAST (*28*), based on a multiple alignment of nucleotide sequences (481-nt) of the viral protein 1 region. The tree is rooted to the most recent common ancestor, visualized in FigTree (http://tree.bio.ed.ac.uk/software/figtree/), and plotted on a temporal *y*-axis scale using the sampling dates. Aichi virus strains from the Netherlands isolated from sewage (red) and surface waters (blue) are indicated. Clusters of sequences of the same sample are represented by triangles (a collapsed branch), and the number of isolates in each triangle is shown in parentheses. B) Bayesian skyline plot obtained by analyzing different Aichi virus sequences sampled at different times. The results are a relative measure for genetic diversity through time. The line represents the median, and the shaded area represents the 95% highest posterior density of the number of isolates.

not be detected by these VP1 primers; this may also be the case with the Aichi virus type found in sewage in 1998. More sequence information for the circulating Aichi virus strains is necessary to elucidate the considerations of a new genotype. Metagenomic studies, in both fecal and sewage samples (*34*,*35*), may facilitate the detection of new Aichi virus genotypes (or other genera of the kobuviruses), which also will facilitate the development of suitable primers for the detection of more Aichi virus genotypes.

Future research might focus on analysis of samples from gastroenteritis outbreaks for which no causative agent can be detected, using the 3C and VP1 primer sets described in this study. The resulting prevalence data could then be compared with the environmental surveillance data and to Aichi virus prevalence rates in fecal samples from persons of different ages with and without clinical illness. The results could elucidate the role of Aichi viruses in disease development and the severity of symptoms. Moreover, the susceptibility of vulnerable groups to Aichi virus infection and disease should be determined because disease was recently detected in elderly hospitalized patients with diarrhea in Sweden (*10*).

In our study, PCR products were cloned before sequence analysis so that we could detect multiple Aichi virus strains in 1 sample, not just the predominant strain. This resulted in the finding of multiple strains in 5 of the analyzed samples: sewage/NL/1989-33; sewage/NL/1991-29; surface/NL/1998-20; sewage/NL/1998-62; and sewage/NL/2011-331. We found a divergent Aichi virus strain in a sewage sample collected in 1998 (sewage/NL/1998-56) by comparing the nucleotide sequences of the 3C region with the known Aichi virus types available in GenBank (Figure 4). A nucleotide difference of ≈20% from the available sequences of genotypes A, B, and C was observed, tentatively leading to the conclusion that this sequence might belong to a new genotype of Aichi virus. More sequence information is needed to substantiate this finding by isolating this Aichi virus strain and subsequently subjecting the virus to whole-genome sequencing, as was described for an Aichi virus isolated in Taiwan in 2010 (*36*). For 3 of the sewage water concentrates from our study, 3C sequences were detected that showed high similarities with the recently discovered canine kobuvirus (*37*,*38*) (Figure 4). Although canine kobuviruses could have ended up in the sewage by run-off, further studies should be performed to gain more information about possible zoonotic transmission of these viruses.

Comparing the Aichi virus nucleotide sequences from the 2 sampling periods, 1987–2000 and 2009–2011, demonstrated that mainly genotype A strains were detected in the samples collected during 1987–2000. Aichi virus genotype B was found in both periods, but the sequences seemed to cluster in 2 distinct branches, which showed a shift in predominance of genotype B Aichi viruses after 2005 (Figure 3). Further analysis of these sequences, using BEAST (*28*), showed evolution of these genotype B strains over time, which resulted in a ladder-like structure, suggesting a continual replacement of lineages over the study period.

In conclusion, our study showed a high prevalence of Aichi viruses in environmental water samples in the Netherlands over an extended period of time, with a possible increase in genetic diversity of genotype B Aichi viruses. The additional sequence data obtained in this study may aid in the analysis of the evolution of Aichi viruses. In addition, the results emphasize the need for further research to understand the relative importance of possible transmission routes of Aichi viruses; that knowledge could allow the implementation of effective control measures.

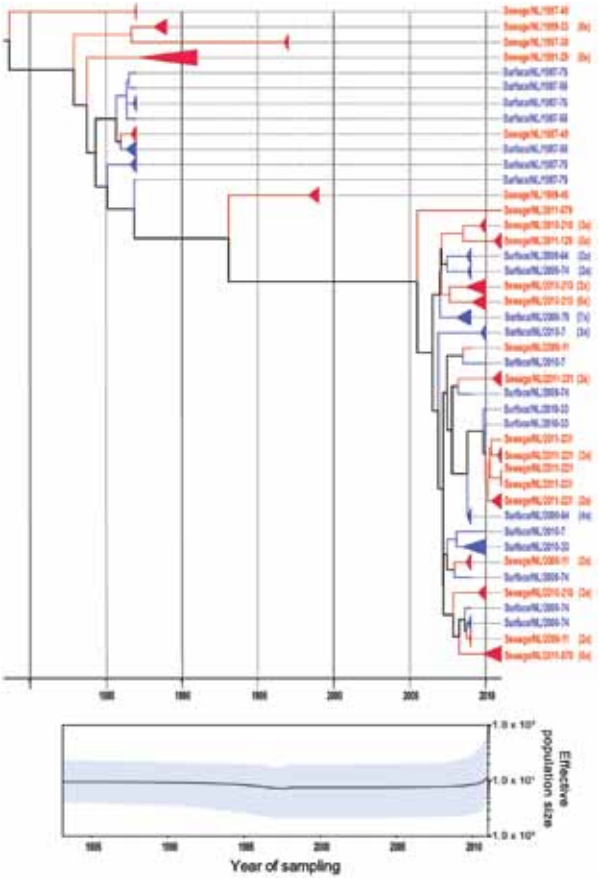

Figure 3. Phylogenetic relationships and genetic diversity over time for 166 sequences of Aichi virus genotype B strains collected in the Netherlands. A) Maximum-clade credibility tree was generated by the Bayesian Markov chain Monte Carlo method in BEAST (*28*), based on a multiple alignment of nucleotide sequences (481-nt) of the viral protein 1 region. The tree is rooted to the most recent common ancestor, visualized in FigTree (http://tree.bio.ed.ac.uk/software/figtree/), and plotted on a temporal *y*-axis scale using the sampling dates. Aichi virus strains from the Netherlands isolated from sewage (red) and surface waters (blue) are indicated. Clusters of sequences of the same sample are represented by triangles (a collapsed branch), and the number of isolates in each triangle is shown in parentheses. B) Bayesian skyline plot obtained by analyzing different Aichi virus sequences sampled at different times. The results are a relative measure for genetic diversity through time. The line represents the median, and the shaded area represents the 95% highest posterior density of the number of isolates.

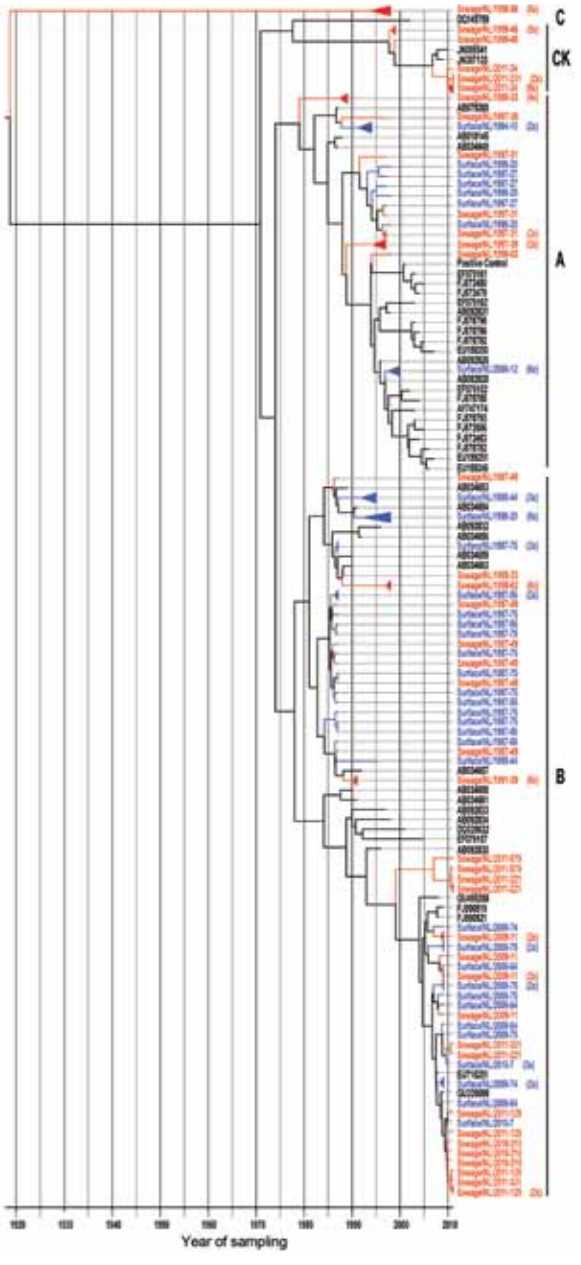

Figure 4. Maximum-clade credibility tree showing the phylogenetic relationships between Aichi virus isolates from the Netherlands and other locations, based on a multiple alignment of nucleotide sequences (139-nt) of the 3C region. The tree was generated by the Bayesian Markov chain Monte Carlo method in BEAST (*28*), rooted to the most recent common ancestor, visualized in FigTree (http://tree.bio.ed.ac.uk/software/figtree/), and plotted on a temporal *y*-axis scale using the sampling dates. Aichi virus strains from the Netherlands isolated from sewage (red) and surface waters (blue) are indicated; reference strains (black) are shown with GenBank accession numbers. Clusters of sequences of the same sample are represented by triangles (a collapsed branch), and the number of isolates in each triangle is shown between brackets. Aichi virus genotypes are shown on the right (A, B, and C). CK, canine kobuviruses.

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Effects and Clinical Significance of GII.4 Sydney Norovirus, United States, 2012–2013

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Describe the epidemiology and most common settings of infection of norovirus outbreaks in general
- Analyze the epidemiology and clinical presentation of the GII.4 Sydney norovirus infection.

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During 2012, global detection of a new norovirus (NoV) strain, GII.4 Sydney, raised concerns about its potential effect in the United States. We analyzed data from NoV outbreaks in 5 states and emergency department visits for gastrointestinal illness in 1 state during the 2012–13 season and compared the data with those of previous seasons. During August 2012–April 2013, a total of 637 NoV outbreaks were reported compared with 536 and 432 in 2011–2012 and 2010–2011 during the same period. The proportion of outbreaks attributed to GII.4 Sydney increased from 8% in September 2012 to 82% in March 2013. The increase in emergency department

visits for gastrointestinal illness during the 2012–13 season was similar to that of previous seasons. GII.4 Sydney has become the predominant US NoV outbreak strain during the 2012–13 season, but its emergence did not cause outbreak activity to substantially increase from that of previous seasons.

Noroviruses (NoVs) are the most common cause of epidemic gastroenteritis worldwide and the leading cause of foodborne outbreaks in the United States (*1*–*3*). In the United States, NoVs cause 19–21 million illnesses and lead to 56,000–70,000 hospitalizations and 570–800 deaths each year (*4*). Severe disease associated with NoV occurs most frequently among older adults, young children, and immunocompromised patients (*4*–*7*). NoV outbreaks occur year round, but activity increases in the United States during the winter months; 80% of reported outbreaks occur during November–April (*8*,*9*).

NoVs belong to the family *Caliciviridae* and can be grouped into at least 5 genogroups (GI–GV), which are further divided into at least 35 genotypes (*2*,*10*). Most NoV outbreaks are attributed to genotype GII.4, which evolve rapidly over time (*11*,*12*). During the past decade, new GII.4 strains have emerged every 2–3 years and replaced previously predominant GII.4 strains. The emergence of new NoV strains is believed to be related, in part, to the antigenic diversity of the novel strain that leads to at least partial escape from preexisting herd immunity acquired against the predominant circulating strain (*12*). These new NoV strains have often, but not always, led to increased outbreak activity (*8*,*10*,*13*,*14*).

In March 2012, a new GII.4 NoV strain was identified in Australia. Named GII.4 Sydney, this emergent strain has since caused acute gastroenteritis outbreaks in New Zealand, Japan, Western Europe, and Canada (*15*–*17*). Preliminary indicators of increased NoV outbreak activity, including an increase in the number of confirmed cases and hospital-related outbreaks in late 2012, were presumed to be associated with emergence of GII.4 Sydney in several of those countries (*15*,*17*,*18*). In the United States, GII.4 Sydney became the predominant NoV strain implicated in outbreaks during the last 4 months of 2012 (*19*).

To assess whether the emergence of GII.4 Sydney strain was associated with an increase in overall NoV disease activity in the United States, we examined data from NoV outbreaks in 5 states and emergency department visits for gastrointestinal illness in 1 state during the 2012–13 season and compared these data with those of the 2 previous seasons. Furthermore, we compared epidemiologic (e.g., setting and mode of transmission) and clinical features of patients in outbreaks attributed to GII.4 Sydney with those of outbreaks attributed to other strains during the 2012–13 season.

Methods

NoV Outbreak Data and Analysis

The Centers for Disease Control and Prevention (CDC) operates 2 surveillance systems for NoV outbreaks in the United States—the National Outbreak Reporting System (NORS) and CaliciNet. NORS is a comprehensive reporting system for all enteric disease outbreaks in the United States, regardless of cause or transmission mode (*9*). NORS data include general outbreak characteristics, patient demographics, symptoms, and clinical outcomes. CaliciNet is an electronic laboratory surveillance network that collects information on genetic sequences of NoVs implicated in outbreaks (*11*). CaliciNet laboratories perform molecular typing of NoV strains by using standardized laboratory protocols for reverse transcription PCR followed by sequence analysis. Sequence data are then uploaded into a central database to monitor national trends in circulating NoV strains. Information about outbreaks is often reported by state health departments to CDC several months after outbreaks occur; therefore, both NORS and CaliciNet data may be subject to substantial reporting delays.

Beginning in August 2012, a network of 5 sentinel states was established to improve the timeliness of NoV outbreak reporting through NORS and CaliciNet and thereby allow near real-time assessment of NoV activity. These 5 states (Minnesota, Ohio, Oregon, Tennessee, and Wisconsin) include ≈33 million residents or 11% of the total US population spread across several regions of the country (*20*). In addition, these 5 states had historically the highest per capita reporting rates for NoV outbreaks and therefore were least likely to be affected by underreporting biases. State health departments that participate in this network–the Norovirus Sentinel Testing and Tracking (NoroSTAT) network–report suspected NoV outbreaks through NORS and CaliciNet within 7 business days of notification of the outbreak to the state health department. NoroSTAT reporting allows NoV strain data uploaded through CaliciNet to be rapidly linked with epidemiologic characteristics of outbreaks reported through NORS by using consistent outbreak identifiers in each system. Aside from more timely and complete data reporting, outbreak reporting practices and case definitions among NoroSTAT participants otherwise remained unchanged.

The present study was restricted to data reported to NORS and CaliciNet by NoroSTAT states on all confirmed and suspected NoV outbreaks with first illness onset dates during August 1, 2012–April 16, 2013. To compare the level of outbreak activity in the 2012–13 season with that in previous years, we extracted data for the same time during the 2 previous seasons (August 1, 2011–April 16, 2012 and August 1, 2010–April 16, 2011) from NORS for the NoroSTAT states. A confirmed NoV outbreak is defined as

≥2 cases of similar enteric illness associated with a common exposure that are laboratory confirmed for NoV by reverse transcription PCR, enzyme immunoassay, or electron microscopy. A suspected NoV outbreak is defined as ≥2 cases of similar enteric illness associated with a common exposure in which NoV was the suspected causative agent, which is determined by each reporting site (e.g., supportive clinical or epidemiologic information or outbreaks with only 1 positive NoV specimen).

NoV genotype and sequence data were extracted from CaliciNet, and epidemiologic data, including demographics, symptoms, and outcome data, were extracted from NORS. Season onset was defined as the week by which at least 10% of the total number of NoV outbreaks during August 1–April 16 of each year had occurred. Seasonal peak in NoV outbreak activity was defined as the month with the highest number of NoV outbreaks. Season duration was defined as the number of weeks between which 10% and 90% of the total number of NoV outbreaks had occurred.

For comparison of GII.4 Sydney outbreak characteristics with those attributed to other NoV strains, outbreaks for which the NoV strain was reported through CaliciNet were grouped into 2 categories: GII.4 Sydney and non–GII.4 Sydney. A third category consisted of outbreaks reported by NoroSTAT states only through NORS (i.e., for which strain data were not available). Data on demographic characteristics, symptoms, and clinical outcomes were not available from all outbreaks. To minimize potential biases from underreporting, we included demographic characteristics, symptoms, and outcomes and analyzed them only when available for >10% of reported illnesses across all reported outbreaks.

To test the increase in proportion of NoV outbreaks attributed to the GII.4 Sydney strain by month during the 2012–13 season, we used the χ^2 test for trend. Categorical variables were compared by calculating rate ratios with 95% CIs. Statistical significance was set at $p<0.05$ by using the mid-*p* exact test. Analyses were performed by using SAS v9.3 (SAS Institute Inc., Cary, NC, USA).

Syndromic Surveillance Data and Analysis

Ohio collects statewide syndromic surveillance data from 178 emergency department and urgent care facilities (referred to collectively herein as emergency departments) into a system called EpiCenter. This system is used by state and local public health agencies to detect, track, and characterize health events, such as pandemic influenza, outbreaks, environmental exposures, and potential bioterrorism, in real-time. The system gathers information on patient symptoms with identification removed and automatically alerts public health officials when an unusual pattern or trend is occurring. We used EpiCenter data for August 1, 2010–April 16, 2013, to measure the weekly proportion of patients with gastrointestinal syndrome, defined as the chief complaint that included the following symptom keywords: abdominal, diarrhea not watery/bloody, diarrhea watery/bloody, nausea, and vomiting. To assess the correlation between the proportion of emergency department visits attributed to gastrointestinal illness and the number of NoV outbreaks, we used the Pearson correlation coefficient.

Results

Norovirus Outbreak Data

During August 1, 2012–April 16, 2013, a total of 637 NoV outbreaks were reported by Minnesota, Ohio, Oregon, Tennessee, and Wisconsin. Season onset was similar in 2012–13 compared with 2011–12 and 2010–11 (October 24 vs. November 7 and October 3, respectively, Figure 1). By April 16, the cumulative number of outbreaks was slightly higher in the 2012–13 season than in the 2011–12 and 2010–11 seasons (637 vs. 536 and 432, respectively). However, the data varied considerably between the states. The cumulative number of outbreaks increased in 3 states (Oregon, Tennessee, and Ohio), Wisconsin data were similar for each season, and Minnesota reported a decreased cumulative number of outbreaks (online Technical Appendix Figure, wwwnc.cdc.gov/

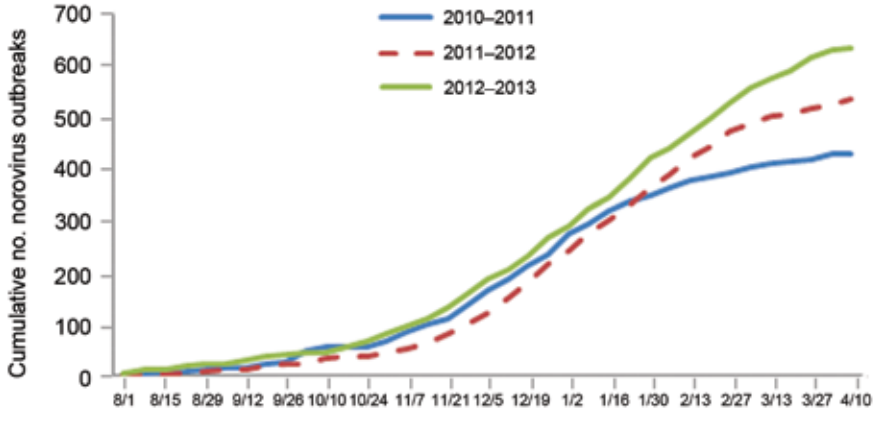

Figure 1. Number of suspected and confirmed norovirus gastroenteritis outbreaks by week of illness onset: Minnesota, Ohio, Oregon, Tennessee, and Wisconsin, August 1, 2010–April 16, 2013 (no. outbreaks = 1,605).

EID/article/19/8/13-0458-Techapp1.pdf). Peak outbreak activity occurred in January 2013 (152 outbreaks) and was 16% higher than the average of peak month outbreak activity in the 2 preceding seasons (148 outbreak in January 2012 and 113 outbreaks in January 2011). Season duration was 21 weeks in 2012–13, compared with 18 weeks in 2011–12 and 22 weeks in 2010–11.

Sequence data were available for 358 (56%) of the 637 outbreaks. Outbreaks with sequence data were more likely to have reported the mode of transmission as foodborne, and transmission was more likely to have occurred at restaurants and less likely to have occurred at schools (data not shown). Other characteristics were similar between the outbreaks with and without sequence data. Of these, 226 (63%) were attributed to the GII.4 Sydney strain. The proportion of outbreaks attributed to GII.4 Sydney increased from 8% in September 2012 to 82% in March 2013 (Figure 2, χ^2 test for trend; $p<0.001$). In December 2012, GII.4 Sydney became the predominant strain, accounting for 44 (66%) of the 67 outbreaks reported that month from which sequence data were available. GII.4 Sydney accounted for most sequenced outbreaks in all 5 states: 16 (57%) of 28 in Minnesota, 50 (56%) of 89 in Ohio, 57 (61%) of 94 in Oregon, 27 (73%) of 37 in Tennessee, and 76 (69%) of 110 in Wisconsin. The proportion of GII.4 Sydney outbreaks in the states with an increased cumulative number of outbreaks in 2012–2013 was not significantly different from the proportion in those states without an increased number of outbreaks (61% vs. 67%, respectively; $p = 0.27$).

Overall, the most commonly identified mode of transmission was person-to-person, which occurred in 481 (75.5%) of 637 outbreaks (Table 1). The proportions of different modes of transmission were similar among GII.4 Sydney and non–GII.4 Sydney outbreaks. Healthcare settings were reported most frequently across all outbreaks. However, GII.4 Sydney outbreaks occurred more frequently in healthcare settings (long-term care facilities and hospitals) than did non–GII.4 Sydney outbreaks (75.2% vs. 47.7%, respectively; rate ratio [RR] 1.58; 95% CI 1.30–1.91). GII.4 Sydney outbreaks occurred less frequently in schools and childcare centers than did non–GII.4 Sydney outbreaks (1.8% vs. 21.2%, respectively; RR 0.08; 95% CI 0.03–0.23). The proportion of outbreaks that occurred at restaurants or banquet facilities was similar for GII.4 Sydney and non–GII.4 Sydney outbreaks. Outbreaks for which sequence data were available were similar with respect to setting and transmission mode to those without sequence data.

Aggregate patient data were available for 310 (49%) of the 637 NoV outbreaks (9,018 patients); 104 of the outbreaks were GII.4 Sydney outbreaks, 74 were non–GII.4 Sydney outbreaks, and 132 were reported only through NORS without genotype data. The proportion of patients >50 years of age was higher in GII.4 Sydney outbreaks than in non–GII.4 Sydney outbreaks (65.9% vs. 43.9%, respectively; RR 1.50; 95% CI 1.39–1.62; Table 2).

Patients from GII.4 Sydney outbreaks reported diarrhea slightly more frequently than patients from outbreaks associated with non–GII.4 Sydney viruses (84.8% vs 75.7%, respectively; RR 1.12; 95% CI 1.09–1.15; Table 2). In contrast, vomiting, fever, and abdominal cramps were reported by a lower proportion of patients from GII.4 Sydney outbreaks than those from non–GII.4 Sydney outbreaks (Table 2).

A higher proportion of patients required outpatient visits among those affected by GII.4 Sydney outbreaks than among those in non–GII.4 Sydney outbreaks (7.9% vs. 4.3%, respectively; RR 1.81; 95% CI 1.33–2.47). We observed 9 (0.4%) deaths among 2,324 patients associated with GII.4 Sydney outbreaks for whom we had mortality data, compared with 3 (0.2%) deaths reported among 1,706 patients associated with non–Sydney outbreaks (RR 2.20, 95% CI 0.60–8.12; Table 2). Hospitalization and emergency department visits occurred at similar proportions in GII.4 Sydney and non–GII.4 Sydney outbreaks.

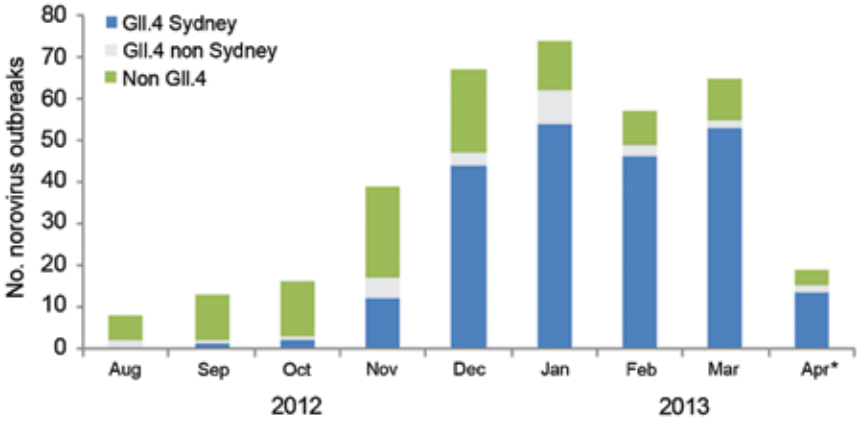

Figure 2. Genotypes of confirmed norovirus gastroenteritis outbreaks in Minnesota, Ohio, Oregon, Tennessee, and Wisconsin, August 1, 2012–April 16, 2013 (no. outbreaks = 358). *Data available for outbreaks during April 1, 2013–April 16, 2013.

Table 1. Number and percentage of norovirus gastroenteritis outbreaks, by genotype, strain, setting, and mode of transmission in Minnesota, Ohio, Oregon, Tennessee, and Wisconsin, August 2012–April 2013*

Outbreak	No. (%) outbreaks with sequence data			No. (%) outbreaks with no sequence data, n = 279	Total no. (%), N = 637
	GII.4 Sydney, n = 226	Non–GII.4 Sydney, n = 132	RR (95% CI)		
Mode of transmission					
Person to person	172 (76.1)	93 (70.5)	1.08 (0.95–1.23)	216 (77.4)	481 (75.5)
Foodborne	35 (15.5)	28 (21.2)	0.73 (0.47–1.14)	28 (10.0)	91 (14.3)
Water	0	1 (0.8)	NA	0	1 (0.2)
Environmental	0	2 (1.5)	NA	0	2 (0.3)
Other/unknown	19 (8.4)	8 (6.1)	1.39 (0.62–3.08)	35 (12.5)	62 (9.7)
Setting					
LTCF/hospital	170 (75.2)	63 (47.7)	1.58 (1.30–1.91)	189 (67.7)	422 (66.2)
School/CCC	4 (1.8)	28 (21.2)	0.08 (0.03–0.23)	49 (17.6)	81 (12.7)
Restaurant/banquet facility	35 (15.5)	25 (18.9)	0.82 (0.51–1.30)	23 (8.2)	83 (13.0)
Other/multiple settings†	14 (6.2)	13 (9.8)	0.63 (0.31–1.30)	15 (5.4)	42 (6.6)
Unknown	3 (1.3)	3 (2.3)	0.58 (0.12–2.85)	3 (1.1)	9 (1.4)

*RR, rate ratio; NA, not available; LTCF, long- term care facility; CCC, childcare center.
†Other settings include the following: private home (n = 15), noncafeteria workplace (n = 7), church (n= 2), other religious location (n = 2), hotel (n = 2), party (n = 3), apple orchard (n = 1), camp (n = 1), football tournament (n = 1), ship (n = 1), indoor playground (n = 1), movie theater (n = 1), trip to relatives (n = 1), other unspecified (n = 1), and multiple settings (n = 3).

Syndromic Surveillance Data

On average, the proportion of total weekly emergency department visits reported through Ohio EpiCenter that were classified into the gastrointestinal syndrome category was 13.3% (median 12.9%, range 11.1%–15.9%; Figure 3). The proportion of weekly gastrointestinal emergency department visits was strongly correlated with NoV outbreaks reported from Ohio (Pearson correlation coefficient; r = 0.68). The magnitude and timing of seasonal increase in the proportion of emergency department visits for gastrointestinal syndrome during the 2012–13 season were similar to those of previous seasons.

Discussion

The global emergence of a novel GII.4 NoV strain (GII.4 Sydney) in late 2012 prompted concern about a possible increase in incidence and severity of NoV disease and outbreaks. In the United States, GII.4 Sydney has become the predominant strain as the 2012–13 season has progressed, replacing the previously predominant GII.4 New Orleans strain (*11*). However, the emergence of GII.4 Sydney does not appear to have caused a substantial increase in the level of outbreak or endemic NoV disease activity compared with the previous 2 seasons.

Scotland and Denmark, like the United States, have reported the emergence of GII.4 Sydney as the predominant cause of NoV outbreaks during October–November 2012 (*16*,*17*). In Scotland and the United Kingdom, the onset of the 2012 winter NoV season was reported to be earlier than usual (*17*,*21*). Earlier descriptions of the GII.4 Sydney strain's emergence in other countries suggested a possible increase in the level of NoV activity compared with that of previous seasons (*15*,*18*). In our study, we found increased peak monthly levels of outbreak activity and cumulative numbers of outbreaks during the 2012–13 season compared with the 2 previous seasons. We also found higher cumulative numbers of outbreaks in 3 of the 5 reporting states during the 20012–13 season. However, these increases were not related to higher proportions of GII.4 Sydney outbreaks when findings were compared with results from states that had no increase in outbreak activity this season. Thus, the observed increase in outbreak activity during the 2012–13 season likely represents random seasonal and state variation rather than a direct result of the emergence of GII.4 Sydney. This conclusion is further supported by syndromic surveillance data from Ohio, which did not show an increase in the proportion of gastrointestinal illness among emergency department patients compared with proportions in previous seasons.

Most outbreaks attributed to the GII.4 Sydney strain occurred in healthcare–related settings and were predominantly transmitted from person to person; these findings were similar to previous data for outbreaks attributed to other GII.4 NoV strains (*10*,*22*). This finding could be caused by age-associated biologic difference in virus infectivity, other virus and host-related factors, or relatively better outbreak reporting in long-term care facilities than in other settings. Additional studies are needed to better define the basis for this observed difference.

Although aggregate patient data were available for less than half of the outbreaks and primarily from those that occurred earlier in the season, some preliminary trends in the affected populations and clinical profiles were observed. GII.4 Sydney outbreaks disproportionally affected older persons, consistent with the observed predilection toward long-term care facilities. Patients affected by GII.4 Sydney were more likely to have diarrheal illness and less likely to have vomiting, fever, and abdominal cramps. Age-associated patterns of illness may explain this observation; however, it differs from a previous

Table 2. Number and percentage of patients in outbreaks of acute gastroenteritis attributed to norovirus, by symptoms, clinical outcomes, and reported strain in Minnesota, Ohio, Oregon, Tennessee, and Wisconsin, August 2012–April 2013*

Characteristic	No. (%) patients linked to outbreaks with sequence data			No. (%) patients linked to outbreaks with no sequence data	No. (%) total
	GII.4 Sydney	Non–GII.4 Sydney	RR (95% CI)		
Demographics					
Sex					
M	576 (31.8)	448 (33.4)	0.95 (0.86–1.06)	355 (31.8)	1,379 (32.3)
F	1,235 (68.2)	895 (66.6)	1.02 (0.97–1.08)	761 (68.2)	2,891 (67.7)
Age, y					
0–4	14 (0.9)	26 (2.3)	0.42 (0.22–0.79)	39 (3.9)	79 (2.2)
5–9	5 (0.3)	156 (13.7)	0.02 (0.01–0.06)	120 (12.0)	281 (7.8)
10–19	109 (7.4)	86 (7.5)	0.98 (0.74–1.28)	107 (10.7)	302 (8.3)
20–49	376 (25.4)	372 (32.6)	0.78 (0.69–0.88)	190 (19.0)	938 (25.9)
50–74	310 (20.9)	174 (15.2)	1.37 (1.16–1.63)	157 (15.7)	641 (17.7)
≥75	666 (45.0)	327 (28.7)	1.57 (1.41–1.75)	387 (38.7)	1,380 (38.1)
Symptoms					
Diarrhea	2,385 (84.8)	1,549 (75.7)	1.12 (1.09–1.15)	1,882 (81.6)	5,816 (81.2)
Vomiting	1,337 (53.0)	1,214 (60.4)	0.88 (0.83–0.92)	1,356 (60.9)	3,907 (57.8)
Fever	1,191 (50.9)	1,126 (58.7)	0.87 (0.82–0.92)	1,030 (56.8)	3,347 (55.1)
Abdominal cramps	995 (48.1)	850 (54.5)	0.88 (0.83–0.94)	544 (44.9)	2,389 (49.3)
Outcome					
Outpatient visit	122 (7.9)	55 (4.3)	1.81 (1.33–2.47)	54 (5.9)	231 (6.2)
Emergency department visit	43 (2.4)	24 (1.8)	1.23 (0.79–2.13)	34 (3.7)	101 (2.5)
Hospitalized	49 (2.2)	22 (1.4)	1.54 (0.93–2.53)	65 (4.4)	136 (2.6)
Death	9 (0.4)	3 (0.2)	2.2 (0.60–8.12)	9 (0.4)	21 (0.3)

*Because of the preliminary nature of the data, information on demographic characteristics, symptoms, or outcomes were available for 310 (49%) of 637 total norovirus outbreaks reported during the study period. The number of patients and the relative proportions of illnesses by symptoms and outcomes from the outbreaks that included such data are reported. RR, rate ratio.

study that concluded that vomiting, fever, and abdominal cramps are more prevalent in patients infected with GII.4 NoV strains than those infected with non–GII.4 strains (*23*). We found higher rates of outpatient visits among patients infected with the GII.4 Sydney strain than among those infected with strains other than GII.4 Sydney, most of whom were infected with non-GII.4 strains. This finding is consistent with the previously reported association between GII.4 strains and severe outcomes (*24*). However, since hospitalization and emergency department visits occurred at similar rates, and given the difference in age of patients in GII.4 Sydney and non–GII.4 Sydney outbreaks, we were unable to associate GII.4 Sydney with differences in clinical severity.

Study limitations include the lack of data from a complete year of GII.4 Sydney transmission and inclusion

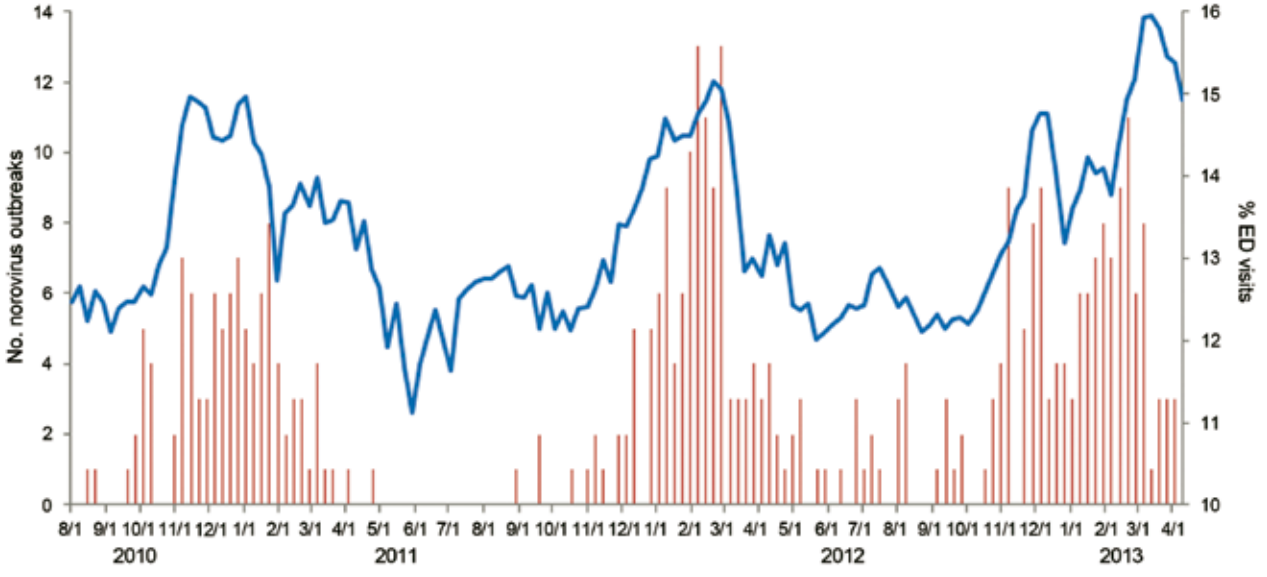

Figure 3. Percentage of emergency department and urgent care (ED) visits for gastrointestinal illness as reported through the Ohio EpiCenter syndromic surveillance system, and number of suspected and confirmed norovirus gastroenteritis outbreaks by week August 1, 2010–April 16, 2013.

of data from only 5 states, which may not be representative of national NoV outbreak activity. To confirm our findings, subsequent analysis of complete NORS and CaliciNet data from all states is necessary when it becomes available. Nevertheless, by using the currently available data reported through NoroSTAT, we ensured stable NoV outbreak definitions and reporting, and timely data allowed us to rapidly assess the magnitude of the current season. Moreover, NoroSTAT currently covers >10% of the US population from states with high per-capita outbreak reporting spread across several geographic regions; thus, it likely serves as a reasonable sentinel system for assessment of overall US NoV activity and the effects of emergent strains. Reporting results of primarily early-season aggregate patient data (demographic, symptom, and clinical outcomes) may introduce bias because these data are likely confounded by outbreak setting, which shifts toward a higher proportion of healthcare-associated outbreaks in the winter. Outbreak identification and reporting may differ across settings, and therefore, genotypes occurring in settings with lower reporting rates may be underrepresented in the analyses. In addition, including suspected NoV outbreaks may have resulted in slightly lower specificity of case definitions; however, the probability of NoV as causative agent is high when no other causative agent is identified because NoV is the most common cause of enteric disease outbreaks (*1*). Last, the definition of suspected NoV infection may have differed between NoroSTAT sites; however, all sites have confirmed stable and standardized outbreak definitions during the study period (2010–2013), which validates comparisons with previous seasons.

In conclusion, GII.4 Sydney has rapidly emerged to become the predominant outbreak strain in the United States; however , timely outbreak surveillance data collected through NORS and CaliciNet and syndromic emergency department data did not indicate that GII.4 Sydney caused a substantial increase in NoV activity during the 2010–13 season compared with the previous 2 seasons. Further analysis of NoV outbreaks reported through NORS and CaliciNet, as well as data on endemic NoV disease, can help verify the strain-specific differences in clinical profile of NoV disease observed in this preliminary assessment. Continuing to expand enhanced real-time outbreak reporting and syndromic surveillance will enable the magnitude and severity of emergent NoV strains, such as GII.4 Sydney, to be evaluated quickly.

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Outbreak-associated *Salmonella enterica* Serotypes and Food Commodities, United States, 1998–2008

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Salmonella enterica infections are transmitted not only by animal-derived foods but also by vegetables, fruits, and other plant products. To clarify links between *Salmonella* serotypes and specific foods, we examined the diversity and predominance of food commodities implicated in outbreaks of salmonellosis during 1998–2008. More than 80% of outbreaks caused by serotypes Enteritidis, Heidelberg, and Hadar were attributed to eggs or poultry, whereas >50% of outbreaks caused by serotypes Javiana, Litchfield, Mbandaka, Muenchen, Poona, and Senftenberg were attributed to plant commodities. Serotypes Typhimurium and Newport were associated with a wide variety of food commodities. Knowledge about these associations can help guide outbreak investigations and control measures.

Salmonella enterica is estimated to cause 1.2 million illnesses each year in the United States and to be the leading cause of hospitalizations and deaths from foodborne disease (*1*). Because of the major public health role of *Salmonella* infections, the US Department of Health and Human Services has made decreasing the nationwide incidence of these infections by 25% a Healthy People 2020 national goal (*2*). Overall, salmonellosis incidence has not decreased in the past decade; the incidence has substantially increased for some serotypes and decreased for others (*2,3*). Focused attention on determining sources of *Salmonella* infections will be vital to reach the 25% target reduction in these infections.

Salmonella serotypes differ in their natural reservoirs and ability to cause human infections (*4–6*); only a small proportion of >2,500 serotypes cause most human infections (*4,7*). In 2009, only 20 serotypes comprised >82% of the ≈36,000 serotyped human-derived *Salmonella* isolates in the United States that were reported to the Centers for Disease Control and Prevention (*3*). A few serotypes have been associated with specific animal reservoirs. For example, serotype Dublin, which caused 103 laboratory-confirmed human infections in 2009 (*3*), is found predominantly in cattle (*5*). However, reservoir sampling alone has limited use in predicting the contribution of a reservoir to the incidence of human illness (*8*).

Outbreak data and case–control studies have linked some serotypes to certain foods or exposures (e.g., serotype Enteritidis to eggs and chicken) (*9–11*). Information obtained during outbreak investigations is a key tool in understanding which foods are common sources of pathogens contributing to foodborne infections. During outbreak investigations, illnesses can be linked to a particular food by using epidemiologic or laboratory evidence (*12*). To our knowledge, no systematic examination of *Salmonella* serotypes and food vehicles implicated in outbreaks has been reported. We analyzed foodborne disease outbreak data to determine associations between food commodities and serotypes to help inform future outbreak investigations, foodborne illness source attribution analyses, and control measures.

Methods

State, local, and territorial health departments voluntarily submit reports of foodborne disease outbreak investigations to the Foodborne Disease Outbreak Surveillance System (FDOSS) of the Centers for Disease Control and Prevention. A foodborne disease outbreak is defined as ≥2 cases of a similar illness resulting from ingestion of a common food. Submitted reports include a description of the pathogen, the implicated food(s), the main ingredients of

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the food, and the contaminated ingredient, if known (*13*). When a *Salmonella* sp. is the etiologic agent, public health laboratories serotype the isolate. A *Salmonella* sp. is considered the confirmed etiology of an outbreak when the same serotype is isolated from ≥2 ill persons or when the bacterium is isolated from an epidemiologically implicated food (*13*).

To standardize the analysis of foods, we used a modified version of an existing classification scheme (*14*) to categorize reported foods into 1 of 20 mutually exclusive food commodities. Foods were classified into a single food commodity if a single ingredient was implicated or if all ingredients in a food product belonged to a single food commodity. We then combined the individual food commodities into 3 broad food commodity groups: 1) aquatic animal–derived food commodities (crustaceans, fish, and mollusks); 2) land animal–derived food commodities (dairy, eggs, beef, game, pork, chicken, turkey, and duck); and 3) plant-derived food commodities (grains–beans, oils–sugars, fruit, nuts, fungi, sprouts, leafy vegetables, root vegetables, and vine–stalk vegetables).

We reviewed all reports of foodborne outbreaks of *Salmonella* infections to FDOSS during 1998–2008 and included in the analysis those outbreaks caused by a single, laboratory-confirmed serotype. We excluded outbreaks in which multiple etiologies were reported, that had an unknown serotype, or that could not be assigned to 1 of the 20 food commodities.

Among all salmonellosis outbreaks and for each *Salmonella* serotype, we calculated the frequency and percentage of outbreaks associated with each food commodity. For each serotype, we also determined the percentage of outbreaks associated with animal-derived food commodities (land and aquatic) and plant-derived food commodities. We calculated the Gini coefficient as a descriptive measure of the magnitude of food commodity diversity, or inequality (*15*) among outbreaks caused by a particular serotype. The Gini coefficient was chosen as a measure of diversity because it provides an easily interpretable range of values from 0 to 1. A Gini coefficient of 0 indicates an equal distribution of outbreaks caused by a serotype across all food commodities, and a value of 1 indicates that all outbreaks were attributed to a single food commodity.

Results

During 1998–2008, a total of 1,491 outbreaks of *Salmonella* infections were reported to FDOSS, and 1,193 (80%) were caused by a single serotype. Of the single-serotype outbreaks, 595 (50%) had an implicated food, and 403 (34%) could be assigned to a single food commodity. Among these 403 outbreaks, 47 serotypes were reported; 23 serotypes caused ≥3 outbreaks. Of the 47 serotypes reported, the 4 most common caused 66% of the 403 outbreaks (Enteritidis 144 [36%], Typhimurium 58 [14%], Newport 40 [10%], and Heidelberg 24 [6%]). Overall, eggs were the most commonly implicated food commodity (112 outbreaks, 28%), followed by chicken (64 outbreaks, 16%), pork (37 outbreaks, 9%), beef (33 outbreaks, 8%), fruit (33 outbreaks, 8%), and turkey (28 outbreaks, 7%) (Table 1, Appendix, wwwnc.cdc.gov/EID/article/19/8/12-1511-T1.htm).

The most commonly implicated food commodity differed by *Salmonella* serotype (Table 1). Eggs were the most common food commodity for outbreaks caused by serotypes Enteritidis (93 [65%] of 144 outbreaks) and Heidelberg (10 [42%] of 24 outbreaks). Egg-associated serotype Enteritidis outbreaks accounted for 23% of all single food commodity outbreaks. Chicken was the most common food commodity for serotypes I 4,[5],12:i:- (3 [75%] of 4 outbreaks) and Typhimurium (15 [26%] of 58 outbreaks). Pork was the most common food commodity for serotypes Uganda (all 4 outbreaks) and Infantis (4 [57%] of 7 outbreaks). Fruit was the most common food commodity for serotypes Litchfield (all 5 outbreaks), Poona (all 4 outbreaks), Oranienburg (2 [50%] of 4 outbreaks), and Javiana (3 [30%] of 10 outbreaks). Turkey was the most common food commodity for serotypes Hadar (3 [38%] of 8 outbreaks) and Saintpaul (3 [33%] of 9 outbreaks). Sprouts were the most common food commodity for serotype Mbandaka (3 [75%] of 4 outbreaks). Food commodities in the aquatic animal group were the most common for serotype Weltevreden (2 [67%] of 3 outbreaks). Animal-derived food commodities were implicated in >90% of outbreaks caused by serotypes Enteritidis, Heidelberg, Hadar, I 4,[5],12:i:-, Uganda, and Weltevreden, whereas plant-derived food commodities were implicated in >50% of outbreaks caused by serotypes Javiana, Litchfield, Mbandaka, Muenchen, Poona, and Senftenberg.

Evaluation of the serotype diversity within food commodity categories (Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/8/12-1511-T2.htm) showed that the 112 egg-associated outbreaks were predominantly caused by *Salmonella* serotypes Enteritidis (83%) and Heidelberg (9%). Of the 64 chicken-associated outbreaks, 64% were caused by serotypes Enteritidis (28%), Typhimurium (23%), and Heidelberg (13%) combined. Among the 37 pork-associated outbreaks, serotypes Typhimurium (22%), Infantis (11%), Newport (11%), and Uganda (11%) were the most common etiology. The most common serotypes causing beef-associated outbreaks were Enteritidis (18%), Newport (18%), and Typhimurium (18%). Of the 33 fruit-associated outbreaks, 57% were caused by serotypes Newport (18%), Litchfield (15%), Enteritidis (12%), and Poona (12%) combined. Among the fruit-associated outbreaks, 17 (52%) were attributed to melons. The most common

serotypes causing melon-associated outbreaks were Litchfield (29%), Poona (24%), Newport (18%), and Javiana (12%). Of the 28 turkey-associated outbreaks, 53% were caused by serotypes Enteritidis (25%), Heidelberg (14%), and Typhimurium (14%) combined. Of the 21 vine-stalk vegetable-associated outbreaks, the most common serotypes were Newport (29%), Braenderup (14%), and Typhimurium (14%). Among the vine-stalk vegetable outbreaks, 19 (90%) were attributed to tomatoes. The most common serotypes causing tomato-associated outbreaks were Newport (32%), Typhimurium (16%), Braenderup (11%), Enteritidis (11%), and Javiana (11%). Of the 16 dairy-associated outbreaks, most were caused by serotypes Typhimurium (56%) and Newport (25%). Eleven outbreaks were associated with aquatic animal–derived food commodities, of which 5 (45%) were caused by serotype Enteritidis. Of the 10 leafy vegetable-associated outbreaks, 50% were caused by serotypes Newport (30%) and Javiana (20%).

Some serotypes were associated with a narrow range of food commodities. Among the 10 serotypes causing the most outbreaks in our study, *Salmonella* serotypes Enteritidis, Hadar, Heidelberg, and Infantis had the lowest diversity, or highest inequality (Gini coefficient ≥0.8), of implicated food commodities (Figure). Outbreaks caused by serotypes Enteritidis, Hadar, and Heidelberg were mostly attributed to eggs and poultry, and serotype Infantis outbreaks were mostly linked to pork. Serotypes Newport and Typhimurium had the greatest diversity (Gini coefficient <0.6), which reflected a wide range of implicated food commodities. Serotypes Braenderup, Javiana, Montevideo, and Saintpaul had modest diversity. Among them, serotype Montevideo outbreaks were mostly attributed to animal–derived food commodities (>80%); 30%–56% of outbreaks caused by serotypes Braenderup, Javiana, and Saintpaul were attributed to animal-derived food commodities.

Discussion

We found notable relationships between *Salmonella* serotypes and food commodities that point to major food reservoirs for different serotypes. Certain serotypes, in particular Enteritidis, Heidelberg, Hadar, and Infantis, caused outbreaks predominantly attributed to specific animal-derived food commodities, a finding that is consistent with results from animal reservoir sampling (*6*). We also identified serotypes that commonly caused outbreaks associated with plant-derived food commodities, particularly the fruit, vine–stalk vegetable, sprouts, and leafy vegetable food commodities. These serotypes that cause plant-associated outbreaks are found relatively infrequently in *Salmonella* reservoir studies of livestock (*6*), which suggests that serotypes with non-livestock reservoirs (e.g., environmental, amphibian, or reptile reservoirs) may be more likely to cause outbreaks by plant-based food vehicles. For example, during an outbreak investigation of serotype Poona infections attributed to cantaloupe consumption, investigators suspected that melons might have been indirectly contaminated through packing equipment or wash water contaminated by reptiles (*16*). Our findings regarding plant-associated serotypes are particularly relevant given recent increases in *Salmonella* outbreaks attributed to fruits or vegetables and a concurrent increase in infections caused by serotype Javiana (*3,17*), a serotype that compared with other common serotypes in this study, caused a higher percentage of plant-derived food commodity–associated outbreaks.

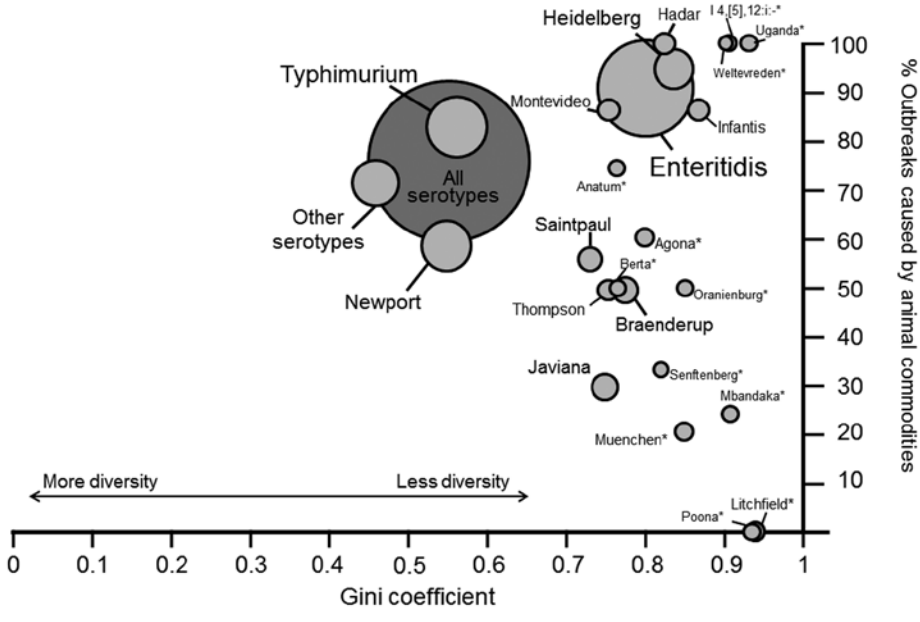

Figure. Gini coefficient and percentage of outbreaks attributed to animal commodities for each *Salmonella enterica* serotype, Foodborne Disease Outbreak Surveillance System, United States, 1998–2008. Size of circle indicates number of outbreaks for each serotype. Animal commodities include land animals (beef, chicken, eggs, game, pork, and turkey) and aquatic animals (crustaceans, fish, and mollusks). *Serotypes with ≤5 outbreaks. The Gini coefficient is a measure of diversity; a value of 0 indicates an equal distribution of outbreaks caused by a serotype across all commodities, and a value of 1 indicates that all outbreaks were attributed to a single commodity.

Our findings of predominant animal-derived food commodities for specific serotypes are supported not only by animal reservoir studies, but also by case–control studies of sporadic illness. Although the percentage of outbreaks attributed to a specific food commodity is not directly comparable to the population–attributable fraction estimated in case–control studies because the units of measure (outbreaks versus illnesses) and the method of estimating the sources of illnesses are different, our results and those of case–control studies show similar dominant food commodity reservoirs for some serotypes. For example, serotype Enteritidis was responsible for a high (83%) proportion of egg-associated outbreaks and ≈25% of chicken and turkey outbreaks; these findings are supported by case–control studies that found eggs and poultry to be common sources of serotype Enteritidis infection (*10,11*).

The high percentages of serotype Heidelberg outbreaks attributed to eggs, chicken, and turkey are also supported by findings from case–control studies and previous reviews (*18,19*). These findings suggest that these products are common vehicles for this serotype. The link we found between serotype Hadar and turkey is consistent with historical data and animal surveillance data showing that serotype Hadar is now the most common serotype isolated from turkey (*6*). The link we found between serotype Infantis and pork is also consistent with animal surveillance data showing that this serotype is commonly isolated from swine but not poultry (*6*). Three of the 4 serotypes with the lowest food commodity diversity measured by the Gini coefficient (Enteritidis, Heidelberg, and Hadar) were predominantly associated with eggs and poultry, suggesting that these serotypes are well adapted to poultry reservoirs and are a well-defined target for control measures.

Two of the most common *Salmonella* serotypes, Typhimurium and Newport, had a wider range of implicated food commodities. Serotype Typhimurium has a well-characterized ability to infect various species (*20*) and can survive for a long time in the environment (*21*); these 2 factors enhance the ability of this serotype to be one of the most common causes of salmonellosis in the United States (*2*). Although we found serotype Typhimurium was associated with several animal commodities, the most common food commodity was chicken (26% of outbreaks), indicating that chicken is a major route of exposure. Among pork-associated outbreaks, Typhimurium was the most common serotype, which corroborates animal data showing that serotype Typhimurium has emerged as the predominant serotype in swine (*6*).

For *Salmonella* serotype Newport, diversity of implicated food commodities might be related to intraserotype genetic variation because several distinct clades have been identified (*22*). Antimicrobial drug resistance data might be helpful for differentiating serotype Newport infections transmitted through animal commodities versus those transmitted by plant-derived food commodities. A sporadic case–control study found associations between infection with multidrug-resistant strains of *Salmonella* serotype Newport and beef and egg consumption, whereas infection with pansusceptible strains was associated with direct or indirect exposure to frogs or lizards (*23*). In a similar manner, strains of serotype Newport causing several outbreaks attributed to beef or dairy products have been multidrug resistant (*24,25*), whereas outbreaks attributed to produce have generally been pansusceptible (*26,27*). Therefore, pansusceptibility might be a marker for serotype Newport strains with environmental reservoirs and a greater potential for transmission though produce. Our findings support the hypothesis that *Salmonella* serotypes with environmental, amphibian, or reptile reservoirs might be more likely to be transmitted by fresh produce.

All outbreaks caused by *Salmonella* serotypes Litchfield and Poona were attributed to fruit. These 2 serotypes were responsible for 25% of fruit outbreaks despite representing only 2% of outbreaks caused by all serotypes in our study. Both serotypes have been established as reptile associated (*28,29*) and reptiles might play a role in fruit contamination (*16*). In a similar manner, 70% of outbreaks caused by serotype Javiana, a serotype associated with reptile and amphibian contact (*30*), were linked to plant-derived food commodities.

Among *Salmonella* serotypes causing small numbers of outbreaks, several had particular animal reservoirs. This result is consistent with reported findings. For example, 2 of 3 serotype Weltevreden outbreaks were associated with aquatic animals, and serotype Weltevreden was the most common serotype found in a survey of imported seafood (*31*). Serotype Agona was responsible for 2 of the 3 outbreaks attributed to grains–beans, both traced to the same facility 10 years apart (*32*). This serotype was introduced into the United States in the 1970s by another dry food product, contaminated fishmeal used in livestock feed (*33*), which suggests good survival of this serotype in dry environments and products.

Salmonella serotype Agona also caused outbreaks attributed to chicken and turkey, consistent with animal surveillance data documenting its frequent isolation in swine, chicken, and turkey since its introduction in animal feed (*6,34*). All 4 serotype Uganda outbreaks were attributed to pork, and all 4 serotype I 4,[5],12:i:- outbreaks were linked to eggs or poultry, suggesting that these food products are reservoirs. Serotype I 4,[5],12:i:- emerged as a cause of human illness in the early 1990s and is now one of the 10 most common serotypes in humans in the United

States (*35*). Serotype Senftenberg is one of the most commonly isolated serotypes from turkeys and chickens (*6*) but was the cause of only a few outbreaks (all nonpoultry) in our study, suggesting that poultry is not the only food serving as a vehicle for transmission of serotype Senftenberg to humans.

Outbreak-associated illnesses represent only a small fraction of all *Salmonella* infections (*1*), and food vehicles responsible for outbreaks might differ from those causing sporadic infections. During the 11 years of our study, changes in product contamination frequency or consumption patterns might be associated with changes in the distribution of serotypes causing illness in the general population or the proportion of sporadic illnesses associated with specific food commodities. In a recent analysis of the distribution of serotypes causing foodborne disease outbreaks (*36*), the proportion of outbreaks caused by serotype Enteritidis decreased from 44% of *Salmonella* outbreaks during 1998–2000 to 24% during 2006–2008, and the percentage of outbreaks caused by *S. enterica* remained relatively constant. That study lacked the statistical power to detect changes over time in the percentages of outbreaks associated with most serotype–food commodity pairs, but found that the percentage of outbreaks caused by *Salmonella* and eggs decreased from 33% during 1998–1999 to 15% during 2006–2008.

Although outbreak data provide one of the only direct connections between food sources and infection, outbreak investigations are frequently unable to confirm the single contaminated food vehicle, limiting our ability to detect major changes over time. In our study, <33% of outbreaks had an implicated food that could be assigned a commodity. Investigators may also report suspected food vehicles on the basis of prior knowledge of the most likely foods associated with the serotype; this reporting technique would bias results toward these typical foods. Although genetic heterogeneity and differences in reservoirs exist within serotypes (*22,37*), our results demonstrate that serotyping provides helpful discrimination among certain serotype–food commodity pairs. Further subtyping of *Salmonella* serotypes could help identify major subtype–food commodity relationships, particularly for common serotypes like Enteritidis and Newport.

This systematic examination of foodborne disease outbreaks by *Salmonella* serotype and implicated food commodity provides major evidence linking serotypes to likely reservoirs and pathways of food contamination. Our analysis could have used outbreak-associated illnesses rather than outbreaks; the attributed sources would have been the same, but the percentages would have differed. However, the goal of this study was to describe major commodity sources by serotype, and this goal was not greatly influenced by the number of outbreak-associated illnesses. Using outbreaks or illnesses for analysis would not provide information about the proportion of sporadic illnesses that can be attributed to specific food commodities; more complex models are needed for such analyses (*14*). The results of our analysis can provide guidance to investigators when forming hypotheses about contaminated food sources during outbreak investigations, and in suggesting the likely contaminated ingredient in outbreaks associated with foods containing ingredients from multiple commodities. Investigators should also remain alert to uncommon or novel food vehicles, which are regularly being identified (*38*). Armed with knowledge of serotype–food commodity associations, public health officials may be able to more quickly form hypotheses, identify likely sources of contamination, and prevent illnesses.

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Comparison of 2 Assays for Diagnosing Rotavirus and Evaluating Vaccine Effectiveness in Children with Gastroenteritis

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We compared rotavirus detection rates in children with acute gastroenteritis (AGE) and in healthy controls using enzyme immunoassays (EIAs) and semiquantitative real-time reverse transcription PCR (qRT-PCR). We calculated rotavirus vaccine effectiveness using different laboratory-based case definitions to determine which best identified the proportion of disease that was vaccine preventable. Of 648 AGE patients, 158 (24%) were EIA positive, and 157 were also qRT-PCR positive. An additional 65 (10%) were qRT-PCR positive but EIA negative. Of 500 healthy controls, 1 was EIA positive and 24 (5%) were qRT-PCR positive. Rotavirus vaccine was highly effective (84% [95% CI 71%–91%]) in EIA-positive children but offered no significant protection (14% [95% CI -105% to 64%]) in EIA-negative children for whom virus was detected by qRT-PCR alone. Children with rotavirus detected by qRT-PCR but not by EIA were not protected by vaccination, suggesting that rotavirus detected by qRT-PCR alone might not be causally associated with AGE in all patients.

Commercially available enzyme immunoassays (EIAs) traditionally have been used to detect rotavirus in children who have acute gastroenteritis (AGE). The rate of rotavirus detection is higher with EIAs than with conventional and semiquantitative real-time reverse transcription PCRs (qRT-PCRs) (*1*–*6*), but some qRT-PCR–positive samples could represent low-level viral shedding from patients with asymptomatic infections or recently resolved rotavirus infections (*6*). qRT-PCR cycle threshold (C_t) values correlate inversely with the amount of viral RNA in a specimen. In a study from the United Kingdom, specimens from patients with AGE that tested positive for rotavirus by EIA had significantly lower qRT-PCR C_t values (higher viral loads) than did qRT-PCR–positive specimens from patients with AGE that tested negative by EIA and from healthy controls; C_t values for the latter 2 groups did not differ (*7*). Another study found that C_t values correlated inversely with severity of disease in patients with AGE and EIA-positive specimens(*8*).

Two rotavirus vaccines (RotaTeq [RV5], Merck, West Point, PA, USA, and Rotarix [RV1] GSK Biologicals, Rixensart, Belgium) are recommended for use worldwide (*9*,*10*). These vaccines have demonstrated high efficacy (>85%) against severe rotavirus-associated AGE in the United States and other high-income countries (*11*–*14*). As vaccine use increases, monitoring vaccine impact is important and requires sensitive and specific detection of rotavirus-associated AGE. Several case–control studies of rotavirus vaccine effectiveness have used patients with AGE who test negative for rotavirus by EIA as a comparison group for patients with AGE who test positive by EIA, and concerns have been raised about whether the rotavirus EIA might fail to detect a proportion of true rotavirus cases and thus lead to bias from misclassification of some cases (*11*–*19*). We compared rates of rotavirus detection by EIA and qRT-PCR among children with and without AGE and examined rotavirus vaccine effectiveness against severe cases of rotavirus-associated AGE, as defined by using different combinations of the EIA and qRT-PCR results.

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Methods

Specimen Collection

Fecal specimens were collected through active surveillance conducted at 3 New Vaccine Surveillance Network sites in the United States (Rochester, NY; Cincinnati, OH; Nashville, TN) year-round during October 2008–October 2009, as described (*18*). In brief, children visiting 1 of the 3 sites who were <5 years of age and had AGE (diarrhea [≥3 loose stools in 24 hours] and/or vomiting [≥1 episode in 24 hours]) for ≤10 days and who lived in 1 of the 3 study areas were enrolled, and a fecal specimen was collected. In addition, during this period, fecal specimens were collected from healthy children <5 years of age who resided in 1 of the same 3 study counties and had a well-child visit or an immunization clinic visit at a community medical practice. These healthy children had neither acute respiratory infection symptoms in the 3 days before nor AGE in the 14 days before the recruitment visit. Parents of all enrolled children were interviewed to collect demographic information and disease history.

Specimen Testing

Fecal specimens were tested for rotavirus by EIA and qRT-PCR. EIA (Premier Rotaclone, Meridian Bioscience, Inc., Cincinnati, OH, USA) testing was done at each study site, and then specimens were frozen and shipped to the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) for further testing. All specimens were retested by EIA (Premier Rotaclone, Meridian Bioscience, Inc.) at CDC. If any EIA result, whether obtained at the study site or at CDC, was positive, then the specimen was classified as rotavirus positive. After preparation of a 10% (vol/vol) suspension of each fecal specimen in phosphate-buffered saline, suspensions were clarified by centrifugation at 3,000 rpm for 10 min. A 100-μL volume of clarified supernatant was added to 300 μL of MagNA Pure LC Total Nucleic Acid Isolation Kit Lysis/Binding Buffer (Roche Applied Science, Indianapolis, IN, USA) to lyse the virus and release nucleic acid. RNA was extracted by using the MagNA Pure 96 Cellular RNA Large Volume Kit (Roche Applied Science) and Cellular RNA LV protocol on the automated MagNA Pure 96 instrument (Roche Applied Science) in accordance with the manufacturer's protocols. The extracted RNA was eluted in 100 μL of elution buffer and stored at −80°C until qRT-PCR testing. RNA was tested for rotavirus by using the NSP3 qRT-PCR designed by Freeman et al. (*3*) and modified to run on an ABI 7500Fast instrument (Applied Biosystems, Foster City, CA, USA) (S. Mijatovic-Rustempasic, KI Tam, TK Kerin, JM Lewis, R Gautam, O Quaye, et al., unpub. data) *21*). C_t values that correlated inversely with the amount of virus in the specimen were used as a proxy for viral load. Lower C_t values indicated higher viral loads. qRT-PCR was run for 45 cycles and was defined as positive if any virus was detected. Standard viral protein 4 and viral protein 7 sequencing procedures, as described, were attempted for all specimens with virus detected by qRT-PCR to identify vaccine strains (*21*).

Analysis

We included in the analysis only children who had sufficient sample volumes for complete testing by EIA and qRT-PCR. Healthy children who were enrolled during a vaccination visit and who had a vaccine strain detected in their feces were excluded from the analyses. We compared sociodemographic characteristics, rotavirus detection rates, and C_t values by using χ^2 statistics for categorical variables and Wilcoxon rank-sum tests for continuous variables.

We calculated vaccine effectiveness using the formula (1 − odds ratio for vaccination) × 100 for children ≥8 months of age. Children are unlikely to receive additional doses of vaccine after 8 months of age. To calculate the adjusted odds ratio, we used unconditional logistic regression and controlled for age at visit, month and year of birth, and month of illness onset. Three laboratory-based rotavirus case definitions were used: EIA positive, qRT-PCR positive, and EIA negative and qRT-PCR positive. Children with AGE who tested negative for rotavirus were used as the control group for the vaccine effectiveness analysis. Three laboratory-based definitions were used for controls: EIA negative, qRT-PCR negative, and EIA negative and qRT-PCR negative. A vaccine dose was considered relevant if it was administered ≥14 days before enrollment. A child was considered fully vaccinated if he or she had received 3 doses of RV5 ≥14 days before enrollment. Children whose immunization record could not be obtained were excluded from the vaccine effectiveness analysis. Because RV1 coverage was extremely low during the study period, children who received RV1 also were excluded from the vaccine effectiveness analysis.

Results

Study Population

Of the 1,145 children whose illnesses met the case definition for AGE during the study period, 815 (71%) had a specimen collected and tested by EIA as part of the surveillance platform (Figure 1). Of these fecal specimens, 648 (80%) were also tested by qRT-PCR, and these 648 children were included in this analysis. Of the 648 specimens tested by both assays, 158 (24%) were positive for rotavirus by EIA. Compared with children whose specimens tested negative by EIA, those whose specimens tested positive for rotavirus by EIA were significantly more likely to be older; be white; have received fewer doses of rotavirus vaccine;

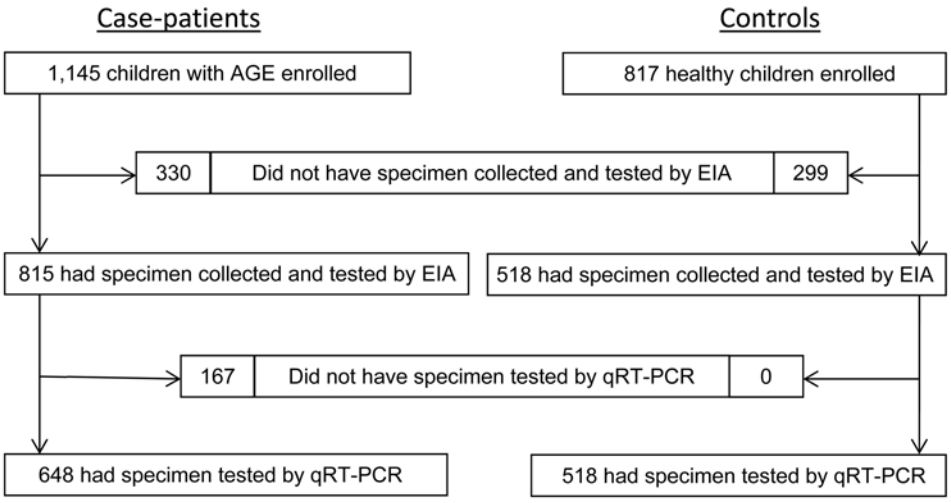

Figure 1. Flowchart of children enrolled in a study of the use of diagnostic assays for rotavirus in children with acute gastroenteritis, 3 New Vaccine Surveillance Network sites (USA), October 2008–October 2009.

have private insurance; live with a child <6 months of age in the household; and have had a specimen collected during January–June, the traditional rotavirus season.

Of the 817 children enrolled as healthy controls, 518 (63%) had a fecal specimen that was tested by EIA and qRT-PCR. Of these, 18 (3%) were enrolled at an immunization visit and had vaccine virus detected in their feces, and they were excluded from further analysis. A total of 500 healthy control children were included in the analysis. Compared with children who had AGE (rotavirus positive or negative by EIA), healthy controls were more likely to be black, fully vaccinated, and have public insurance and less likely to have been breast-fed, attend day care, and have had a specimen collected during January–June. Healthy controls were younger than children positive for rotavirus by EIA and similar in age to children negative for rotavirus by EIA (Table 1).

Comparison of EIA and qRT-PCR for Rotavirus Detection

AGE Cases

For the 158 specimens from children with AGE whose specimens tested positive for rotavirus by EIA, the median C_t value was 18 (range 11–40; Figure 2). An RV5 vaccine strain was detected in 1 specimen from an unvaccinated child that tested positive for rotavirus by EIA and had a C_t value of 16. No virus was detected by qRT-PCR in 1 (1%) specimen from a child whose fecal sample tested positive by EIA. Specimens from an additional 65 (10%) children were positive by qRT-PCR alone, with a median C_t value of 36 (range 23–45), which was significantly higher than the median C_t value for EIA-positive children ($p<0.001$) (Table 2). Rotavirus was detected by qRT-PCR in specimens that were EIA negative and collected during January–June (39 [11%] of 347) and outside the rotavirus season during July–December (26 [18%] of 143).

No vaccine strains were detected among children with AGE whose specimens tested negative for rotavirus by EIA but positive by qRT-PCR. Wild-type rotavirus strains were detected in 8 (12%) of the 65 specimens with any virus detected, whereas a genotype could not be determined for the remaining 57 (88%) specimens for which virus was detected by qRT-PCR.

Healthy Controls

From the 500 healthy control children, 1 specimen tested positive for rotavirus by EIA but not by qRT-PCR. Overall, virus was detected by qRT-PCR in specimens from 24 (5%) healthy children; the median C_t value of 32 (range 21–44) was significantly higher than that for EIA-positive children ($p<0.001$) and significantly lower than that for EIA-negative children ($p = 0.02$) (Table 2).

Of the 24 healthy controls whose specimens had rotavirus detected by qRT-PCR, 11 (46%) had vaccine virus detected, of which 9 contained an RV5 strain and 2 contained the RV1 strain. Six of these 11 children were unvaccinated, including both children for whom the RV1 strain was detected; 3 had received 1 dose of RV5 (70, 75, and 78 days before enrollment); and 2 had received 2 doses of RV5, with the second dose received 28 and 64 days, respectively, before enrollment. Wild-type virus was detected by qRT-PCR in 13 (3%) of the 500 healthy controls. The median C_t values for children with a vaccine virus and a wild-type virus were similar (34 and 30, respectively [$p = 0.05$]). Wild-type rotavirus was detected by qRT-PCR during the traditional January–June rotavirus season (8 [3%] of 317 specimens) and outside the rotavirus season during July–December (5 [3%] of 183 specimens).

Table 1. Sociodemographic characteristics of patients enrolled in a study of the use of diagnostic assays for rotavirus in children with acute gastroenteritis, 3 New Vaccine Surveillance Network sites (USA), October 2008–October 2009*

	Children with AGE			Healthy controls		
Characteristic	Rotavirus EIA+, n = 158	Rotavirus EIA–, n = 490	p value†	All, n = 500	p value‡	p value§
Median age, mo (IQR)	23 (13–30)	12 (5–23)	<0.001	12 (4–20)	<0.001	0.14
Race			0.04		<0.001	<0.001
White	74 (47)	177 (36)		113 (23)		
Black	45 (28)	196 (40)		293 (59)		
Asian	1 (1)	6 (1)		7 (1)		
Other	38 (24)	111 (23)		84 (17)		
Unknown	0	0		3 (1)		
Hispanic ethnicity	27 (17)	95 (19)	0.56	74 (15)	0.48	0.07
Premature birth	14 (9)	53 (11)	0.47	51 (10)	0.61	0.74
Ever breast-fed	110 (70)	310 (63)	0.16	286 (57)	0.006	0.04
Attended day care	55 (35)	150 (31)	0.28	88 (18)	<0.001	<0.001
No. doses rotavirus vaccine received			<0.001		<0.001	0.02
0	105 (66)	171 (34)		178 (36)		
1	9 (6)	57 (12)		53 (11)		
2	8 (5)	72 (15)		74 (15)		
3	22 (14)	164 (34)		187 (37)		
Ineligible	8 (5)	15 (3)		6 (1)		
Unknown	6 (4)	10 (2)		1 (0)		
Data missing	0	1 (0)		1 (0)		
Insurance status			0.01		<0.001	<0.001
Public	86 (54)	335 (68)		430 (86)		
Private	58 (37)	117 (24)		49 (10)		
Public and private	3 (2)	14 (3)		6 (1)		
None	10 (6)	23 (5)		14 (3)		
Unknown	1 (1)	1 (0)		1 (0)		
Maternal education			0.33		0.07	0.48
Less than high school	44 (28)	134 (27)		141 (28)		
High school	40 (25)	153 (31)		170 (34)		
More than high school	74 (47)	203 (41)		189 (38)		
Age of other child in household						
<6 mo	12 (8)	18 (4)	0.04	26 (5)	0.26	0.24
6–23 mo	23 (15)	50 (10)	0.13	67 (13)	0.71	0.12
2–4 y	51 (32)	145 (30)	0.52	153 (31)	0.69	0.73
<5 y	74 (47)	190 (39)	0.07	214 (43)	0.37	0.20
Season specimen collected			<0.001		<0.001	0.01
January–June	141 (89)	347 (71)		316 (63)		
July–December	17 (11)	143 (29)		183 (37)		
Study site			0.34		0.13	0.72
Nashville, TN	39 (25)	149 (30)		163 (33)		
Rochester, NY	54 (34)	146 (30)		140 (28)		
Cincinnati, OH	65 (41)	195 (40)		197 (39)		

*Values are no. (%) except as indicated. AGE, acute gastroenteritis; EIA, enzyme immunoassay; +, positive; –, negative; IQR, interquartile range.
†Children with specimens positive vs. negative for rotavirus by EIA.
‡Children with specimens positive for rotavirus by EIA vs. healthy children.
§Children with specimens negative for rotavirus by EIA vs. healthy children.

Vaccine Effectiveness Using Different Definitions for Cases and Controls

Using only the EIA result to define cases and controls among AGE patients ≥8 months of age, we found that the 3-dose vaccine effectiveness against rotavirus disease that required emergency department care or hospitalization was 84% (95% CI 71%–91%) (Table 3). When cases were defined by using only the qRT-PCR result, the 3-dose vaccine effectiveness estimate decreased slightly to 75% (95% CI 58%–86%), but this estimate did not differ significantly from that estimated by using the EIA result. When cases were restricted to children whose specimens tested negative by EIA but for whom virus was detected at any level by qRT–PCR, the 3-dose vaccine effectiveness estimate was not statistically significant (14% [95% CI -105% to 64%]).

Discussion

The rate of rotavirus detection was higher by qRT-PCR than by EIA. Rotavirus was detected by qRT-PCR in fecal specimens from an additional 10% of children with AGE who tested negative by EIA. However, several lines of evidence suggest that rotavirus detected by qRT-PCR alone might not have been the causative agent in some patients with AGE. First, C_t values of fecal specimens from children with AGE for whom rotavirus was detected only by qRT-PCR were significantly higher (lower viral loads)

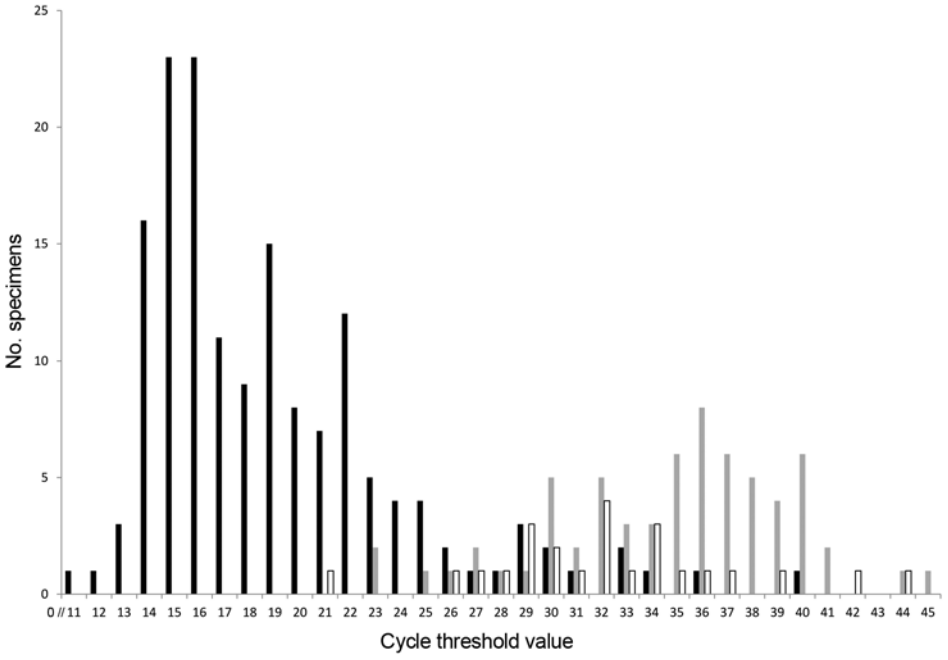

Figure 2. Frequency distribution of Ct values for specimens in which rotavirus was detected by qRT-PCR, 3 New Vaccine Surveillance Network sites (USA), October 2008–October 2009. For 1 (1%) acute gastroenteritis EIA+ specimen, 425 (87%) acute gastroenteritis EIA– specimens, and 476 (95%) healthy control specimens, no virus was detected by qRT-PCR. Ct, cycle threshold; qRT-PCR, semiquantitative reverse transcription PCR; EIA, enzyme immunoassay; +, positive; –, negative. Black bars indicate acute gastroenteritis patients with EIA+ specimens, n = 157; gray bars indicate acute gastroenteritis patients with EIA- specimens, n = 65; white bars indicate healthy controls, n = 24.

than C_t values of specimens from children for whom rotavirus was detected by EIA (36 vs. 18). Second, full genotypes could not be determined for most (88%) specimens for which virus was detected by qRT-PCR only, probably because of the low level of viral shedding. Last, rotavirus vaccine showed limited effectiveness against virus identified by qRT-PCR alone, but this result may partially be a function of the small number of cases in this group. In contrast, 3-dose vaccine effectiveness was high (83%–84%) for children whose samples were positive by EIA and comparable to vaccine effectiveness determined by prelicensure trials and other case–control studies in the United States that similarly identified rotavirus-positive cases by EIA (*11–13*). These findings, together with the easier implementation of commercial EIAs than qRT-PCRs, support the use of EIA for identifying cases and controls to estimate vaccine effectiveness, even though a few rotavirus infections might be missed by the EIA, particularly in specimens for which C_t values are high (low viral loads). qRT-PCR may be useful for identifying cases and controls during vaccine effectiveness studies. However, because such assays also detect low levels of rotavirus circulating in the population but not associated with illness, further work is needed to define a cutoff C_t value below which the detected virus is likely to cause illness. This C_t value would help to identify the few cases missed by the EIA and exclude cases with low-level background shedding.

Previous studies have compared different methods of detecting rotavirus in fecal specimens. These studies should be directly compared with caution because they used different commercial assays and different PCR techniques; however, trends in patterns of detection can be compared. Similar to researchers in the United Kingdom, we found significantly lower C_t values (higher viral loads) in fecal specimens from patients with AGE that tested positive for rotavirus by EIA than in qRT-PCR–positive

Table 2. Comparison of laboratory results in a study of the use of diagnostic assays for rotavirus in children with AGE, 3 New Vaccine Surveillance Network sites (USA), October 2008–October 2009*

	Children with AGE			Healthy controls		
Laboratory result	Rotavirus EIA+, n = 158	Rotavirus EIA–, n = 490	p value†	All, n = 500	p value‡	p value§
Virus detected by qRT-PCR	157 (99)	65 (13)	<0.001	24 (5)	<0.001	<0.001
Of those with virus detected						
Median C_t value (range)	18 (11–40)	36 (23–45)	<0.001	32 (21–44)	<0.001	0.02
G and P type determined	155 (99)	8 (12)	<0.001	12 (50)	<0.001	<0.001
Vaccine strain detected	1 (1)	0	0.52	11 (46)	<0.001	<0.001

*Values are no. (%) except as indicated. AGE, acute gastroenteritis; EIA, enzyme immunoassay; +, positive; –, negative; qRT-PCR, semiquantitative reverse transcription PCR; C_t, cycle threshold.
†Children with specimens that are EIA+ vs. EIA– for rotavirus.
‡Children with specimens EIA+ for rotavirus vs. healthy children.
§Children with specimens EIA– for rotavirus vs. healthy children.

Table 3. VE using different case and control definitions in a study of the use of diagnostic assays for rotavirus in children ≥8 months of age with acute gastroenteritis, 3 New Vaccine Surveillance Network sites (USA), October 2008–October 2009*

Definition, no. doses	No. (%) cases	No. (%) controls	% VE (95% CI)†
EIA+ cases and EIA– controls	128	302	
0	98 (77)	115 (38)	NA
1	6 (5)	15 (5)	51 (–38 to 83)
2	4 (3)	43 (14)	90 (70–97)
3	20 (16)	129 (43)	84 (71–91)
EIA+ case and qRT-PCR– controls	128	266	
0	98 (77)	99 (37)	NA
1	6 (5)	13 (5)	47 (–53 to 82)
2	4 (3)	40 (15)	89 (66–96)
3	20 (16)	114 (43)	83 (68–91)
qRT-PCR+ cases and qRT-PCR– controls	164	266	
0	114 (70)	99 (37)	NA
1	8 (5)	13 (5)	47 (–38 to 80)
2	7 (4)	40 (15)	85 (64–94)
3	35 (21)	114 (43)	75 (58–86)
EIA- and qRT-PCR+ cases vs. EIA- and qRT-PCR- controls	36	266	
0	16 (44)	99 (37)	NA
1	2 (6)	13 (5)	21 (–309 to 80)
2	3 (8)	40 (15)	47 (–108 to 87)
3	15 (42)	114 (43)	14 (–105 to 64)

*VE, vaccine effectiveness; EIA, enzyme immunoassay; +, positive; –, negative; NA, not applicable; qRT-PCR, semiquantitative reverse transcription PCR.
†Controlling for age (in months), month and year of birth, and month of illness onset in the analysis.

specimens from patients with AGE whose feces tested negative for rotavirus by EIA or from healthy controls (*7*). Among UK children <5 years of age who had AGE, use of conventional RT-PCR increased the rotavirus detection rate from 17% by ELISA to 54% by PCR (*6*). However, rotavirus also was detected in 23% of healthy controls by PCR, compared with only 1% of those in whom virus was detected by ELISA. In a study in the United States, rotavirus detection rates for patients with AGE were similar between conventional RT-PCR and EIA (53% and 49%, respectively), but in 18% of healthy controls, rotavirus was detected by conventional RT-PCR, whereas no healthy controls were positive by EIA (*4*). We found a much lower rotavirus detection rate among healthy children (5%) than was found in the previous studies in the United States and United Kingdom (18% and 23%, respectively). The low detection rate among healthy controls in our study also might be partially attributable to the eligibility criteria for healthy controls that required a child to be 14 days without AGE before enrollment and the fecal specimen obtained within 5 days enrollment. In addition, unlike the previous studies, our study was conducted after the introduction of rotavirus vaccine into the US immunization program at a time when rotavirus activity had declined substantially (*22–24*). The lower detection rate of rotavirus in healthy children in our study may reflect this decrease in rotavirus activity after vaccine introduction; that is, fewer children may have been shedding virus from a previous infection, some may have had an asymptomatic infection, and infected children who had been vaccinated were possibly clearing the virus more quickly.

We detected vaccine virus in 2% of healthy controls, all of whom were either unvaccinated or had not been vaccinated within 4 weeks before illness onset; the source of vaccine virus for these children is unknown. These vaccine strains were detected only by qRT-PCR because no healthy children in whom a vaccine strain was detected were positive for rotavirus by EIA. RV5 virus also was detected in the fecal specimen from 1 unvaccinated child with AGE; the source of vaccine virus for this patient was a recently vaccinated sibling, as described (*25*). This symptomatic patient was positive by both EIA and qRT-PCR.

Our study had some limitations. First, if children were seen for medical care late in their illness or if specimen collection was delayed, rotavirus might have been the cause of symptoms in some children whose specimens tested negative for rotavirus by EIA but showed low levels of qRT-PCR–detected virus. However, 99% of EIA-negative specimens that had low levels of qRT-PCR–detected virus were collected from children within 7 days after they were brought for treatment, and RV5 was not effective against AGE detected by qRT-PCR only, arguing against this possibility. Second, an internal positive control was not used in this study to monitor for false-negative qRT-PCR results possibly resulting from PCR inhibitors in feces that were carried over into the RNA extracts. We believe that the numbers of such samples would have been small because we detected only 1 EIA-positive, qRT-PCR-negative sample in this study. Third, the enrollment of some healthy controls during an immunization visit resulted in oversampling of children shedding vaccine virus. Although we excluded recently vaccinated children in whom vaccine virus was

detected, all detected viruses had to be sequenced to identify children who were shedding vaccine virus. However, in a true random sample of healthy children, we would expect some children to be recently vaccinated, so we might have underestimated the proportion of healthy children in whom vaccine virus can be detected. Last, because these data are from an industrialized country in which rotavirus vaccination is routine, our findings might not apply to developing countries where the severity of infection, rates of asymptomatic viral shedding, and performance of the EIA may differ.

In conclusion, our study, which was performed after rotavirus vaccine was introduced, supports the use of EIA for vaccine effectiveness evaluations in patients with AGE, even though EIA may fail to detect some true rotavirus shedding at lower levels. Although qRT-PCR increases the sensitivity of rotavirus detection, some of these cases may be in children with low-level viral shedding from a resolved or asymptomatic wild-type rotavirus infection and not true disease. The use of qRT-PCR with a cutoff C_t value should be further examined as a possible diagnostic tool in a range of settings, including in developing countries.

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Extended-Spectrum ß-Lactamase– and AmpC-Producing Enterobacteria in Healthy Broiler Chickens, Germany

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During 2010, we evaluated the presence of extended-spectrum ß-lactamase– and AmpC-producing enterobacteria in broiler chickens at slaughter. Samples (70 carcasses and 51 ceca) from 4 flocks were analyzed by direct plating and after enrichment. Extended-spectrum ß-lactamase producers were found in 88.6% and 72.5% of carcasses and ceca, respectively; AmpC producers were found in 52.9% and 56.9% of carcasses and ceca, respectively. Most isolates were identified as *Escherichia coli*; *Enterobacter cloacae* (cecum) and *Proteus mirabilis* (carcass) were found in 2 samples each. Molecular characterization revealed the domination of CTX-M genes; plasmidic AmpC was CIT-like. Phylogenetic grouping of *E. coli* showed types A (31.5%), B1 (20.2%), B2 (13.5%), and D (34.8%). These findings provide evidence that healthy broilers in Germany are a source for the dissemination of transmissible resistance mechanisms in enterobacteria brought from the rearing environment into the food chain during slaughtering.

Antimicrobial drug resistance is a threat for therapy failure in human medicine. The presence of enterobacteria, especially *Escherichia coli* that produces extended-spectrum ß-lactamases (ESBLs), has increased during past decades in terms of the worldwide distribution of such resistance traits and of the evolution of different genes (*1*). Resistance genes of the ESBL type are mostly plasmid associated and therefore can spread among bacteria. Although chromosomal AmpC genes exist in several enterobacteria and *E. coli*, plasmid-bound types also exist that can be transferred among bacteria. These types can lead to the overall distribution of antimicrobial resistance, although the carrier bacteria are not pathogenic per se but might lead to opportunistic infections in predisposed patients because ESBL-producing *E. coli* are associated with, for example, urinary tract infection and severe systemic disease. *E. coli* infections can be nosocomial, community acquired, or foodborne. The main ESBL types are TEM, SHV, and CTX-M. Rates of CTX-M infections have increased during the last decade compared with rates of TEM and SHV infections. These enzymes confer resistance to ß-lactam antibacterial drugs, particularly cephalosporins, and may be accompanied by co-resistance to drugs of other classes (*1*,*2*). Because of the ESBL resistance and associated co-resistance, empiric oral antibacterial therapy appears to be limited, especially in the community setting (*3*,*4*).

Sources of infection can be diverse. In addition to human sources of transmission in hospitals and communities, animals pose a reservoir for different pathogenic bacteria with zoonotic potential. Especially with food-producing animals, animals and humans are directly linked. Foodborne pathogens usually do not result in clinical infection of the animal host. Thus identification of sources is possible only by extensive field research in primary production and regular testing of end products. ESBL-producing enterobacteria were shown in different sources of food-producing animals at the farm and from products (*5*–*7*).

Several studies have focused on the characterization of ESBL or AmpC producers from food-production animals by testing flocks at the farm; others have focused only on fecal samples at later production steps (*8*–*11*). Less is known about the actual prevalence or diversity within single healthy broiler flocks at the slaughterhouse and the effect on meat contamination. The processing of meat contributes to overall transmission of bacteria from contamination during slaughtering and dressing, including transmission of resistant bacteria introduced at slaughter by colonized animals onto the meat product.

Our objective was to assess the prevalence of ESBL and AmpC producers in the broiler chicken–production chain in Germany in different species of the *Enterobacteriaceae* family. We focused on individual broiler flocks at the slaughterhouse level to show the introduction of enterobacteria to the slaughtering operation and transmission to the product. In addition to determining different resistance phenotypes and molecular characterization of the isolates, we evaluated the number of presumptive ESBL producers found in meat.

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Materials and Methods

Sampling

From August through November 2010, we collected samples from housing of broiler chicken flocks at 2 different rearing sites (farms A and B) in Germany during fattening in 2 rearing periods (cycles). Each of the 4 flocks comprised up to 40.000 birds per house. To estimate flock status, we collected 2 pairs of sock swab specimens per housing 1 week before slaughter. In brief, moistened boot covers were put on the boots of specimen collectors, and specimens were collected by walking through the housing.

At the slaughterhouse, broiler carcasses and cecal samples were collected from the same flocks; the carcasses were collected after being chilled. Each flock was slaughtered at day 42 ± 1 d. For farm A, a total of 39 cecal samples and 40 carcasses were collected; for farm B, 12 cecal samples were available for cycle 1, and a total of 30 carcasses were collected for both cycles.

Isolation

Sample preparation and isolation of ESBL- or AmpC-producing enterobacteria were conducted as follows. Sock swabs were rinsed in peptone water (Merck, Darmstadt, Germany). Cecal contents were diluted at a ratio of 1 to 10 in peptone water. Broiler carcasses were rinsed in 500 mL maximum recovery diluent (Oxoid, Wesel, Germany). Rinsates (0.1 mL) were plated onto MacConkey agar (Merck) containing 1 mg/L cefotaxime or ceftazidime (Sigma-Aldrich, Munich, Germany) and onto Brilliance ESBL Agar (Oxoid) directly; plates were incubated aerobically for 24 h at 37°C ± 0.5°C. We mixed 100 mL of carcass rinse with 100 mL of double-strength peptone water (Merck). Samples in peptone water were incubated overnight at 37°C ± 0.5°C. Enrichment cultures were streaked onto MacConkey agar and Brilliance ESBL Agar and incubated as before.

Lactose-fermenting or -nonfermenting colonies of different morphology were collected from MacConkey agar and Brilliance ESBL Agar and screened for ESBL production by disk diffusion by using cefpodoxime (10 μg), aztreonam (30 μg), ceftazidime (30 μg), cefotaxime (30 μg), and ceftriaxone (30 μg) disks (Oxoid) and confirmed by microdilution test according to Clinical Laboratory Standards Institute (CLSI) methods (*12*) by using Micronaut-S ß-lactamase V test plates (Merlin Diagnostika, Bornheim-Hersel, Germany). These plates contained cefepime, cefpodoxime, ceftazidime, and cefotaxime, each with and without the addition of clavulanic acid, aztreonam, piperacillin/tazobactam, and meropenem, and meropenem/EDTA. Isolates were identified to species level by using the API 20E test kit (BioMérieux, Nürtingen, Germany).

Isolates showing cefoxitin resistance were considered presumptive AmpC producers. Production of AmpC was confirmed according to methods of Black et al. (*13*). Coproduction of ESBL was confirmed by adding 200 mg/L cloxacillin (Sigma-Aldrich, Munich, Germany) to Mueller-Hinton agar (Oxoid) using disks (Oxoid) according to CLSI ESBL confirmatory tests with disks of cefotaxime and ceftazidime, with or without clavulanic acid. An increase of the inhibition zone around disks containing cephalosporine and clavulanic acid by at least 5 mm confirmed ESBL production (*14*). Other antimicrobial agents tested by microdilution (Merlin Diagnostika, Bornheim-Hersel, Germany) were ciprofloxacin, nalidixic acid, chloramphenicol, tetracycline, trimethoprim–sulfamethoxazole, gentamicin, and streptomycin.

MICs were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (*15*) clinical breakpoints and, for cefoxitin, nalidixic acid, and tetracycline, according to CLSI breakpoints (*12*). Epidemiologic cutoff values dividing wild-type from non–wild-type strains were evaluated according to EUCAST (*16*). The EUCAST breakpoints were chosen because of more conservative values (*17*).

Molecular Characterization of Isolates

A subset of 76 *E. coli* isolates, confirmed ESBL or AmpC by phenotypic methods, was grouped phylogenetically by using modified triplex PCR (*18*). In brief, gene fragments of *chuA* and *yjaA* and a DNA fragment (TSPE4.C2) were amplified with primers ChuA.1 (5′-GACGAACCAACGGTCAGGAT-3′) and ChuA.2 (5′-TGCCGCCAGTACCAAAGACA-3′), fragment 279 bp; YjaA.1 (5′-TGAAGTGTCAGGAGACGCTG-3′), and YjaA.2 (5′-ATGGAGAATGCGTTCCTCAAC-3′), fragment 211 bp; and TspE4C2.1 (5′-GAGTAATGTCGGGGCATTCA-3′) and TspE4C2.2 (5′-CGCGCCAACAAAGTATTACG-3′), fragment 152 bp. PCR conditions were 3 min of initial denaturation, followed by 35 cycles at 94°C for 15 s, 60°C for 30 s, and 72°C for 45 s and final extension at 72°C for 5 min.

A representative selection of 78 isolates from cecal contents and carcasses, depending on sample type and phenotype was analyzed. The isolates were confirmed to show either an ESBL or an AmpC phenotype in the antimicrobial susceptibility tests. The presence of ß-lactamase genes was confirmed by PCR with primers and conditions as reported: bla_{TEM} TEM-F: ATAAAATTCTTGAAGACGAAA, TEM-R: GACAGTTACCAATGCTTAATC, fragment 1,080 bp, annealing 50°C (*19*); bla_{SHV} SHV-F: GGGTTATTCTTATTTGTCGC, SHV-R: TTAGCGTTGCCAGTGCTC, fragment 930 bp, annealing 56°C (*20*); and bla_{CTX-M} CTX-M-F: SCSATGTGCAGYACCAGTAA, CTX-M-R: ACCAGAAYVAGCGGBGC fragment 585

bp, annealing 58°C (*21*). AmpC presumptive isolates were tested for the presence of AmpC gene groups by multiplex PCR according to Pérez-Pérez and Hanson (*22*): MOX-MF: GCTGCTCAAGGAGCACAGGAT, MOX-MR: CACATTGACATAGGTGTGGTGC, fragment 520 bp; CIT-MF: TGGCCAGAACTGACAGGCAAA, CIT-MR: TTTCTCCTGAACGTGGCTGGC, fragment 462 bp; DHA-MF: AACTTTCACAGGTGTGCTGGGT, DHA-MR: CCGTACGCATACTGGCTTTGC, fragment 405 bp; ACC-MF: AACAGCCTCAGCAGCCGGTTA, ACC-MR: TTCGCCGCAATCATCCCTAGC, fragment 346 bp; EBC-MF: TCGGTAAAGCCGATGTTGCGG, EBC-MR: CTTCCACTGCGGCTGCCAGTT, fragment 302 bp; FOX-MF: AACATGGGGTATCAGGGAGATG, FOX-MR: CAAAGCGCGTAACCGGATTGG, fragment 190 bp; annealing 64°C.

Statistical Analysis

The 78 isolates were analyzed statistically to define phenotypic clusters, according to the resistance pattern. Isolates were characterized as susceptible, non–wild type, or clinically resistant according to aforementioned breakpoints for the individual antimicrobial agents and presence or absence of *bla* genes (TEM, SHV, CTX-M) and plasmidic AmpC genes. Data were analyzed by using BioNumerics software, version 5.1 (Applied Maths, Sint-Martens-Latem, Belgium) applying Pearson correlation and clustering with UPGMA (unweighted pair-group method with arithmetic mean). The cutoff for similarity was set to 80%.

Results

All 4 broiler flocks tested positive for ESBL- or AmpC-producing enterobacteria in 2 consecutive rearing cycles. All farm-level ESBL-positive isolates were identified as *E. coli*; in AmpC-producing enterobacteria, we found *E. coli*, *Enterobacter cloacae*, or *Proteus mirabilis*. The rate of ESBL-positive isolates was higher than AmpC-positive isolates in 3 flocks. Only in flock 1 on farm B was the rate of ESBL-confirmed isolates comparatively low (1 isolate was identified as an ESBL producer); AmpC-producers dominated that cycle (data not shown). These results identify healthy broiler flocks as reservoirs of antimicrobial-resistant enterobacteria for further distribution along the food production chain in Germany.

ESBL and AmpC Producers at Slaughter

At slaughter, ESBL- and AmpC-producing enterobacteria were found in 88.6% and 52.9% of the 70 carcasses and 72.5% and 56.9% of the 51 ceca, respectively. Most isolates were identified as *E. coli*; an AmpC-producing *P. mirabilis* isolate was detected in 1 carcass from each farm. The isolate from farm A could not be characterized further because the isolate died during storage. Furthermore, during the first cycle of farm B, *E. cloacae* strains were isolated from 2 cecal samples. Those isolates shared the same phenotype; thus only 1 isolate was characterized. At the flock level, up to 100% of the analyzed carcasses and cecal samples tested positive for ESBL-producing *E. coli* (Table 1). Overall, the presence of ESBL- or AmpC-positive enterobacteria on carcasses mirrored the presence in cecal contents.

We tested samples of carcasses after direct plating and after enrichment. Direct plating allowed us to estimate numbers of suspected ESBL-producing *E. coli* isolates. After direct plating, 24 (38.7%) carcasses were positive. The calculated limit of detection for the direct plating was 3.7 log CFU/carcass, compared with 0.7 log CFU/carcass for the enrichment culture. The number of presumptive ESBL-producing *E. coli* isolates in samples from direct plating was 3.7–4.2 log CFU/carcass.

Antimicrobial Resistance, ß-Lactamase Genes, and Phylogenetic Typing

Testing of antimicrobial agents found 3 different resistance phenotypes. The first phenotype was ESBL only, with reduced susceptibility to at least 1 of the tested cephalosporins with sensitivity to clavulanic acid. The second was AmpC only, with cefoxitin resistance and absence of a clavulanic acid MIC reduction. The third was ESBL in the presence of AmpC production, which could not be identified directly but was shown by confirmation of the clavulanic acid effect on Mueller-Hinton agar containing 200 mg/L cloxacillin.

The resistance phenotype differed by mechanism of resistance. For all isolates, we demonstrated resistance to cefpodoxime and breakpoints above the epidemiologic cutoff value for cefotaxime and ceftazidime. Although clinical resistance to cefotaxime was more prevalent in ESBL producers, the rate of ceftazidime resistance was higher in AmpC producers and in SHV-containing isolates. Some CTX-M gene–carrying isolates only were resistant

Table 1. Prevalence of ESBL- or AmpC-producing enterobacteria in broiler chickens at slaughter, Germany, 2010*

Farm, rearing cycle leading to slaughter	Carcass			Cecum		
	No. samples	ESBL, no. (%)	AmpC, no. (%)	No. samples	ESBL, no. (%)	AmpC, no. (%)
A, 1	20	20 (100)	11 (55)	20	16 (80)	10 (50)
A, 2	20	17 (85)	9 (45)	19	19 (100)	7 (36.8)
B, 1	10	6 (60)	3 (30)	12	2 (16.7)	12 (100)
B, 2	20	19 (95)	14 (70)	0	0	0

*ESBL, extended-spectrum β-lactamase.

to cefepime (Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/08/12-0879-T2.htm; Figure, Appendix, wwwnc.cdc.gov/EID/article/19/8/12-0879-F1.htm). Clinical resistance and indication of other mechanisms according to cutoff values for non–ß-lactam agents differed in the isolates. Clinical resistance for tetracycline was found in >50% of isolates and to nalidixic acid and chloramphenicol in 47% and 38% of *E. coli* isolates, respectively. Because no clinical MIC for streptomycin is defined by CLSI or EUCAST (*12*,*15*), isolates were considered resistant according to epidemiologic cutoff values. Resistance was found in 76 (60%) *E. coli* isolates. Multiresistant isolates showed resistance to nalidixic acid, tetracycline, and chloramphenicol (streptomycin) in most cases.

We analyzed *bla* genes by PCR. We found all 3 *bla* genes (bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$), with $bla_{CTX\text{-}M}$ being the most prevalent.

A total of 74 isolates tested by PCR were positive for at least 1 resistance gene. A single resistance gene was detected in 42 (54%) isolates; 32 (41%) isolates showed 2 or 3 resistance genes.

We identified ESBL producers that carried an additional plasmidic AmpC in 9 (12%) isolates from different samples. The $bla_{CTX\text{-}M}$ was identified in 89% typed ESBL producers; bla_{TEM} and bla_{SHV} were found in 27% and 47%, respectively. In AmpC producers, only CIT-like genes were found in 36 (75%) cefoxitin-resistant or AmpC-positive isolates. The CIT group of AmpC ß-lactamases includes CMY with CMY-2 as the most prevalent member (*23*,*24*). Eight isolates of *E. coli* indicating an AmpC phenotype tested negative for plasmidic AmpC.

Phylogenetic typing revealed the 4 groups of *E. coli*. The types found most often were D and A, followed by B1 and, to a lesser extent, B2 (Table 3). The distribution was similar in carcasses of both flocks. In ceca, this distribution also was reflected for farm A; the limited number of isolates from farm B provided evidence only of B2 and D.

Comparison of isolates showed 2 major groups with 7 clades (A–G) and 14 clusters at a cutoff at 80% similarity (I–XIV) and 7 isolates that did not cluster with other strains (Figure, Appendix). The major groups were identified by the phenotype of resistance or susceptibility to clavulanic acid. The clades included strains of different origin because isolates from both farms and/or different sample types clustered.

The 2 other enterobacteria found clustered in separate branches. *P. mirabilis* isolates clustered with *E. coli* isolates in cluster IV, which might be due to the resistance inferred by the same resistance gene of CIT type possibly resulting from interspecies plasmid transfer. The single *E. cloacae* strain did not cluster with other isolates but clustered in the group with clavulanic acid–resistant isolates. This strain was positive in the AmpC test. Absence of plasmidic AmpC indicates inducible enzymes. In addition, the strain contained TEM ß-lactamase.

Discussion

Bacteria in food-producing animals are spread through the food chain, which is important in terms of food shelf life and for transmission of pathogenic bacteria to the consumer. High numbers of bacteria can reduce shelf life and increase early food spoilage. Foodborne pathogens and bacteria with zoonotic potential are in focus worldwide because of immense health loss and costs that arise from foodborne infection associated with bacteria, such as *Salmonella* sp. and *Campylobacter* spp. (*25*).

Antimicrobial resistance is also of concern because of the limitation and even the risk for loss of effective antimicrobial treatment of infections; evidence exists that resistance from enterobacteria, such as *Salmonella* sp. and *E. coli* of animal origin can be transferred to humans (*26*,*27*). Food-producing animals may play a role in this process, and the food production chain needs to be evaluated to identify the potential for transmission pathways. Animals infected with antimicrobial-resistant strains of bacteria seem to be linked directly with human bacterial strains of *E. coli* (*28*–*30*).

Studies that focus on ESBL-producing *E. coli* at slaughter have found prevalences of 42.1% in feces of broilers and of 60% in chicken carcasses at the retail level in Portugal, but these studies have not included a flock attribution analysis (*9*,*31*). Results from different studies are sometimes difficult to compare because of different isolation and testing methods.

Total *E. coli* or coliforms usually are found in 100% of broiler carcasses at slaughter, at concentrations of 2.5 log CFU/mL for *E. coli* and 2.8 log CFU/mL for coliforms in rinse (100 mL/carcass), resulting in ≈4–5 log CFU/carcass (*32*). The percentage of samples positive for ESBL

Table 3. Phylogenetic group distribution of extended spectrum β-lactamases– and AmpC-producing *Escherichia coli* in broiler chickens, Germany, 2010

	At farm, no. (%)		At slaughter, no. (%)				Total, no. (%)
			Ceca		Carcass		
Phylogenetic group	Farm A	Farm B	Farm A	Farm B	Farm A	Farm B	
A	4 (44)	1 (25)	4 (22)	0	9 (30)	10 (40)	28 (32)
B1	2 (22)	2 (50)	5 (28)	0	8 (27)	1 (4)	18 (20)
B2	1 (11)	0	2 (11)	2 (67)	3 (10)	4 (16)	12 (14)
D	2 (22)	1 (25)	7 (39)	1 (33)	10 (33)	10 (40)	31 (35)

enterobacteria after direct plating and their numbers in this study were lower than those in reports of total *E. coli*, but in the view of possible genetic transfer between bacteria, even low numbers of bacteria harboring mechanisms of antibacterial drug resistance are relevant.

Moreno et al. (*11*) compared the proportion of *E. coli* with reduced susceptibility to expanded-spectrum cephalosporins with the total *E. coli* in the feces of healthy food animals. The resistant population in broilers was 4.3%. Horton et al. (*33*) estimated shedding densities of presumptive CTX-M *E. coli* for cattle, pigs, and chicken in the United Kingdom, where the latter showed higher shedding rates than did the red meat species. Although those studies described the numbers or proportions of resistant *E. coli* in feces of healthy animals, our study focused on total enterobacteria expressing the ESBL phenotype on carcasses at the end of slaughtering.

Fecal contamination, a known problem during broiler production, can lead to contamination of the meat with foodborne pathogens, such as *Salmonella* sp. and *Campylobacter* spp. (*34*,*35*). The role of shedding of bacteria through feces, which leads to contamination of carcasses during slaughtering, was evident in our study. Consequently, a high prevalence of ESBL-producing bacteria in colonized flocks could be shown. As a result, a considerable proportion of broilers were surface contaminated with ESBL-producing bacteria during slaughter.

In connection with the farm isolates, it is evident that strains from the 4 phylogenetic groups were already present during rearing of the poultry. A similar distribution also was found in other countries, where type A or type D dominated in poultry, and type B2 was present at a lower rate (*29*,*36*). Especially groups B2 and, to a lesser extent, type D are of public health concern. Those are supposed to contain strains of higher pathogenic character resulting from more virulence traits. B2 strains can be found in diseased and in healthy poultry and could have zoonotic potential through direct bird-to-human transmission or as genetic reservoir (*37*,*38*). This fact is even more important when increased virulence is paired with antibacterial drug resistance.

The sampled farms were geographically separate, and their poultry were supplied by different companies; however the farms were situated in a region with a high density of food animal production, which could indicate a common source, or selective pressure. At the cluster level, the picture was more diverse, and several clusters contained isolates unique to the individual farms. Isolates from the different sample types (cecal contents, carcasses) clustered, which indicates fecal contamination during the slaughtering process.

Co-resistance to non–ß-lactam antibacterial drugs was most often associated with ESBL genes and was less prevalent in AmpC isolates (Table 2, Appendix; Figure, Appendix). Resistance to nalidixic acid found in several clusters (I, III, V, VIII–IX, and XIII), was usually combined with reduced susceptibility to ciprofloxacin. This resistance was present in isolates of various ESBL or AmpC gene combinations. High levels of nalidixic acid–resistant isolates are considered a first step in mutation to fluorquinolone-resistant strains (*8*). Reduced susceptibility to ciprofloxacin in the tested isolates, together with nalidixic acid resistance, indicates this process. Resistance to chloramphenicol and tetracycline, on the other hand, was most often linked with isolates carrying bla_{SHV} alone or in combination with resistance to nalidixic acid. The co-resistance and the rate of reduced susceptibility are comparable to findings in a recent study by Dierikx et al. (*5*) and may be caused by similar treatment regimens in conventionally reared broilers in Germany and the Netherlands. Phylogenetic groups were not linked to definite resistance patterns, but isolates of the clinically important B2 group were found with either SHV- or CIT-like genes. Overall, the clustering showed a diversity of resistance phenotypes and *bla* gene combinations. These were present in different *E. coli* isolates according to the phylogenetic typing.

The ESBL gene families identified showed distributions comparable with distributions recently reported in Europe and other continents. TEM-52, SHV-12, and CTX-M-1 are the most often reported types from the food animal reservoir (*2*,*39*). CIT-like was the only AmpC type found. The absence of AmpC genes in some phenotype-confirmed isolates might indicate a different mechanism of resistance, probably attributable to overexpression of chromosomal AmpC, which usually results from mutations in the promoter/attenuator region (*40*).

Cephalosporin-resistant enterobacteria isolates were prevalent in the broiler flocks studied. Furthermore, colonization of broilers during rearing correlated with considerable contamination of broiler meat at the slaughterhouse. Isolates in the animals' feces are distributed to the carcasses during the slaughtering operation by fecal contamination. This is a vital point when assessing the transmission potential through the food chain. Therefore, broilers seem to be an important reservoir for enterobacteria with transmissible mechanisms of resistance. In addition to ESBL-producing strains, a considerable number of isolates contained plasmidic AmpC of CIT type.

Phylogenetic characterization of *E. coli* isolates identified possible extraintestinal pathogenic group B2 strains with low prevalence. Their presence, together with the various resistance phenotype, should be observed further to evaluate the distribution and effect on public health.

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Duration of Immunity to Norovirus Gastroenteritis

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The duration of immunity to norovirus (NoV) gastroenteritis has been believed to be from 6 months to 2 years. However, several observations are inconsistent with this short period. To gain better estimates of the duration of immunity to NoV, we developed a mathematical model of community NoV transmission. The model was parameterized from the literature and also fit to age-specific incidence data from England and Wales by using maximum likelihood. We developed several scenarios to determine the effect of unknowns regarding transmission and immunity on estimates of the duration of immunity. In the various models, duration of immunity to NoV gastroenteritis was estimated at 4.1 (95% CI 3.2–5.1) to 8.7 (95% CI 6.8–11.3) years. Moreover, we calculated that children (<5 years) are much more infectious than older children and adults. If a vaccine can achieve protection for duration of natural immunity indicated by our results, its potential health and economic benefits could be substantial.

Noroviruses (NoVs) are the most common cause of acute gastroenteritis (AGE) in industrialized countries. In the United States, NoV causes an estimated 21 million cases of AGE (*1*), 1.7 million outpatient visits (*2*), 400,000 emergency care visits, 70,000 hospitalizations (*3*), and 800 deaths annually across all age groups (*4*). Although the highest rates of disease are in young children, infection and disease occur throughout life (*5*), despite an antibody seroprevalence >50%, and infection rates approach 100% in older adults (*6*,*7*).

Frequently cited estimates of the duration of immunity to NoV are based on human challenge studies conducted in the 1970s. In the first, Parrino et al. challenged volunteers with Norwalk virus (the prototype NoV strain) inoculum multiple times. Results suggested that the immunity to Norwalk AGE lasts from ≈2 months to 2 years (*8*). A subsequent study with a shorter challenge interval suggested that immunity to Norwalk virus lasts for at least 6 months (*9*). In addition, the collection of volunteer studies together demonstrate that antibodies against NoV may not confer protection and that protection from infection (serologic response or viral shedding) is harder to achieve than protection from disease (defined as AGE symptoms) (*10*–*14*). That said, most recent studies have reported some protection from illness and infection in association with antibodies that block binding of virus-like particles to histo-blood group antigen (HBGA) (*13*,*14*). Other studies have also associated genetic resistance to NoV infections with mutations in the 1,2-fucosyltransferase (*FUT2*) gene (or "secretor" gene) (*15*). Persons with a nonsecretor gene (*FUT2*–/–) represent as much as 20% of the European population. Challenge studies have also shown that recently infected volunteers are susceptible to heterologous strains sooner than to homotypic challenge, indicating limited cross-protection (*11*).

One of many concerns with all classic challenge studies is that the virus dose given to volunteers was several thousand–fold greater than the small amount of virus capable of causing human illness (estimated as 18–1,000 virus particles) (*16*). Thus, immunity to a lower challenge dose, similar to what might be encountered in the community, might be more robust and broadly protective than the protection against artificial doses encountered in these volunteer studies. Indeed, Teunis et al. have clearly demonstrated a dose-response relationship whereby persons challenged with a higher NoV dose have substantially greater illness risk (*16*).

Furthermore, in contrast with results of early challenge studies, several observations can be made that, when taken together, are inconsistent with a duration of immunity on the scale of months. First, the incidence of NoV in the general population has been estimated in several countries as ≈5% per year, with substantially higher rates in children (*5*). Second, Norwalk virus (GI.1) volunteer studies conducted over 3 decades, indicate that approximately one third of genetically susceptible

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persons (i.e., secretor-positive persons with a functional *FUT2* gene) are immune (Table 1) (*18,20,22*). The point prevalence of immunity in the population (i.e., population immunity) can be approximated by the incidence of infection (or exposure) multiplied by the duration of immunity. If duration of immunity is truly <1 year and incidence is 5%, <5% of the population should have acquired immunity at any given time. However, challenge studies show population immunity levels on the order of 30%–45%, suggesting that our understanding of the duration of immunity is incomplete (*8*,*11*,*17*,*18*). HBGA–mediated lack of susceptibility may play a key role, but given the high seroprevalence of NoV antibodies and broad diversity of human HBGAs and NoV, HBGA–mediated lack of susceptibility cannot solely explain the discrepancy between estimates of duration of immunity and observed NoV incidence. Moreover, population immunity levels may be driven through the acquisition of immunity of fully susceptible persons or through boosting of immunity among those previously exposed.

In this study, we aimed to gain better estimates of the duration of immunity to NoV by developing a community-based transmission model that represents the transmission process and natural history of NoV, including the waning of immunity. The model distinguishes between persons susceptible to disease and those susceptible to infection but not disease. We fit the model to age-specific incidence data from a community cohort study. However, several factors related to NoV transmission remain unknown (e.g., the role asymptomatic persons who shed virus play in transmission). Therefore, we constructed and fit a series of 6 models to represent the variety of possible infection processes to gain a more robust estimate of the duration of immunity. This approach does not consider multiple strains or the emergence of new variants, so we are effectively estimating minimum duration of immunity in the absence of major strain changes.

Methods

Model Design

We developed a deterministic dynamic transmission model with age structure that tracks the population with respect to NoV infection and immunity status (Figure 1; online Technical Appendix, wwwnc.cdc.gov/EID/article/19/8/13-0472-Techapp1.pdf for model equations). Here we describe the basic structure of the model (model A), which forms the basis for 5 other iterations (models B–F, described below). The models track 5 classes of persons: 1) susceptible to infection and disease (S), 2) exposed but not yet symptomatic (E), 3) infected with symptoms (I), 4) infected but asymptomatic (A), and 5) immune to disease, but not infection (R). In model D, we included an additional class for genetically resistant persons (G).

We assume that maternal immunity is negligible because the youngest age class includes children ages 0–4 years; as such, newborns in all models except model D enter directly into S class. In model D, genetically resistant persons bypass the S class and remain resistant for life, although they make contacts and are included in calculations of incidence for model-fitting purposes, because all persons (not just those susceptible) were included in the empirical studies to which the model was fit. All persons in the S class can be infected at rate $\lambda(t)$ (the force of infection) and move into the E class. They then progress from the E class into the I class (symptomatic) at a rate inversely proportional to the incubation period ($1/\mu_s$). We are thus assuming that when a susceptible (S) person becomes infected, disease will later develop and that all first infections are symptomatic. Persons then recover at a rate inversely proportional to duration of illness ($1/\mu_a$), at which point they are shedding asymptomatically (A). Infection then ends at a rate inversely proportional to duration of shedding ($1/\rho$), after which the person is assumed to have cleared the infection and is recovered from symptoms and that the person's immune system protects from further disease (R).

Table 1. Summary of literature review of Norwalk virus volunteer challenge studies*

	All			Secretor positive			Secretor negative		
Study	No. challenged	No. (%) infected	No. (%) AGE	No. challenged	No. (%) infected	No. (%) AGE	No. challenged	No. (%) infected	Strain
Dolin 1971 (*10*)	12		9 (75)						SM
Wyatt 1974 (*11*)†	23		16 (70)						NV, MC, HI
Parrino 1977 (*8*)†	12		6 (50)						NV
Johnson 1990 (*17*)†	42	31 (74)	25 (60)						NV
Graham 1994 (*12*)	50	41 (82)	34 (68)						NV
Lindesmith 2003 (*18*)	77	34 (44)	21 (27)	55	35 (64)	21 (38)	21	0	NV
Lindesmith 2005 (*19*)	15	9 (60)	7 (47)	12	8 (67)		3	1 (33)	SM
Atmar 2008 (*20*)	21	16 (76)	11 (52)	21	16 (76)	11 (52)			NV
Leon 2011 (*21*)‡	15	7 (47)	5 (33)	15	7 (47)	5 (33)			NV
Atmar 2011 (*14*)‡	41	34 (83)	29 (71)	41	34 (83)	29 (71)			NV
Seitz 2011 (*22*)	13	10 (77)	10 (77)	13	10 (77)	10 (77)		1 (5.6)	NV
Frenck 2012 (*23*)	40	17 (42)	12 (30)	23	16 (70)	12 (52.1)	17		GII.4

*AGE, acute gastroenteritis; SM, Snow Mountain virus; NV, Norwalk virus; MC, Montgomery County virus; HI, Hawaii virus; GII.4, genogroup 2 type 4.
†Only includes initial challenge, not subsequent re-challenge.
‡Only includes placebo or control group.

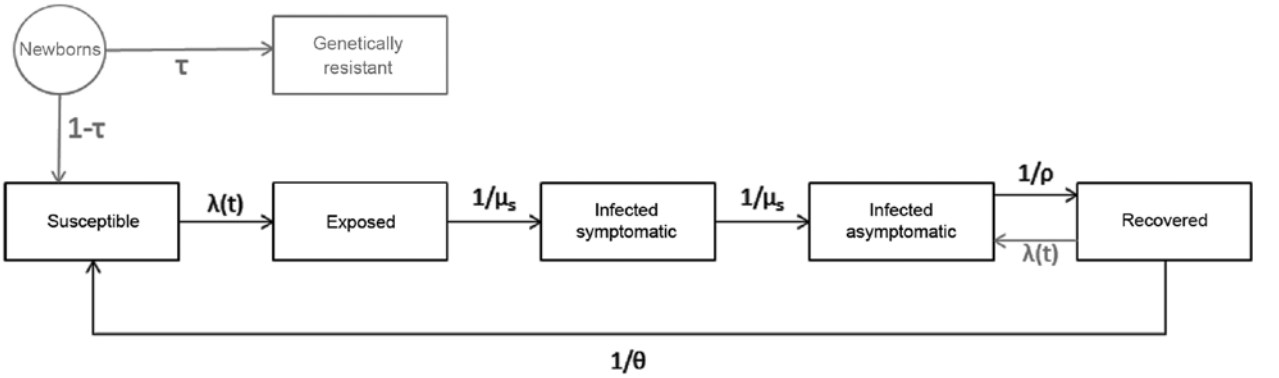

Figure 1. Model schematic illustrating the immunity and infection states of the population with respect to norovirus (NoV) infection and the flows between those states. Persons are born directly into the susceptible pool, become exposed at the force of infection, and then progress through symptomatic and asymptomatic stages before arriving in the recovered compartment, which represents immunity to disease, but not necessarily to infection. As such, from the recovered compartment, persons can become asymptomatically infected at the force of infection or can become susceptible to disease once again through the waning of immunity. For the sake of simplicity, deaths from all categories equal to the incoming births are not shown but are included in the model code. In 1 iteration of the model (scenario E), a compartment is included that represents a class of persons who are born with genetic resistance (in gray to represent absence in all other model iterations) to NoV infection.

Consistent with the understanding of NoV host response, in our model, NoV-specific immunity is not lifelong and we allow 2 pathways out of R class. First, persons can become asymptomatically infected by cycling back into the A class at the same force of infection to which S persons are subjected [λ(t)]. As such, R class represents a type of immunity in which persons are subject to infection but not disease—they can become asymptomatically infected and shed virus in stool specimens, but symptoms of AGE do not develop. Persons in R class can also lose their immunity to disease through the waning process, whereby they become fully susceptible again at a rate of 1/θ. θ is a fitted parameter (described below). Births and deaths are assumed to be equal and occur at a constant rate throughout the year. Static model inputs are detailed in Table 2.

In this baseline model (Figure 1), we assume that only symptomatic (I) persons contribute to transmission, so l(t) is a function of the number of susceptible persons, the age-specific contact rate β_i, the prevalence of infection I(t), and the probability of transmission, given contact (see online Technical Appendix). We allow for children <5 years old to have a different, presumably higher, level of infectiousness (q_1) than older children and adults (q_2) (Table 3).

Model Scenarios

Our first model incorporated several simplifications (e.g., that the entire population is genetically susceptible) for which considerable uncertainty exists (e.g., that immunity to 1 strain of NoV protects against other strains). Therefore, we set up several scenarios to explore the effects on duration of immunity estimates of pre- and

Table 2. Fixed input parameters for each model scenario for duration of immunity to norovirus gastroenteritis*

Parameter	Symbol	Model A	B	C	D	E	F	Source
Life expectancy, y	NA	76	76	76	76	76	76	CDC FastStats (*24*)
Duration of incubation, d	μ_s	1	1	1	1	1	1	Atmar et al., 2008 (*20*)
Duration of symptoms, d	μ_a	2	2	2	2	2	2	Atmar et al., 2008 (*20*)
Duration of asymptomatic infection, d	ρ	10	10	10	10	10	10	Rockx et al., 2002 (*25*)
Relative infectiousness during incubation period	NA	0	0.05	0.25	0	0	0	Sukhrie et al., 2010 (*26*)
Relative infectiousness during asymptomatic infection period	NA	0	0.05	0.25	0	0	0	Sukhrie et al., 2010 (*26*)
Proportion of population genetically resistant	τ	0	0	0	0.2	0	0	Lindesmith et al., 2003 (*18*)
Strains included		All	All	All	All	GII.4 only	All	Rosenthal et al., 2011 (*27*)
Boosting of immunity by asymptomatic infection?	NA	Yes	Yes	Yes	Yes	Yes	No	

*NA, not applicable; CDC, Centers for Disease Control and Prevention. Model A, only symptomatic infectiousness; model B, presyymptomatic and postsymptomatic infectiousness (low); model C, presymptomatic and postsymptomatic infectiousness (high); model D, innate genetic resistance; model E, genogroup 2 type 4 (GII.4); model F, no immune boosting by asymptomatic infection.

Table 3. Duration of immunity, fitted parameter estimates, and log-likelihood and basic reproductive number for models of duration of immunity to norovirus gastroenteritis

Parameter	Symbol	Model A	Model B	Model C	Model D	Model E	Model F
Duration of immunity, y	θ	5.1 (3.9–6.5)	5.1 (4.0– 6.7)	8.7 (6.8–11.3)	4.1 (3.2–5.1)	7.6 (5.6–8.0)	5.1 (3.9–6.6)
Probability of transmission per infected contact, 0–4 y	q_1	0.25 (0.21–0.31)	0.18 (0.15–0.21)	0.37 (0.14–0.91)	0.35 (0.27–0.44)	0.23 (0.19–0.25)	0.25 (0.21–0.31)
Probability of transmission per infected contact, ≥5 y	q_2	0.050 (0.042–0.055)	0.036 (0.032–0.039)	0.094 (0.078–0.114)	0.062 (0.057–0.066)	0.051 (0.47–0.056)	0.050 (0.046–0.054)
Negative log likelihood		615.497	613.905	663.052	616.597	611.509	615.375
Annual incidence, %†		5.2	5.3	5.5	5.1	3.8	5.2
Basic reproductive number (all ages)	R_0	1.79	1.64	7.16	1.88	1.73	1.79
Basic reproductive number (0–4 y)	R_0	4.33	3.98	15.22	4.84	3.98	4.33

*Model A, only symptomatic infectiousness; model B, presyymptomatic and postsymptomatic infectiousness (low); model C, presymptomatic and postsymptomatic infectiousness (high); model D, innate genetic resistance; model E, genogroup 2 type 4 (GII.4); model F, no immune boosting by asymptomatic infection.
†Compared with an observed annual incidence 4.5% from Phillips et al. (*5*), except for model E, which should be compared with norovirus GII.4-specific incidence of 3.2%.

postsymptomatic infectiousness, genetic resistance within a portion of the population, and whether immunity to NoV is strain specific (Table 2).

Model A: Symptomatic Individuals Infectious

In model A, described in the previous section, only symptomatic individuals are infectious. This model provides the basis for the 5 following iterations.

Model B: Presymptomatic and Postsymptomatic Infectiousness (Low)

Presymptomatic persons (E) have been observed to transmit NoV (*28*), although how often this occurs is not known. Also, exposed, but not-yet-symptomatic, persons (E) are 5% as infectious as symptomatic persons (*26*). Because they incubate the virus for only 1 day (1/2 as long as the symptomatic phase), they are 2.5% as infectious as a symptomatic case-patient over the course of their incubation period. Persons may shed virus after resolution of symptoms and may also become infected and shed virus without exhibiting symptoms. Again, their importance in transmission has not been quantified. Sukhrie et al. have demonstrated that asymptomatic shedders can transmit the virus, but they do so at lower levels than symptomatic persons (*26*,*29*). In this scenario, asymptomatic (A) and presymptomatic (E) persons are 5% as infectious as symptomatic persons. Because the mean duration of shedding is 10 days, asymptomatic and presymptomatic persons have a cumulative infectiousness of 25% compared with symptomatic persons (Table 2).

Model C: Presymptomatic and Postsymptomatic Infectiousness (High)

This model has the same structure as model B. However, persons in the exposed (E) and asymptomatic (A) compartments are 25% as infectious as symptomatic persons.

Model D: Innate Genetic Resistance

In model D, we assume that 20% of the population is completely resistant to infection and disease (i.e., they have the nonsecretor phenotype), and therefore play no role in the transmission process (Figure 1) (*18*). They do, however, continue to make contact with other persons and are included in empirical incidence estimates, so the whole population is included in this model, even though 20% cannot become infected. This model includes a separate class of persons born with complete genetic resistance (G).

Model E: Genogroup 2 Type 4 (GII.4)

In Models A–D, we assume that all NoVs are antigenetically indistinguishable, since the degree of strain specificity of NoV immunity is not well understood. Model E tests the sensitivity of that assumption by including only GII.4 infections, which have been the predominant circulating strain for the past decade. We multiplied incidence data by 0.72 (an estimate of the proportion of all NoV AGE caused by GII.4 viruses) (*32*) to represent only GII-4 cases and subsequently refitted the model. This model assumes that GII.4 viruses are antigenically distinct from non-GII.4 NoVs and that all GII.4 viruses are antigenically indistinguishable (*33*).

Model F: No Immune Boosting by Asymptomatic Infection

Persons do not move from the recovered (R) to asymptomatic (A) compartments. The only pathway out of the R class is through waning of immunity to become susceptible (S) again.

Data and Model Fitting

We fit the model to age-specific incidence from the Study of Infectious Intestinal Disease in England (*5*) and

the size of the adult (defined as 15– 44 years of age) population immune at endemic equilibrium by allowing the transmission probabilities (q_is) and duration of immunity (θ) to vary during the fitting process. Size of the immune population was estimated from a literature review of challenge studies (Table 1).

We calculated the log-likelihood of the data under each model by assuming Poisson distributions with mean equal to the number of model-predicted cases for symptomatic NoV incidence in each age group and number of immune persons in the adult age group (see online Technical Appendix). Both incidence and population immune were treated as count data, on the basis of the size of the study population in the study in England (*5*) and the cumulative number of subjects included in challenge studies. The best-fitting parameter set maximized the log-likelihood of the age-stratified time series for the given set of estimated and fixed parameters (*34*). We calculated 95% CIs for each parameter (in each model) and generated a likelihood profile by holding a given parameter constant at a series of values and refitting the model. The upper and lower values were found by using the likelihood ratio test to determine at which parameter value the model converged on a significantly worse fit.

Because seasonality is a defining characteristic of NoV infection, we added seasonal forcing variables to visually inspect whether US outbreak patterns as described by Yen et al. (*35*) could be captured. We allowed the transmission coefficient (β_1) to vary by 6% over the course of the year. However, because including seasonality did not qualitatively change our estimate of the duration of immunity, we excluded it in favor of a more parsimonious model.

Results

All models provided a qualitatively good fit to the crude incidence data, ranging from 5.1% (models D and E) to 5.5% (model C) per year, compared with the observed 4.5% per year (Table 3; Figure 2; online Technical Appendix Figure). All models also captured the decreasing incidence by age; model B was best able to represent the overall incidence and the high incidence in children <5 years of age (21.4% observed; 19.3% fitted), and model B roughly captured the incidence in the groups ≥45 years of age. Model C provided a worse fit than models A, B, D, or F. Model E could not be readily compared because it is fitted to a different incidence case count. Although model B was not a significantly better fit than A, D, or F, it did have the smallest negative log-likelihood, so we used model B for subsequent results, unless stated otherwise.

The R_0 (basic reproductive number) for all models ranged from 1.64 to 1.88, except in model C, which had an R_0 of 7.16. R_0 for children 0–4 years of age was 15.22, substantially higher than for persons >5 years (R_0 = 0.89) (model B, Table 3).

In model A, the duration of immunity to NoV was estimated at 5.1 years (95% CI 3.9–6.5; Table 3). The duration of immunity estimated in model B was essentially the same as in model A at 5.1 years (95% CI 4.0–7.6). When the infectiousness of asymptomatic persons was increased in model C, estimate of duration of immunity increased substantially, to 8.7 (95% CI 4.0–11.3). Duration of immunity estimated in model D, in which transmission was effectively restricted to 80% of the population, was 4.1 years (95% CI 3.2–5.1). In model E, which was essentially fitted to a lower incidence to reflect only GII.4 transmission, duration of immunity was estimated at 7.6 years (95% CI 5.6–8.0). Model F, which did not allow subclinical infection to boost immunity, resulted in a duration of immunity estimate of 5.1 years (95% CI 3.9–6.6). Note that the transmission parameters (q_is) fell into 3 relative patterns: lower (model B), middle (models A, E, and F), and high (models C and D). These differences in transmissibility partly explain why the duration of immunity estimates are not more divergent between models.

With mild seasonal forcing (6% seasonal variation in transmission probabilities), the model captures fluctuations in disease incidence similar to those reported from outbreaks in 30 US states during 2007–2010 (Figure 3). Seventy-three percent of cases were estimated to occur during October–March, compared with 73% observed in the United States during October–March from 2007 to 2010.

Discussion

The goal of this study was to gain a better estimate of the duration of immunity to NoV AGE, and our results

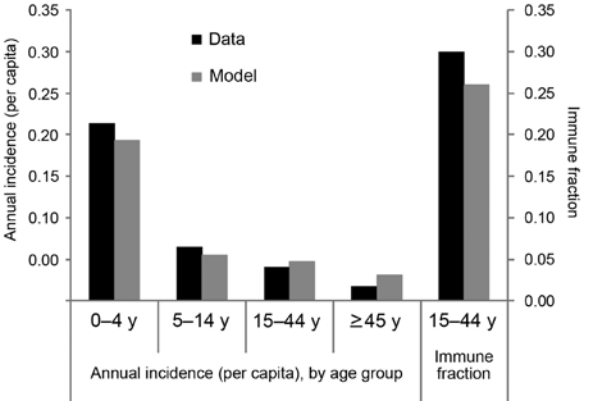

Figure 2. Age-specific annual incidence of norovirus gastroenteritis, observed (black) and model predicted (gray). These results are for model B (which includes presymptomatic and postsymptomatic infectiousness).

suggest that it is longer than was previously understood. We modeled a range of possible infection and immunity processes to capture the unknown aspects of the transmission process, and from these models, we estimate a mean duration of immunity ranging from ≈4 to 8 years. Variations in duration of immunity between models can be traced to inclusion of presymptomatic and postsymptomatic states in the model scenarios. The best-fit models converge on a much higher infectiousness and R_0 for young children (<5 years) than for older children and adults. This finding is consistent with observational studies that found contact with a symptomatic child to be the prime risk factor for NoV infection for children and adults (*5*,*30*) and suggests that young children have a key role in the transmission of NoV to all age groups. Children have relatively high rates of contact with both other children and adults, and because of their lower levels of hygiene, they are likely to be more infectious than adults, given contact (*31*).

In our study, the models produced strong quantitative fits to the empirical data on incidence and population immunity, as well as on seasonality. Results suggest that parameter estimates are not overly sensitive to structural uncertainties, such as the role of asymptomatic shedding in disease transmission, at least within the range of the fixed parameters we have considered. The possible exeption is model C, which resulted in a much higher R_o than the other models and previous estimates, suggesting that asymptomatic persons are unlikely to be as infectious as they were parameterized to be in this scenario. Because the exact structure of immunity in the general population is unknown, our study sought to elucidate that structure rather than identify exact values for the various parameters included in each model scenario.

Several caveats should be borne in mind when interpreting these results. First, perhaps most critical, our model assumes that immunity is to disease (e.g., symptoms) rather than to infection. As such, so-called immune persons are still subject to becoming infected but they do not show symptoms. Infection without symptoms is a common outcome of exposure, as shown by volunteer studies and point prevalence of asymptomatic infection detected in the general population, which can be as high as 30% (*5,18,19*). In effect, our model allows for boosting of immunity by cycling between the recovered (R) and infectious asymptomatic (A) compartments. However, our estimates of duration of immunity pertain to time spent in the immune state from time of most recent symptomatic infection. If a person repeatedly became asymptomatically infected (moves from R to A class), that person would effectively be immune to disease for longer than a person without successive asymptomatic/subclinical infections. The duration of immunity estimates are therefore conservative with respect to total time a person is protected from disease.

Second, with this single-strain model, we assume that all NoVs are antigenically indistinguishable and that infection with 1 NoV provides protection against all others. This is not strictly true (*11*), but data are not available on cross-protection to a range of NoV strains circulating at a particular time. As an extreme simplification of this process, we modeled GII.4 viruses on the assumption that they comprise 72% of observed incidence and are an antigenically homotypic genotype, essentially acting as a separate virus. However, GII.4 viruses are antigenically distinct from other GII viruses, and every few years, new GII.4 strains emerge that escape acquired population immunity. Over the past 15 years, at least 2 immune escape variants of GII.4 have emerged (in 2002 and 2006) (*33*). Although our estimate of duration of immunity (>4 years) may be compromised by this assumption, that novel GII.4s emerge once every 4 years or so would still suggest a role for the duration of immunity on the scale of years. Immunity gained through exposure to the prevalent strain would persist past emergence of a new strain, even though such protection could be effectively useless against the new strain.

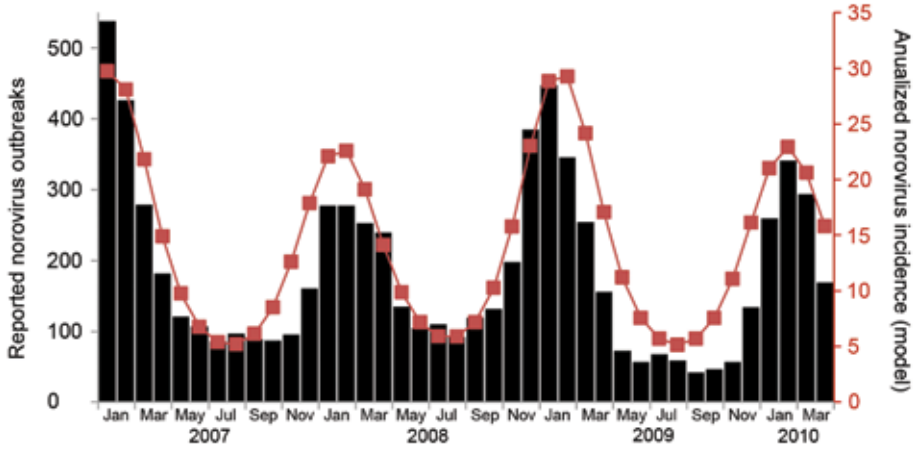

Figure 3. Norovirus gastroenteritis outbreak patterns from 30 US states, January 2007–April 2010 (black bars) and predicted annualized monthly incidence for all age groups (red line). These results are for model B (which includes presymptomatic and postsymptomatic infectiousness) and, for this illustration, seasonal forcing (*35*).

These findings could ultimately have implications for vaccine policy. Empirical studies strongly document that children have the highest incidence of disease. Our results suggest that young children play a dominant role in the transmission process. Therefore, vaccinating young children is likely to result in both the greatest direct and indirect benefits. This conclusion is at odds with the current direction of vaccine development, which is increasingly focused on demonstrating safety and efficacy in older age groups (*36*,*37*). Future modeling studies could explicitly examine the potential direct and indirect benefits of vaccinating different age groups. Moreover, severe disease disproportionately occurs among the elderly (despite their lower incidence of disease), but the elderly are difficult to successfully immunize for both programmatic and immunologic reasons. Therefore, future modeling studies should address the question of whether severe disease outcomes could best be prevented directly, by vaccinating the elderly, or indirectly, by vaccinating children (*38*–*40*). Our study provides estimates of the infectiousness of children <5 years of age and adults (with the former being much more infectious) on which to base such simulations.

Because these results suggest a longer duration of protection than previously estimated, they support the continued development of NoV vaccines. A short duration of protection (<1 year, for example) would be a major impediment for widespread use of a NoV vaccine because it would have to be given frequently, and the distribution would be expensive and logistically difficult (e.g., willingness for annual vaccination). However, if duration of immunity and possibly vaccine protection are indeed on the order of 5 years, as this study suggests, the cost-benefits and health gains per person vaccinated could be substantially greater than previously estimated (*41*).

Our findings represent a substantial departure from current estimates of the duration of immunity to NoV. As noted, our models make several potentially influential simplifying assumptions. However, these models, grounded in observational evidence on age-specific incidence, seasonality of disease, and levels of population immunity, may be more realistic than results of re-challenge studies, which have formed the basis of current estimates. Specifically, this analysis suggests that the large dose or type (GI.1) delivered to volunteers in the classic challenge studies was unrepresentative of natural exposure to common contemporary strains. Because a robust duration of protection is likely crucial for the success of vaccines, future trials could consider following-up at least a subset of participants for several years either for natural disease or by challenge, providing an empirical test of these modeling results.

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Accuracy of Diagnostic Methods and Surveillance Sensitivity for Human Enterovirus, South Korea, 1999–2011

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The epidemiology of enteroviral infection in South Korea during 1999–2011 chronicles nationwide outbreaks and changing detection and subtyping methods used over the 13-year period. Of 14,657 patients whose samples were tested, 4,762 (32.5%) samples were positive for human enterovirus (human EV); as diagnostic methods improved, the rate of positive results increased. A seasonal trend of outbreaks was documented. Genotypes enterovirus 71, echovirus 30, coxsackievirus B5, enterovirus 6, and coxsackievirus B2 were the most common genotypes identified. Accurate test results correlated clinical syndromes to enterovirus genotypes: aseptic meningitis to echovirus 30, enterovirus 6, and coxsackievirus B5; hand, foot and mouth disease to coxsackievirus A16; and hand, foot and mouth disease with neurologic complications to enterovirus 71. There are currently no treatments specific to human EV infections; surveillance of enterovirus infections such as this study provides may assist with evaluating the need to research and develop treatments for infections caused by virulent human EV genotypes.

Human enteroviruses (EVs) belong to the family *Picornaviridae*, genus *Enterovirus*, and are classified into 4 species, EV-A, B, C, and D (*1–3*). More than 90 serotypes are currently recognized by the International Committee on Taxonomy of Virus Classifications. EV-A (17 serotypes), EV-B (56 serotypes), EV-C (16 serotypes), and EV–D (3 serotypes) species classifications are based on similarities in virus capsid protein (VP) genes (*4–6*). Among them, 65 serotypes are known to cause infections in humans, including polioviruses, echoviruses (E), coxsackieviruses A (CA) and B (CB), and EV types 68–71 (*7,8*).

Most EV infections (hand, foot and mouth disease [HFMD]; gastroenteritis; and acute hemorrhagic conjunctivitis) are asymptomatic or mild, and infected persons can recover without specific medication (*5,8–10*). However, the neurotropism of some EVs can cause serious central nervous system complications such as aseptic meningitis, encephalitis, and flaccid paralysis (*9,11,12*). Although some EVs cause severe and potentially life-threatening illness, there is currently no antiviral treatment available for EV infection (*9*).

Laboratory diagnosis of EV infection is based on detection of the virus in clinical specimens such as fecal or rectal swab samples, cerebrospinal fluid (CSF), nasopharyngeal secretions collected by throat swab, and blood (*11,13*). Detection of EV is usually performed by isolation of the virus in cell culture, reverse transcription PCR (RT-PCR), or real-time RT-PCR (*11,14–16*). Currently, RT-PCR is used routinely worldwide to diagnose EV infection because of its sensitivity, specificity, and ability to detect highly conserved 5′ noncoding regions of the human EV genome (*15,17,18*). For determining subtype, the neutralization test is the standard diagnostic tool and is generally reliable, but it is also labor-intensive, time-consuming, and may fail to identify an isolate (*16,19*). Therefore, RT-PCR amplification of the VP1 coding region, then amplicon sequencing, is a sufficient mechanism for molecular typing of EVs (*20*).

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Since 1993, the national enterovirus surveillance system of the Korea Centers for Disease Control and Prevention (KCDC) has monitored and characterized human EV infection in patients with EV-related diseases. Three basic detection methods for diagnosis have been used in this system since surveillance began. During 1993–2004 (phase I), cell culture methods were used; during 2005–2007, RT-PCR was used (phase II); and from 2008–2011 (phase III), real-time RT-PCR was the standard detection method used. Before 2005, genotyping was performed by using the neutralization test, but since then, as documented for phases II and III of this study, VP1 sequencing was used to genotype EV. In this study, we obtained the clinical and epidemiologic data regarding enterovirus infections, including outbreaks and sporadic cases, during 1999–2011 in South Korea, and focused on the improvement of surveillance sensitivity as diagnostic methods developed.

Materials and Methods

Surveillance System and Data Sources

The KCDC national enterovirus surveillance system consists of 180 clinics managed by pediatrics physicians (35 primary clinics, 105 secondary hospitals, and 40 tertiary hospitals nationwide), and the number of clinics participating in the surveillance system varied each year. Participating physicians collected specimens from patients whose illnesses included meningitis, encephalitis, influenza-like illness, HFMD, herpangina, and gastroenteritis, and documented patient age, date of specimen collection, symptoms, and suspected diagnosis. KCDC registered information on a website (http://enterovirus.macrogen.com/cdclab/) originally set up in 2009. Analysis of the specimens, including typing of relevant EVs and other characterizations, was done at the National Polio Laboratory of KCDC.

Patients and Samples

In total, 17,349 clinical samples from 14,657 patients with suspected enteroviral disease were collected during January 1999–December 2011. The average ages of the patients from primary clinics, secondary hospitals, and tertiary hospitals were 13, 5, and 8 years, respectively. Sample types investigated were as follows: 9,012 fecal samples; 5,045 CSF samples; 1,979 throat swab samples; 516 blood samples; and 804 other samples, including urine, saliva, pericardial fluid, and skin swab. Fecal samples are the most common samples obtained from patients suspected of having enterovirus infections. CSF samples were collected from the patients in secondary and tertiary hospitals who had meningitis or encephalitis, and throat swab samples were collected from those with influenza-like illness.

EV Detection in Clinical Samples

Over 13 years, 3 methods were used to detect EV in South Korea. We identified the periods during which the methods were used as phases I, II, and III.

Phase I (1999–2004): Cell Culture

During 1999–2004, clinical samples were processed by using the World Health Organization (WHO) polio laboratory manual as follows (*21*). Fecal material was made into a suspension (10%) by dilution with 0.1mmol/L phosphate-buffered saline, and 10% (vol/vol) chloroform was added. The mixture was then vigorously shaken for 5 min and centrifuged at 500 × *g* for 15 min. Following centrifugation, the supernatant was transferred to a new tube and then injected into cells. Pharyngeal swab samples were collected in virus transport medium; CSF, serum, and pericardial fluids were directly injected into cells. Rhabdomyosarcoma and L20B cell lines were used to isolate the EVs.

Phase II (2005–2007): RT-PCR

During 2005–2007, viral RNA was extracted from each sample by using magnetic beads (GM-Autoprep Kit, Seoul, South Korea) according to the manufacturer's instructions, and the purified viral nucleic acid was processed by using Freedom EVO (Tecan, Männedorf, Switzerland). RT-PCR was performed by using primers designed in a previous study (*14*).

Phase III (2008–2011): Real-time RT-PCR

During 2008–2011, one-step real-time RT-PCR was performed by using a dually labeled fluorogenic EV-specific probe and primers. A highly conserved 5′ noncoding region was the target of a previously described 196-bp region (*15,22*).

Characterization of EV

Phase I: Neutralization Test

Cell culture isolates were identified by using neutralization tests consisting of standard polyclonal antiserum typing according to WHO recommendations (*21,22*). Two reference-typing serum sources were used for microneutralization tests: the Lim-Benyesh–Melnick equine antiserum pools supplied by the WHO Collaborating Centre for Virus Reference and Research and the RIVM pools (National Institute of Public Health and the Environment, Bilthoven, the Netherlands).

Phases II and III: Partial Sequencing of the VP1 Genomic Region

For genotyping, the VP1 amplicon (375 bp) was amplified by seminested RT-PCR and then sequenced according

to the US CDC protocol (*24*). To determine the EV genotype, we compared the sequence homology between the amplified PCR products and the VP1 sequences available from GenBank. The sequences obtained were identified in terms of closest homology by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Prevalence of EV and Improvement of Surveillance Sensitivity

Of 17,349 specimens collected during 1999–2011, a total of 5,220 (30.1%) were laboratory confirmed as EV positive (Figure 1). Fecal or rectal swab samples, the most commonly collected sample type, accounted for 9,012 (51.9%) of all samples collected, and 3,213 (35.7%) of those samples were positive for EV (Figure 1). For other sample types, 19.0% (958/5,045) of CSF, 36.0% (713/1,979) of throat swab samples or secretions, 16.7% (86/515) of blood, and 31.3% (250/798) of other samples (i.e., urine, saliva, pericardial fluid, and skin swabs) were confirmed positive for EV.

The annual prevalence of EV during 1999–2011 is shown in Table 1. The EV detection rate varied each year, ranging from 3.8% in 2001 to 54.2% in 2008. A total of 4,762 (32.5%) of 14,657 patients were infected during 1999–2011. By use of the cell culture method during phase I (1999–2004), 20.5% were detected; by use of RT-PCR during phase II (2005–2007), 26.4% were detected; and by use of real-time RT-PCR during phase III, (2008–2011), 39.2% were identified.

Distribution of Enterovirus Infection by Season and Age

The EV detection rate varied throughout each year (Figure 2); the number of EV cases increased during late spring, summer, and the beginning of autumn (May–September) (Figure 2). The peak months of detection were as follows: July in 1999, May in 2000 and 2001, July in 2002, October in 2003, September in 2004, August in 2005, July in 2006, June in 2007, July in 2008–2010, and June in 2011 (Figure 2). Low detection rates (<10%) were generally observed during late autumn into early spring (October–April) except during January in 2000, February in 2001 and 2004, October in 2003 and 2007, and November in 2007 (Figure 2).

Age was known for 12,296 of the 14,657 patients studied. Age distribution of the 4,209 patients whose ages were known and test results were positive, as shown in Table 2, was 980 (23. 3%) patients <1 year of age, 1,846 (43.9%) 2–5 years of age, 937 (22.3%) 6–10 years of age, 285 (6.8%) 11–20 years of age, 60 (1.4%) 21–30 years of age, 68 (1.6%) 31–40 years of age, 17(0.4%) 41–50 years of age, 13 (0.3%) 51–60 years of age, and 3 (0.07%) >60 years of age.

Clinical Manifestations and Genotypes of EV

During the period studied, 44 different genotypes were detected among 3,128 EV-positive samples (Table 3). The 5 main genotypes were enterovirus (EV) 71, echovirus (E) 30, coxsackieviruses B (CB) 5, E6, and CB2, accounting for 14.9%, 12.5%, 9.3%, 8.4%, and 6.0% of the total EV, respectively. The 5 most frequently observed genotypes during each phase were phase I: E6, E13, E9, polio Sabin

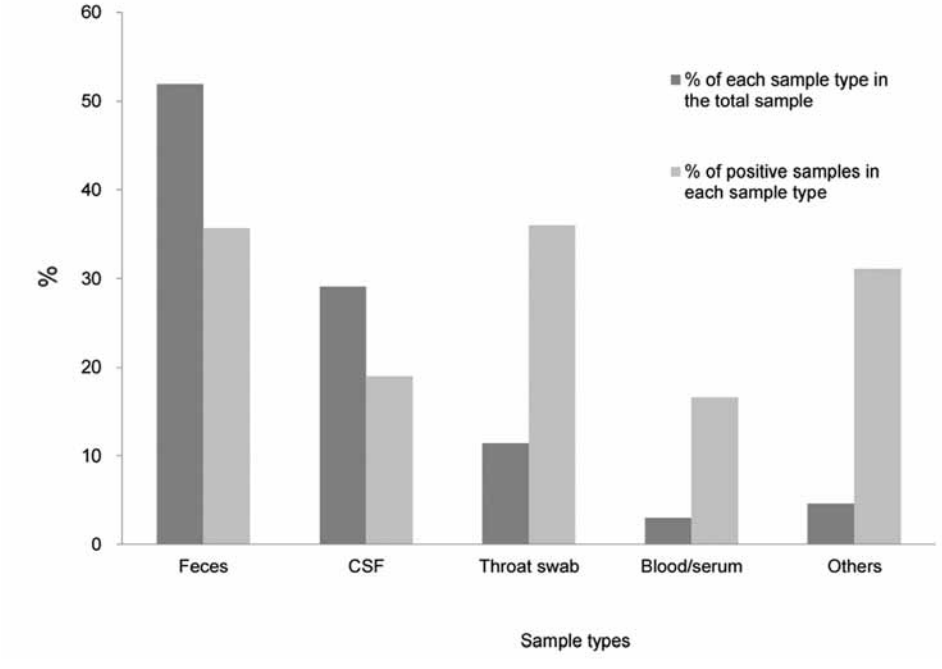

Figure 1. Specimens submitted for detection of enterovirus (n = 17,349) and proportions with positive results. Other samples included urine, saliva, pericardial fluid, and skin swab.

Table 1. Analysis of diagnostic methods for detecting human EV and surveillance outcomes, South Korea, 1999–2011*

Year	No. samples	No. (%) positive	Average % positive
Phase I†			20.5
1999	372	133 (35.8)	NA
2000	261	30 (11.5)	NA
2001	676	26 (3.80)	NA
2002	1,272	361 (28.4)	NA
2003	264	66 (25.0)	NA
2004	314	33 (10.5)	NA
Phase II‡			26.4
2005	890	382 (42.9)	NA
2006	1,059	238 (22.5)	NA
2007	1,131	193 (17.1)	NA
Phase III§			39.2
2008	2,332	1,264 (54.2)	NA
2009	2,766	869 (31.4)	NA
2010	1,477	566 (38.3)	NA
2011	1,843	601 (32.6)	NA
Total	14,657	4,762 (32.5)	NA

*EV, enterovirus; NA, not applicable.
†During phase I, human EV was detected and serotyped by using cell culture.
‡During phase II, human EV was detected by reverse transcription PCR (RT-PCR) and genotyped by sequencing of virus capsid protein (VP) 1 region.
§During phase III, human EV was detected by real-time RT-PCR and genotyped by sequencing of the VP1 region.

strain, and CB2; phase II: CB5, E18, CB3, CB2, and E25; and phase III: EV71, E30, E6, CA16, and CB5. In addition, 39 polioviruses had been detected before 2006 and confirmed as being related to the polio Sabin strain (data not shown).

EV genotypes are described in 4 major categories on the basis of associations with groups and clinical signs and symptoms; these are described as follows: 1) aseptic meningitis; 2) HFMD and/or herpangina; 3) HFMD with neurologic complications; and 4) other manifestations, including sepsis, acute gastroenteritis, hepatitis, pneumonia, and myopericarditis (Figure 3). The clinical manifestations of 1,624 (34.1%) patients whose samples tested positive were as follows (Figure 3): aseptic meningitis was diagnosed for 1,063 (65.5%) patients, HFMD for 155 (9.5%) patients, HFMD with neurologic complications for 295 (18.2%) patients, and other pathogenesis for 111(6.8%) patients. The genotypes of EV detected in 5 other cases during 1999–2011 are shown in in Figure 3. Aseptic meningitis was frequently associated with E30 (225/1,063, 21%), E6 (159/1,063, 15%), and CB5 (123/1,064, 12%) (Figure 3, panel A). Among HFMD cases, infection with CA16 was identified for 37% (58/155), CA10 for 16% (24/155), and E30 for 9% (14/155) of the patients (Figure 3, panel B). Regarding HFMD with neurologic complications, EV71 was the dominant genotype in 84% (247/295) of the cases and CA16 in 5% (14/295) (Figure 3, panel C). For patients with sepsis, acute gastroenteritis, hepatitis, pneumonia, and myopericarditis cases, E25, E18, and E6 were identified for 12% (13/111), 11% (12/111), and 9% (10/111), respectively (Figure 3, panel D).

Discussion

We have presented longitudinal data reflecting changing patterns of enterovirus prevalence over a 13-year period in the South Korea while explicitly noting the changing laboratory methodology over the period. The results

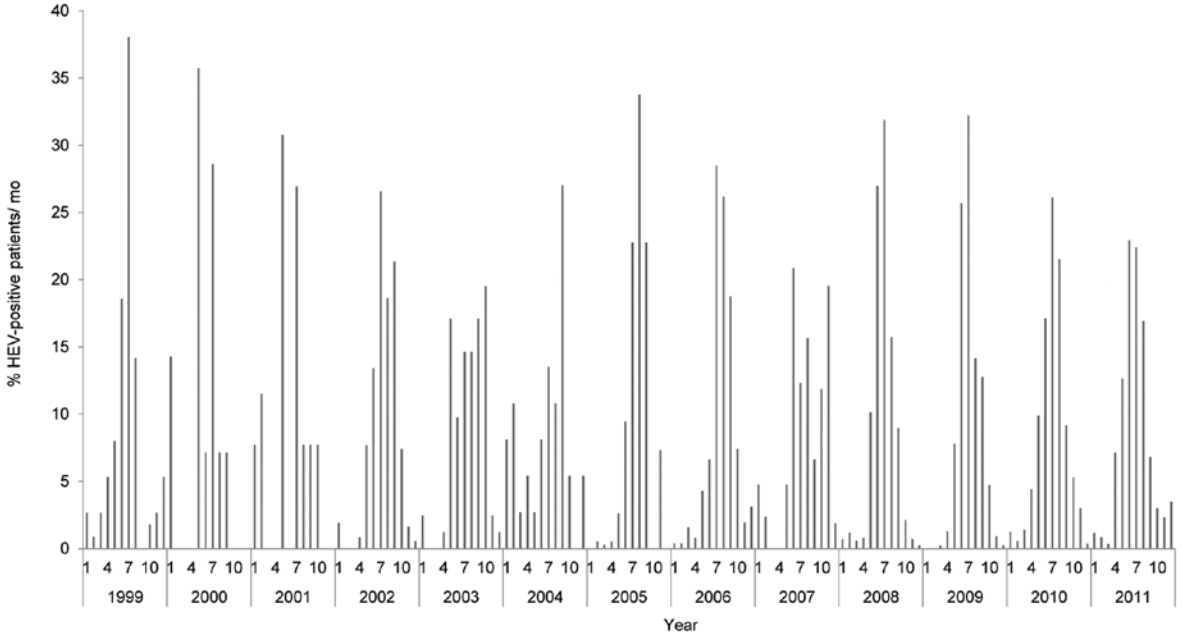

Figure 2. Seasonal pattern of enterovirus circulation during 1999–2011. Bars indicate percentage of patients positive for human enterovirus per month.

Table 2. Age distribution of human enterovius patients, South Korea, 1999–2011

Age, y*	No. positive/total persons in age group													Total (%)
	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	
0–1	23/48	5/51	1/131	30/145	7/35	12/315	130/252	73/318	52/336	260/624	10/40	145/579	232/877	980 (23.3)
2–5	36/86	3/33	9/90	143/369	27/44	8/46	113/202	65/189	78/230	309/529	529/1,628	288/470	238/429	1,846 (43.9)
6–10	26/76	2/34	6/71	73/240	5/20	8/70	63/204	31/96	1/75	300/446	264/619	82/172	76/174	937 (22.3)
11–20	13/38	1/19	1/49	48/162	2/26	0/6	12/64	10/60	1/26	70/158	63/425	29/150	35/168	285 (6.8)
21–30	2/7	0/3	1/11	9/25	0/2	0/10	2/11	6/13	0/8	31/69	0/14	4/21	5/91	60 (1.4)
31–40	1/6	0/2	0/3	5/13	6/8	0/4	3/10	1/11	0/11	39/58	3/16	8/30	2/56	68 (1.6)
41–50	2/9	0/3	0/3	6/16	4/6	0/3	2/8	0/10	0/1	0/12	0/11	0/9	3/15	17 (0.4)
51–60	0/4	0/3	0/3	4/6	2/3	2/3	0/6	1/7	0/1	1/7	0/7	1/9	2/2	13 (0.3)
>60	0/5	0/1	0/3	3/6	0/2	0/2	0/4	0/8	0/3	0/8	0/5	0/5	0/0	3 (0.1)
Total	103/279	11/149	18/364	321/982	53/146	30/279	325/761	187/712	132/691	1,010/1,911	869/2,765	557/1,445	593/1,812	4,209

*Age known for 12,296 persons.

of prevalence and distribution of genotypes of EV in this study reflected nationwide outbreaks and detection and subtyping methods for EV surveillance.

We described 4 EV outbreaks in South Korea during 1999–2011: aseptic meningitis caused by EV71 in 2000, by E13 in 2002, by CB5 in 2005, and by E30 in 2008 (*25–27*). EV detection rates in 2002, 2005, and 2008 were relatively higher than those in other years, and the dominant genotypes found during these years were outbreak-associated genotypes.

The prevalence increased as detection technology changed from cell culture (phase I), to RT-PCR (phase II), to real-time RT-PCR (phase III). Overall increases were probably caused by a combination of outbreaks of EV infection and enhanced sensitivity of detection methods. Because of a greatly advanced molecular detection method, molecular-based methods enable detection of uncultivable EV by use of small sample quantities and specific primer sets. Consistent with our findings, Roth et al. reported higher sensitivity by using the RT-PCR method rather than cell culture for fecal and CSF samples (*11*). In our study, although there are no data from parallel testing to address the issue of relative sensitivity of the 3 detection methods used over this period in this study, it is possible that increased prevalence during phases II and III could have been accounted for by the enhanced sensitivity of detection methods.

We described the prevalence, seasonal trend, and epidemiologic data for human EV infection collected by the national enterovirus surveillance system during 1999–2011 in South Korea. Our laboratory identified EV from fecal, CSF, nasopharyngeal, blood, and other sample types such as urine from persons who had an array of symptoms. Although feces is the most convenient specimen type for detecting EVs for surveillance purposes, detecting EV in fecal samples is not the most specific way to confirm the cause of an individual patient's symptoms (*4*). In this study, a higher frequency of EV detection from fecal samples, in contrast to CSF, was observed; this finding is in agreement with the finding of a previous study that used both cell culture and RT-PCR (*11*). However, Antona et al. showed that when compared with other specimens, the highest percentage of positive detection was found in CSF specimens (*1*). This finding could be influenced by the fact that different detection methods were used for each sample type: Antona et al. used cell culture for fecal samples and RT-PCR for CSF.

Genotyping has been shown to greatly improve epidemiologic investigation of common EV types when compared with seroneutralization testing (*28*). During phase I of this study, CA types and some E and EV types did not propagate well in cell culture and, therefore, were underdiagnosed. Confirmation of EV genotype by sequencing was systematically conducted after 2005 in South Korea; untypeable EV decreased but reemerged as real-time RT-PCR methods were introduced for detection during 2008 (data not shown). These findings could be related to a low quantity of EV in the sample, which can be detected by using real-time PCR but not by PCR-based VP1 sequencing.

As far as clinical aspects are concerned, the prevalent ages and clinical manifestation are consistent with results from previous studies that showed that the majority of cases occurred in children <10 years of age (*1,29,30*). In addition, a link between clinical syndromes and genotypes was in accordance with previous studies (*1,2, 4–6,31*); aseptic meningitis by E30, E6, and CB5; HFMD by CA16; and HFMD with neurologic complications by EV71. In this study, EV71 was the most frequent type of EV detected during 1999–2011 in South Korea. This finding is probably because our expanded surveillance detected more patients with neuro-

Table 3. Five most frequent human enterovirus genotype rankings during the epidemic seasons in South Korea, by year, 1999–2011*

Year	Genotype, no. (%)				
	Rank 1	Rank 2	Rank 3	Rank 4	Rank 5
Phase 1†					
1999, n = 85	CB2, 21 (24.7)	E6, 15 (17.7)	CB3, 10 (11.7)	E11, 8 (9.4)	E30, 8 (9.4)
2000, n = 30	EV71, 12 (40)	Polio Sabin strain, 9 (30)	CB2, 2 (2.7)	E6, 2 (2.7)	E11, 2 (2.7)
2001, n = 26	CB5, 12 (46.2)	CB3, 4 (15.4)	Polio Sabin strain, 3 (11.6)	CB2, 3 (11.6)	CB1, 3 (11.6)
2002, n = 272	E13, 70 (25.7)	E9, 59 (21.7)	E6, 53 (19.5)	E7, 24 (8.8)	CB3, 17 (6.3)
2003, n = 54	CB4, 16 (19.5)	E6, 10 (12.2)	E30, 7 (8.5)	CB1, 7 (8.5)	Polio Sabin strain, 5 (6.1)
2004, n = 29	Polio Sabin strain, 7 (24.1)	E30, 6 (20.7)	CB2, 5 (17.3)	CB1, 4 (13.8)	E6, 3 (10.3)
Phase 2‡					
2005, n = 369	CB5, 159 (43.1)	E18, 127 (34.4)	CB3, 47 (12.7)	E9, 25 (6.8)	CB1, 7 (1.9)
2006, n = 238	E25, 56 (23.5)	E30, 48 (20.2)	E5, 28 (11.8)	CA16, 20 (8.4)	CB4, 18 (7.6)
2007, n = 180	CB2, 62 (34.4)	CA9, 28 (15.6)	EV71, 21 (11.7)	E16, 14 (7.8)	CA10, 10 (5.6)
Phase 3§					
2008, n = 626	E30, 299 (47.8)	E6, 170 (27.2)	CA10, 33 (5.3)	CB3, 29 (4.6)	CB1, 27 (4.5)
2009, n = 288	EV71, 127 (44.1)	CB1, 70 (24.3)	CA2, 23 (8.0)	CA5, 20 (6.9)	CA14, 17 (5.9)
2010, n = 402	EV71, 190 (47.3)	CA6, 65 (16.2)	CB5, 32 (8.0)	CA9, 28 (7.0)	CA10, 19 (4.7)
2011, n = 529	EV71, 118 (22.3)	CA16, 109 (20.6)	CB5, 72 (13.6)	CB2, 70 (13.2)	E18, 42 (7.9)
Total, 1999–2011, n = 3,128	EV71, 476 (14.9)	E30, 390 (12.5)	CB5, 290 (9.3)	E6, 261 (8.4)	CB2, 186 (6.0)

*CB, coxsackievirus B; E, echovirus; EV, enterovirus; CA, coxsackievirus A.

logic disease. Since 1997, multiple cases of EV71 infection have been associated with severe aseptic meningitis and pulmonary edema in the Asia–Pacific region, including Taiwan, Malaysia, Singapore, and Japan (*26,32–37*). In addition, E30 was the second most common genotype detected during this period in this study and in previous studies from other countries; Asia and European countries reported that E30 was the predominant genotype (*1,4,11*).

EV infections have been known to increase in summer and early autumn in countries in temperate climates (*1, 2, 13*). As expected in a temperate climate, our surveillance data revealed a seasonal pattern of distribution, with transmission peaking in the summer and decreasing in the period from autumn to spring.

Our study has limitations that may affect the interpretation of its findings. First, although the patients were from almost all regions of South Korea, the number of patients and strains obtained from each is unequal. This variability is related to the level of cooperation and workload related to surveillance among different hospitals and local public health institutes. Second, some EV types that cannot propagate well in cell culture were underdiagnosed during 1999–2005, when ≈23.1% of isolates were recorded as untypeable (data not shown). Third, EV71 has been the most frequently detected type since 2009. It is likely that because HFMD with neurologic complications was actively monitored by our surveillance, EV serotypes associated with this clinical manifestation may have been overdetected.

This study focused on EV epidemiology in South Korea over a 13-year period by using a nationwide EV surveillance system. This surveillance provides valuable data on the epidemiologic pattern and clinical manifestations associated with specific genotypes and provides vital information that can be used to control annual EV epidemics. The public health impacts of EVs vary: some of the viruses are benign and some cause serious illness. Although it is

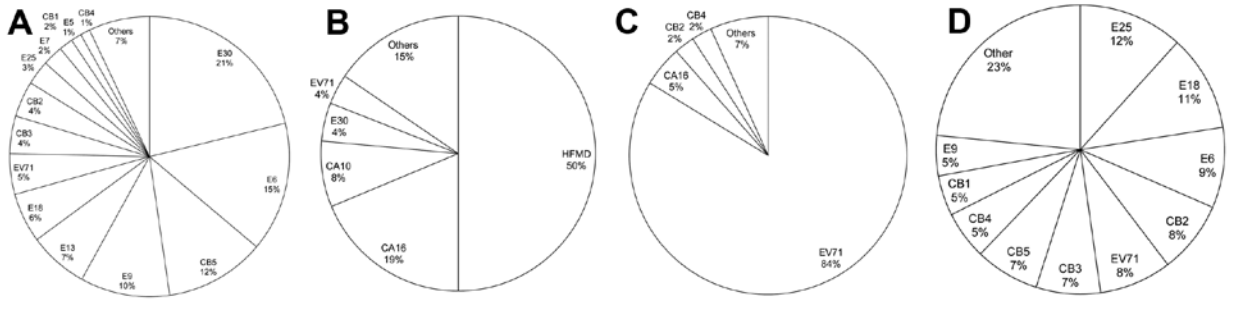

Figure 3. Distribution of nonpolio enterovirus genotypes by clinical manifestation. Graphics show percentage of each genotype from the total isolates of A) aseptic meningitis; B) hand, foot and mouth disease or herpangina; C), hand, foot and mouth disease with neurologic complications; and D), other pathogenesis including sepsis, acute gastroenteritis, hepatitis, pneumonia, and myopericarditis. CA, coxsackievirus A; CB, coxsackievirus B; E, echovirus; EV, enterovirus; HFMD, hand, foot and mouth disease.

appropriate in some instances to use cell cultures, we recommend the use of real-time RT-PCR for samples from patients who have typical symptoms of infection with the more virulent genotypes described here. Evaluation of findings from surveillance of enterovirus infections will contribute to development of prevention and treatment plans.

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Impact of 2003 State Regulation on Raw Oyster–associated *Vibrio vulnificus* Illnesses and Deaths, California, USA

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US vibriosis rates have increased since 1996, and many *Vibrio vulnificus* infections are fatal. In April 2003, California implemented a regulation restricting the sale of raw oysters harvested from the Gulf of Mexico during April 1–October 31, unless they were processed to reduce *V. vulnificus* to nondetectable levels. We analyzed California cases of *V. vulnificus* infection before and after the regulation's implementation and compared case data with data from other states. The annual number of reported *V. vulnificus* infections and deaths in California with patient's sole exposure to raw oysters dropped from 0 to 6 cases and 0 to 5 deaths per year during 1991–2002, before implementation, to 0 during 2003–2010, after implementation (p = 0.0005 for both). In other states, median annual numbers of similar cases and deaths increased slightly after 2002. The data strongly suggest that the 2003 regulation led to a significant reduction in reported raw oyster–associated *V. vulnificus* illnesses and deaths.

A recent review of surveillance data indicated that rates of *Vibrio* spp. infections in the United States increased from 1996 to 2010, and, of the 3 most commonly reported species, *V. vulnificus* caused the most hospitalizations and deaths (*1*). *V. vulnificus* is a gram-negative, halophilic bacterium that occurs naturally in marine and estuarine waters. Human infection usually results from exposure to the organism by consumption of raw or undercooked shellfish, usually oysters, or by a wound coming into contact with seawater. Illness typically is manifest as primary septicemia (following ingestion) or as wound infection with or without septicemia (following wound exposure) (*2–5*). Persons at risk for severe *V. vulnificus* disease are those with preexisting liver disease, alcoholism, diabetes, hemochromatosis, or an immunocompromising condition. Patients with primary septicemia often are in shock when they come to medical attention, and the fatality rate has been reported to be >50% (*3,4*). Most patients with primary septicemia report recent consumption of raw oysters, usually from the Gulf of Mexico (*2–4*).

Most oysters harvested in the United States are from the Gulf Coast region (*6*). Surveys regarding raw oysters in the US market have repeatedly found that Gulf Coast oysters have higher frequency and levels of *V. vulnificus* bacteria than oysters from the North Atlantic or Pacific Coasts, especially during the summer months (*7,8*). However, raw oysters can be treated with a postharvest processing method to reduce *V. vulnificus* to "nondetectable" levels, which is defined nationally as a most probable number of <30 organisms/gm oyster meat (*9,10*). Three postharvest processing methods are commercially available: 1) individual quick freezing, by which half-shell oysters were rapidly frozen, 2) mild heat–cool pasteurization, by which oysters are heated in warm water and then dipped them in cold water to stop the process, and 3) high hydrostatic pressure processing, in which oysters are subjected to pressure ≤45,000 pounds per square inch.

In 1991, California adopted a regulation to decrease oyster-associated *V. vulnificus* infections and deaths. Restaurants and other food establishments that sold or served raw Gulf Coast oysters were required to provide the following written warning to prospective customers about the potential harmful effects of consuming raw oysters: "Eating raw oysters may cause severe illness and even death in persons who have liver disease (for example, alcoholic cirrhosis), cancer, or other chronic illnesses that

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weaken the immune system." In 1996, the Los Angeles County Department of Health Services reported that, despite this regulation, *V. vulnificus* cases and deaths due to eating raw oysters were ongoing, especially among the Spanish-speaking Hispanic population. A survey of 103 restaurants serving raw Gulf Coast oysters showed that >50% either had no warning sign or a poorly visible sign (*11*). In 1997, California updated the raw oyster regulation to require provision of the written warning both in English ("Warning") and in Spanish ("Aviso Importante"), with specific wording and formatting requirements for a prominently posted sign, a boxed statement prominently placed on each menu, or a tent card for each dining table (*12*).

Despite implementation of these updated regulations, oyster-associated *V. vulnificus* infections and deaths continued. This situation led the state of California to enact an emergency regulation on April 14, 2003, restricting the sale, in California, of raw oysters harvested from the Gulf of Mexico from April 1 through October 31, unless the oysters were treated with a scientifically validated process to reduce *V. vulnificus* to nondetectable levels (defined for California as <3 most probable number of organisms/gm/oyster meat) (*12*). California is the only state with this restriction on the sale of raw summer Gulf Coast oysters.

To assess the public health effects of the 2003 California emergency regulation, we analyzed records for California cases of raw oyster-associated foodborne *V. vulnificus* infection before (1991–2002) and after (2003–2010) implementation of the regulation. We then compared the data with data for cases reported from other states.

Methods

Vibrio infection surveillance in the United States was initiated in 1988 by the Gulf Coast states of Alabama, Florida, Louisiana, and Texas, the US Centers for Disease Control (now US Centers for Disease Control and Prevention [CDC]), and the US Food and Drug Administration. By the early 2000s, most states were reporting cases of *Vibrio* infection to CDC's Cholera and Other *Vibrio* Illness Surveillance (COVIS) system, and in 2007, vibriosis became nationally notifiable. For each case, information collected on the COVIS form includes demographics, clinical symptoms, underlying illness, history of seafood consumption, exposure to seawater, and *Vibrio* species. In California, *Vibrio* infections have been reportable since 1988 (and the same COVIS form has been used). When shellfish exposure is reported, the local environmental health specialists and the Food and Drug Branch of the California Department of Public Health attempt to trace back the shellfish to its harvest site.

Cases reported to COVIS are classified into foodborne, nonfoodborne, or unknown transmission routes on the bases of the reported exposure (seafood consumption, marine/estuarine water contact, unknown) and specimen site (gastrointestinal, blood, or other normally sterile site; skin or soft tissue, other nonsterile site; unknown). We defined a case as foodborne if the patient reported seafood consumption as the only exposure. We also considered cases foodborne if both of these conditions are met: 1) the exposure is unknown or the patient reported seafood consumption and exposure to marine/estuarine water, and 2) *Vibrio* isolates were obtained only from a gastrointestinal site or from multiple sites, including a gastrointestinal site but not a skin or soft tissue site.

We examined reports from 1991 to 2010 of California cases of oyster-associated *V. vulnificus* infection for patient's death, age, sex, race/ethnicity, history of liver disease, or alcoholism or other underlying conditions, and for oyster preparation and harvest site. We initially examined the large group of cases in patients who consumed any oysters, raw or cooked, with or without other seafood, with mode of transmission classified either as foodborne or as unknown (e.g., because the patient had both food and water exposure and only a blood isolate). We then narrowed the analysis to only foodborne cases among patients who reported consuming only raw oysters.

For comparison, we examined reports from 1991 to 2010 of cases of foodborne *V. vulnificus* infection from the rest of the United States for resulting death and oyster harvest site, focusing on cases among patients who reported consuming only raw oysters. Data were analyzed by using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC, USA). We used the Wilcoxon-Mann-Whitney 2-sample test to compare the distribution of the annual number of cases before (1991–2002) and after (2003–2010) implementation of the 2003 emergency regulation.

Results

During 1991–2010, California reported 88 patients with *V. vulnificus* infection. Among them, 61 (69%) had a history of eating any oysters, raw or cooked, with or without other seafood, in the 7 days before illness began and had a mode of transmission classified as foodborne or as unknown. Thirty-nine (64%) of these patients died. The median annual number of cases dropped from 5.5 (range 1–9; total 57 cases) during 1991–2002, before implementation, to 0 (range, 0–2; total 4 cases) during 2003–2010, after implementation of the 2003 regulation ($p = 0.0005$). The median annual number of deaths dropped from 2.5 (range 1–6; total 38 deaths) to 0 (range 0–1; total 1 death) after implementation of the 2003 regulation ($p = 0.0001$).

Twenty-seven case-patients with foodborne *V. vulnificus* infection reported consuming only raw oysters (i.e., no other seafood); 20 (74%) of these patients died. The median annual number of patients who consumed only raw

oysters dropped from 2 (range 0 to 6) during 1991–2002, before implementation, to 0 (none in the entire time) during 2003–2010, after implementation of the 2003 regulation (p = 0.0005) (Figure 1). The median annual number of deaths among patients who consumed only raw oysters decreased from 1 (range 0 to 5) to 0 (none in the entire time) after implementation of the 2003 regulation (p = 0.0005).

The 27 patients who consumed only raw oysters had a median age of 48 years (range 27–72); 24 (89%) were men, and 23 (85%) were Hispanic. All had an underlying condition predisposing them to severe infection, including 22 (81%) with liver disease, cirrhosis, or hepatitis. The oyster harvest site was known (for 19) or suspected (for 2) for 21 (78%) patients who consumed only raw oysters; all oysters were traced to the Gulf of Mexico.

During 1991–2010, states other than California reported 231 cases of foodborne *V. vulnificus* infection in patients who reported consuming only raw oysters; 106 (46%) of these patients died. The median annual number of non-California patients who reported consuming only raw oysters was 10.5 (range 2–21) during 1991–2002 and 15 (range 9–19) during 2003–2010 (p = 0.02) (Figure 2). The median annual number of these non-California patients who died was 5 (range 1–12) during 1991–2002 and 6.5 (range 4–7) during 2003–2010 (p = 0.17). The oyster harvest site was known for 151 (65%) of these patients; 145 (96%) of the oysters were traced to the Gulf of Mexico.

Discussion

The data strongly suggest that the dramatic and sustained drop in reported raw oyster–associated *V. vulnificus* illnesses and deaths in California was related to the 2003 California regulation that restricts the sale of raw oysters harvested from the Gulf Coast during the 7 warmest months to oysters treated with postharvest processing. This conclusion is supported by the lack of decline after 2002 in the number of foodborne *V. vulnificus* cases and deaths associated with consuming only raw oysters among persons living in other states, none of which has a similar raw oyster restriction. The significant reduction after 2002 in the larger number of California patients who consumed raw or cooked oysters, with or without other seafood, suggests that many of these illnesses were also due to raw oysters.

Evidence suggests that the proportion of persons eating raw oysters in California did not decrease after the 2003 regulation. Surveys of persons in the California counties of Alameda, Contra Costa, and San Francisco who participated in the Foodborne Diseases Active Surveillance Network (FoodNet) showed that in 2006–2007, ≈2% of persons interviewed reported eating raw oysters in the previous 7 days (*13*), compared with 2% in 2002–2003 (*14*). The FoodNet surveys also did not show any significant difference between the proportion of Hispanic and non-Hispanic White persons who reported eating raw oysters. Thus, it is not known why the proportion of case-patients who were Hispanic (85%) was much higher than the proportion of the state's Hispanic population (32% in 2000 US Census [*15*]). The higher prevalence of chronic liver disease among the Hispanic populations may be a contributing factor (*16*).

To decrease the risk of *V. vulnificus* infection, persons in high-risk groups and others who want to decrease the risk of illness should not eat raw, unprocessed oysters, especially those harvested from the Gulf Coast during the summer months. Summer-harvested oysters from the Mid-Atlantic region, however, should also be of concern because they have been shown to have *V. vulnificus* levels

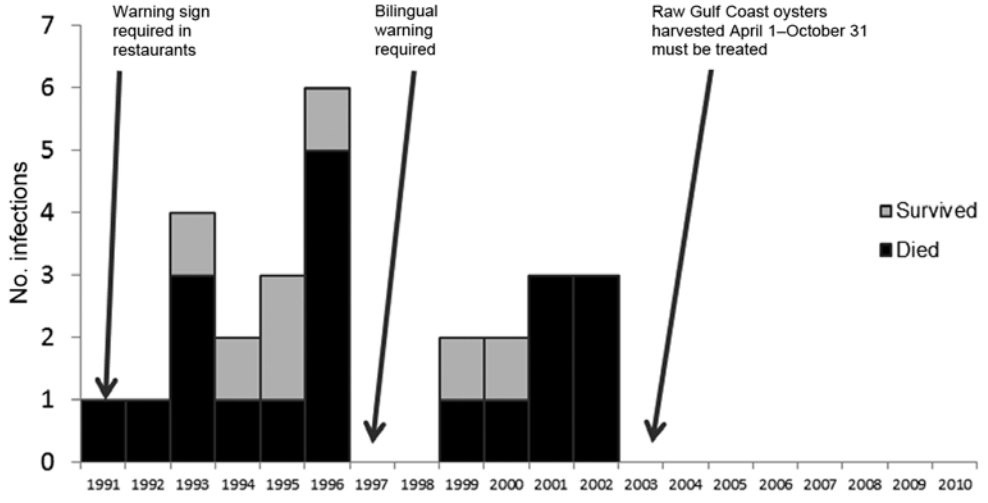

Figure 1. *Vibrio vulnificus* infections among 27 California patients who consumed only raw oysters, by year, 1991–2010. Arrows indicate enactment of different requirements.

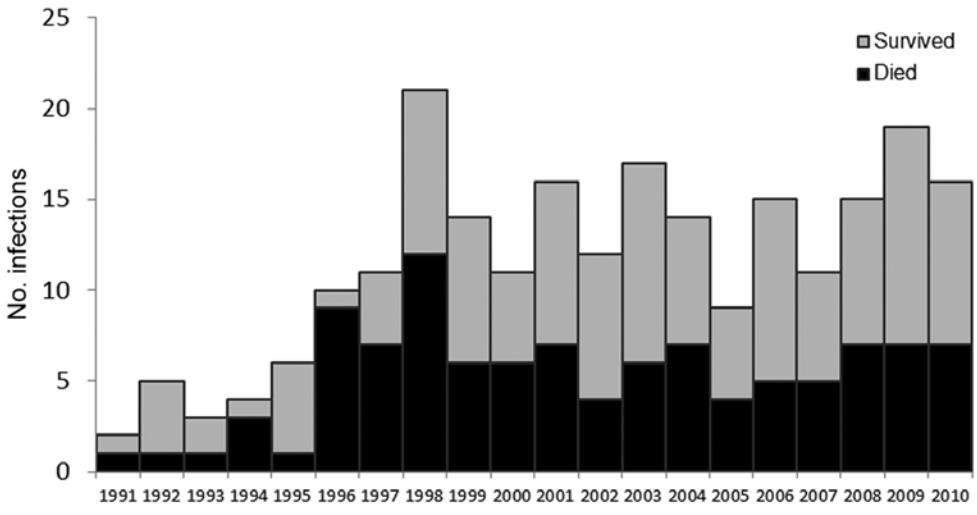

Figure 2. *Vibrio vulnificus* infections among 231 persons who consumed only raw oysters, by year, United States (excluding California), 1991–2010.

nearly as high as those from the Gulf Coast (*7,8*). Persons at high risk for disease should also avoid seawater exposure if they have a fresh wound and should seek medical care as soon as possible if signs of wound infection develop after such exposure. Clinicians' high awareness of the risk factors for *V. vulnificus* infection along with prompt diagnosis and treatment can substantially improve patient outcomes (*2–5*).

Our study had some limitations. First, the surveillance system is based on passive reporting, and some cases might not have been reported. If cases occurred after 2003 that were not reported to public health, the decline might not have been so significant. However, any underreporting would most likely have occurred both before and after 2003, and *V. vulnificus* disease is severe enough that most cases are likely recognized and reported. Second, because vibriosis did not become officially nationally reportable until 2007, some of the increase of reported cases nationally after 2002 could have been due to increased reporting. All states, however, have been voluntarily reporting vibriosis since before 2003, and FoodNet population-based surveillance data, albeit based on a smaller national catchment area, also showed increased incidence of *V. vulnificus* cases during 1996–2010 (*1*). Furthermore, although we show a significant drop in *V. vulnificus* cases for which patients had only raw oyster exposure in California after implementation of the 2003 regulation, a small but undefined risk for *V. vulnificus* infection remains among persons in California who eat raw oysters.

A variety of approaches have been used to address oyster-associated cases of severe *V. vulnificus* infection and those that lead to death, including consumer education, time and temperature control regulations for raw oysters, and postharvest processing. In 2001, the Interstate Shellfish Sanitation Conference (a national organization with participants from the US Food and Drug Administration, the US Environmental Protection Agency, the shellfish industry, Gulf Coast states, and others), as part of its proposed *Vibrio vulnificus* Risk Management Plan, pushed to increase education of at-risk oyster consumers in participating states (*17*). In 2004, an Interstate Shellfish Sanitation Conference survey of raw oyster consumers in California, Florida, Louisiana, and Texas "found no significant increase in overall consumer knowledge about the risk of eating raw oysters or the proportion of high-risk consumers who stopped eating them" when compared with results of a similar survey in 2002 (*18*). In May 2010, time- and temperature-control regulations (e.g., within how many hours after harvest oysters must be refrigerated and cooled) were enacted in Florida, Louisiana, and Texas, but compliance has not been evaluated (*18*).

Educational outreach to high-risk populations is a time-honored public health approach, and some have credited that approach with success in reducing the incidence of vibriosis associated with raw oyster consumption, such as in Florida (*19*). However, the survey of raw oyster consumers mentioned above suggests difficulty in reaching or convincing high-risk consumers. Implementation of California's warning regulations was not followed by a reduction in the number of reported cases or deaths caused by *V. vulnificus*. The higher than expected proportion of Hispanic patients also suggests that the 1997 regulation to reach Spanish-speaking consumers was not effective. Not until after the 2003 emergency regulation was implemented did the number of cases and deaths drop significantly. A similar regulation to restrict the sale of raw summer-harvested Gulf Coast oysters to those treated by postharvest processing, if implemented nationwide, would likely decrease *V. vulnificus* illnesses and deaths due to eating unprocessed raw oysters.

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Macrolide Resistance of *Mycoplasma pneumoniae*, South Korea, 2000–2011[1]

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In Korea, *Mycoplasma pneumoniae* was detected in 255/2,089 respiratory specimens collected during 2000–2011; 80 isolates carried 23S rRNA gene mutations, and 69/123 culture-positive samples with the mutation were resistant to 5 macrolides. During 2000–2011, prevalence of the mutation increased substantially. These findings have critical implications for the treatment of children with mycoplasma pneumonia.

Mycoplasma pneumoniae is 1 of the most common causes of community-acquired pneumonia in children and young adults (*1*). Epidemics of mycoplasma pneumonia typically occur every 4–7 years; however, epidemics have occurred every 3–4 years in South Korea (*2*,*3*). The first-line treatment for mycoplasma pneumonia is macrolide antimicrobial drugs, but macrolide-resistant infections have been recognized in conjunction with an increase in cases in children in Japan, China, Germany, France, Israel, and the United States (*1*,*4*–*10*). Because of the risk to children administered tetracycline and fluoroquinolone (non-macrolide drugs), *M. pneumoniae* resistance to macrolide drugs has critical implications for the treatment of mycoplasma pneumonia in children. This study was conducted to identify the prevalence of macrolide resistance among *M. pneumoniae* strains isolated from children with lower respiratory tract infections (LRTIs) during 4 consecutive epidemics (2000–2011) in South Korea.

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The Study

A total of 2,089 respiratory samples were tested for the presence of *M. pneumoniae*. Of these, a total of 378 were archived samples collected during epidemics in 2000 (71 samples), 2003 (112 samples), and 2006 (195 samples), and 1,711 were samples collected and tested during August 2010–December 2011. Specimens from the 2010–2011 epidemic were collected from children at Seoul National University Children's Hospital, Seoul National University Bundang Hospital, and Seoul Eulji Hospital. All samples were obtained from children (median age 5 years, range 6 months–18 years) with a diagnosis of community-acquired LRTI.

P1 adhesin was amplified by PCR for the detection of *M. pneumoniae* from the 378 archived samples. *M. pneumoniae* was cultivated by using pleuropneumonia-like organism broth and agar for the 1,711 samples collected during 2010–2011. Media were incubated aerobically at 37°C for 6 weeks. Plates were observed daily to identify change in the color of the broth from red to transparent orange. When the color changed, the samples were subcultured on agar plates. Spherical *M. pneumoniae* colonies were observed by using a microscope.

For the cultured *M. pneumoniae* isolates, we amplified domain V of the 23S rRNA gene by PCR; for the archived samples, we extracted DNA. For PCR, we used primers MP23SV-F 5′-TAACTATAACGGTCCTAAGG-3′ and MP23SV-R 5′-ACACTTAGATGCTTTCAGCG-3′. The PCR products were sequenced to identify mutations. Sixty-four of the *M. pneumoniae*–positive samples from 2000 and 2003 had been previously tested for mutations in the 23S rRNA gene.

Minimum inhibitory concentrations (MICs) were measured by using the microdilution method in triplicate for the following antimicrobial agents: erythromycin, clarithromycin, azithromycin, roxithromycin, josamycin, tetracycline, doxycycline, levofloxacin, moxifloxacin, and ciprofloxacin. MIC was defined as the lowest antimicrobial drug concentration at which the media color did not change at the time when the color of the positive control media (containing *M. pneumoniae* strains only) changed (*11*).

M. pneumoniae was detected in 255 (12.2%) of 2,089 clinical samples; 132 (51.8%) of the positive samples were among the 378 archived samples, and 123 (48.2%) were among the 1,711 samples from 2010–2011. For the 132 archived samples, *M. pneumoniae* was detected by PCR: 30 (22.8%) were among the 71 samples from 2000, 34 (25.8% were among the 112 samples from 2003, and 68 (51.5%) were among the 195 samples from 2006. For the

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123 samples from 2010–2011, *M. pneumoniae* was detected by culture.

Overall, 80 (31.4%) of the 255 *M. pneumoniae*–positive samples carried mutations in the 23S rRNA gene. Of these, 78 had the A2063G transition and 2 exhibited the A2064G transition. The prevalence of the 23S rRNA mutation increased significantly over the 4 consecutive epidemics, as follows: 2000 epidemic, 0 of 30 samples; 2003 epidemic, 1 (2.9%) of 34 samples; 2006 epidemic, 10 (14.7%) of 68 samples; and 2010–2011 epidemic, 25 (47.2%) of 53 samples in 2010 and 44 (62.9%) of 70 samples in 2011 ($p<0.001$ for trend) (Figure).

Among 123 samples culture-positive for *M. pneumoniae*, 69 that carried the 23S rRNA mutation exhibited significantly higher MIC_{50} (MIC for 50% of strains) and MIC_{90} when tested with 5 macrolides, compared with 54 strains that lacked the mutation. For example, the MIC_{50} and MIC_{90} of erythromycin were 16 μg/mL and 128 μg/mL, respectively, for strains with the 23S rRNA mutation and 0.001 μg/mL and 0.002 μg/mL, respectively, for strains without the mutation ($p<0.0001$) (Table 1). All 123 *M. pneumoniae* strains were susceptible to nonmacrolide antimicrobial drugs, including tetracycline, doxycycline, levofloxacin, ciprofloxacin, and moxifloxacin (Table 2).

Macrolide resistance is associated with point mutations in domain V of the *M. pneumoniae* 23S rRNA gene, especially those corresponding to A2063G or A2064G transitions (*4*,*5*,*7*). Thus, emergence of macrolide-resistant strains may result in treatment failure of *M. pneumoniae* infections (*4*).

We did not detect macrolide resistance among *M. pneumoniae* strains collected during 2000; thereafter, the prevalence of macrolide resistance remained low through the 2003 epidemic. Macrolide resistance then increased to 14.7% during the epidemic of 2006 and to 56.1% during the epidemic of 2010–2011, as indicated by substantially higher MICs against macrolide agents in association with the presence of the 23S rRNA gene mutation in *M. pneumoniae* isolates.

Macrolide resistance has been detected with increasing frequency in many parts of the world, highlighting the importance of knowing the geographic distribution and temporal patterns of macrolide-resistant *M. pneumoniae*. After the first isolation of a macrolide-resistant strain in 2001, Japan reported a dramatic increase in macrolide resistance among children with mycoplasma pneumonia, and in 2011 resistance was >80% (*12*,*13*). China identified an 83%–92% prevalence of macrolide-resistant *M. pneumoniae* isolates (*6*,*9*). In contrast, France identified only 2 resistant *M. pneumoniae* isolates during 1994–2006, and the United States reported a 30% prevalence of macrolide-resistant strains (*10*). Israel reported that in 2010, ≈30% of *M. pneumoniae* isolates carried an A2063G transition in domain V of the 23S rRNA gene (*8*). In Italy, 26% of *M. pneumoniae*–infected children harbored strains with point mutations in domain V of the 23S rRNA gene (*14*). Thus, there is great variability in the prevalence of macrolide resistance in *M. pneumoniae* isolates.

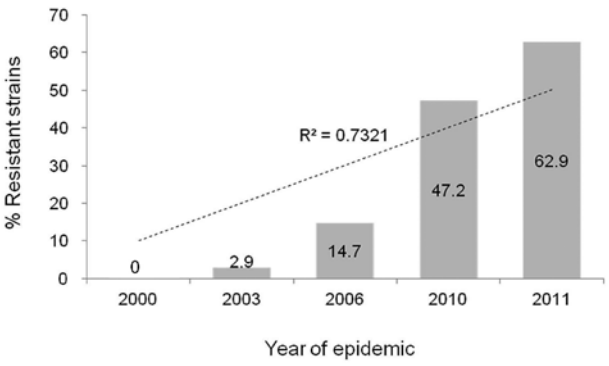

Figure. Increased prevalence of macrolide resistance of *Mycoplasma pneumoniae* strains isolated from children during epidemics of lower respiratory tract infections, South Korea, 2000–2011. During the 2000 epidemic, 0 of 30 strains were resistant, but during the epidemics of 2003 and 2006, 1 of 34 and 10 of 68 strains, respectively, showed resistance. During the 2010–2011 outbreak, 25 of 53 (2010) and 44 of 70 (2011) strains were resistant. Numbers on the bars are the percentages of resistant strains for each year.

Conclusions

A key finding of this study is the increasing prevalence of macrolide resistance over time. Several factors may have led to this increase. First, the increased use of macrolide antimicrobial drugs may be responsible for the development and spread of macrolide resistance. A recent study showed

Table 1. MICs of macrolide antimicrobial drugs for 123 *Mycoplasma pneumoniae* strains in a study of macrolide resistance, South Korea, 2000–2011*

	Strains with 23S rRNA mutation, n = 69			Strains without 23S rRNA mutation, n = 54		
Macrolides	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Erythromycin	2 to >128	16	128	0.001 to 0.004	0.001	0.002
Clarithromycin	8 to >128	64	128	0.001 to 0.002	0.001	0.002
Roxithromycin	0.008 to 128	8	32	0.001 to 0.008	0.001	0.004
Azithromycin	1 to 64	8	16	0.001 to 0.001	0.001	0.001
Josamycin	1 to 8	4	8	0.001 to 0.016	0.001	0.008

*MIC_{50} and MIC_{90} are minimum inhibitory concentrations at which 50% and 90% of the isolates, respectively, were inhibited by the drug. In each instance, $p<0.0001$.

Table 2. MICs of tetracyclines and fluoroquinolones for *Mycoplasma pneumoniae* strains in a study of macrolide resistance, South Korea, 2000–2011*

Antimicrobial drug	Strains with 23S rRNA mutation, n = 69			Strains without 23S rRNA mutation, n = 54		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Tetracyclines						
Tetracycline	0.016 to 0.5	0.06	0.25	0.016 to 0.5	0.06	0.25
Doxycycline	0.002 to 0.125	0.06	0.06	0.004 to 0.125	0.03	0.06
Fluoroquinolones						
Levofloxacin	0.016 to 0.5	0.25	0.25	0.016 to 0.5	0.25	0.5
Ciprofloxacin	0.125 to 1.0	0.5	1.0	0.06 to 1.0	0.5	1.0
Moxifloxacin	0.008 to 0.06	0.016	0.06	0.004 to 0.06	0.016	0.06

*MIC_{50} and MIC_{90} are minimum inhibitory concentrations at which 50% and 90% of the isolates, respectively, were inhibited by the drug.

a correlation between increased use of oral macrolides and an increase in macrolide resistance by selective pressure in *M. pneumoniae* and other respiratory pathogens (*13*). A comprehensive trend analysis of the national data showed an increase in macrolide use in the community (expressed in defined daily doses [DDD]/1,000 inhabitants/day) during 2005–2009 (*15*). Penicillins and cephalosporins are the 2 most frequently used classes of oral antimicrobial drugs. There were decreasing trends in penicillin use and a subtle increase in cephalosporin use during 2005–2009. Macrolide use remained steady until 2007; however, there was an increase of >30% in DDD/1,000 inhabitants/day between 2007 (2.5 DDD/1,000 inhabitants/day) and 2009 (3.3 DDD/1,000 inhabitants/day). Our data on macrolide use do not fully explain the 10-year change in *M. pneumoniae* resistance to macrolide drugs because data were available only for 2005–2009. In addition, the spread of resistant strains could have been facilitated by other factors, such as high population density or geographic closeness with the 2 neighboring countries, where resistant strains were highly prevalent.

We found an increasing prevalence of the 23S rRNA gene mutation in *M. pneumoniae* isolates during 2000–2011 in South Korea. We did not address the clinical issues regarding antimicrobial drug choices for macrolide-resistant mycoplasma pneumonia or compare the clinical outcomes for macrolide-resistant and macrolide-sensitive infections; nevertheless, we believe that the evidence of a recent increase in macrolide resistance provides guidance for additional clinical investigations and new therapeutic strategies. The incidences of macrolide-resistant *M. pneumoniae* infection should be carefully monitored, particularly among children, for whom treatment can be challenging. Further studies are needed to evaluate the clinical significance of macrolide-resistant *M. pneumoniae* pneumonia.

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Recombinant Coxsackievirus A2 and Deaths of Children, Hong Kong, 2012

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A natural recombinant of coxsackievirus A2 was found in 4 children with respiratory symptoms in Hong Kong, China, during the summer of 2012. Two of these children died. Vigilant monitoring of this emerging recombinant enterovirus is needed to prevent its transmission to other regions.

Human coxsackieviruses belong to the family *Picornaviridae* and genus *Enterovirus*. These viruses are divided into groups A and B on the basis of their pathogenicity in suckling mice (flaccid paralysis caused by group A viruses and spastic paralysis caused by group B viruses) (*1*). Human infections with enteroviruses such as coxsackievirus A (CVA) are generally mild, but severe complications were more often reported for infections caused by enterovirus 71 (EV71) (*1,2*). We report infection of 4 children with recombinant coxsackievirus A2 in Hong Kong.

The Study

On June 10, 2012, a previously healthy 4-year-old boy in Hong Kong had fever, cough, and rhinorrhea. His condition deteriorated rapidly and he lost consciousness. He was admitted to Pamela Youde Nethersole Eastern Hospital in Hong Kong, cardiac asystole developed, and he died ≤4 hours after admission. Autopsy showed areas of dull red consolidation on the upper lobes of the lungs. Postmortem histologic examination showed focal areas of alveolar damage and hyaline membrane formation with lymphocytic infiltrates. There were no gross or histologic changes indicative of myocarditis or encephalitis in any part of his brain, including the brainstem.

Tissue samples of heart, lung, spleen, and rectum, and nasopharyngeal and rectal swab specimens were positive for enterovirus by virus culture in a rhabdomyosarcoma cell line. These samples were also positive by reverse transcription PCR (RT-PCR) with pan-enterovirus primers (5′-CAAGCACTTCTGTBWCCCCGG-3′ and 5′-GAAACACGGACACCCAAAGTAGT-3′) specific for the 5′ untranslated region (5′-UTR) as described (*3–5*).

Because sequence analysis of the 5′-UTR amplicon showed that this enterovirus strain was closely related to other human enterovirus A strains, another set of consensus primers (5′-TGCCCACAYCARTGGATHAA-3′ and 5′-CCTGACCACTGNGTRTARTA-3′) specific for the viral protein 2–viral protein 3 region of human enterovirus A was used for typing. CVA2 strain 430895 was identified. No other pathogens were detected in these specimens.

On June 19, 2012, a previously healthy 2-month-old girl who lived in Hong Kong had an upper respiratory tract infection for 3 days. She was cyanotic and unresponsive, and showed cardiorespiratory arrest at admission to the emergency department of Queen Elizabeth Hospital in Kowloon. Despite attempted resuscitation, she died ≤1 hour after admission. No epidemiologic link was found between this patient and the previous patient when public health officials interviewed the parents about exposure histories. Mild perivascular lymphocytic infiltrates were observed in postmortem lung samples. No gross or histologic evidence of encephalitis or myocarditis was found at postmortem examination. Although airway samples were positive for community-acquired methicillin-resistant *Staphylococcus aureus* and cytomegalovirus, no characteristic histologic changes indicative of such infections were found in lung tissues. One intestinal sample from this patient was positive for CVA2 strain 2260165 by RT-PCR.

Two other closely related CVA2 strains were detected in 2 children with respiratory symptoms in early June 2012. A 4-year-old boy was admitted to Pamela Youde Nethersole Eastern Hospital and a 10-month-old boy was admitted to Queen Mary Hospital for fever and upper respiratory tract infection. Both patients recovered. Their CVA2 strains, 431135 and 431306, respectively, were detected by RT-PCR in nasopharyngeal aspirates.

To understand the molecular basis for the possible pathogenetic mechanism of these CVA2 strains, we analyzed their complete genome sequences. The genome sequences of the 4 CVA2 strains were amplified and sequenced as described (*4,5*) and deposited in GenBank under accession nos. JX867330–JX867333. The genomes of the 4 CVA2 strains are 7.4 kbp and have a G + C content of 48.8%–48.9% (excluding the 3′ polyadenylated tract). They have sequence identities of 99.6%–99.9%.

Nucleotide and amino acid sequence identities between the 4 CVA2 strains and other human enterovirus A strains were compared (Table). Sequences of the capsid region (P1) of the 4 CVA2 strains showed ≥81.6% nt and ≥96.9%

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Table. Pairwise sequence identities between coxsackievirus A2 strains from 4 children in Hong Kong, China, 2012*, and other representative human enterovirus A strains.

	Nucleotide identity, %		Amino acid identity, %	
Region	CVA2F	Other human enterovirus A strains	CVA2F	Other human enterovirus A strains
5′-UTR	84.9–85.3	71.1–87.2	NA	NA
Polyprotein	79.3–79.4	69.1–78.4	96–96.1	76–88.4
P1	81.6–81.7	64.8–69.1	96.9–97	67.4–74.6
VP4	80.2–80.7	61.8–70.8	97.1	63.8–81.2
VP2	81.6	66.1–70.3	97.6	72.9–79.2
VP3	82.4–82.6	66.1–72.8	97.1–97.5	70.7–83.3
VP1	81.1–81.2	60.1–65.9	95.9–96.3	56.7–67.3
P2	78–78.2	70.5–83.8	96.9–97.1	78.9–98.4
2A	78–78.2	65.3–82	96	69.3–98.7
2B	73.7–74.1	66–84.2	94.9	76.8–99
2C	79.2–79.4	71.9–86.3	97.9–98.2	83–98.8
P3	77.8–77.9	72–87.8	94.4–94.6	81.1–97.9
3A	79.8–80.6	69–86.4	94.2–95.3	68.6–98.8
3B	74.2	62.1–93.9	90.9	68.2–95.5
3C	77.4	72.3–87.4	95.1	84.2–98.9
3D	77.7–77.9	72.2–89.6	94.2–94.4	82.7–98.5
3′-UTR	83.3–84.5	38.9–96.3	NA	NA

*CVA2F, coxsackievirus A prototype strain Fleetwood; UTR, untranslated region; NA, not applicable; P1, capsid protein 1; VP, viral protein; P2, nonstructural protein 2; P3, nonstructural protein 3.

aa identities with the CVA2 prototype strain Fleetwood (CVA2F), suggesting that these strains belonged to the same serotype as CVA2. The 5′-UTR and nonstructural regions (P2 and P3) of the 4 CVA2 strains had highest sequence identities with those of other human enterovirus A strains but not with CVA2F.

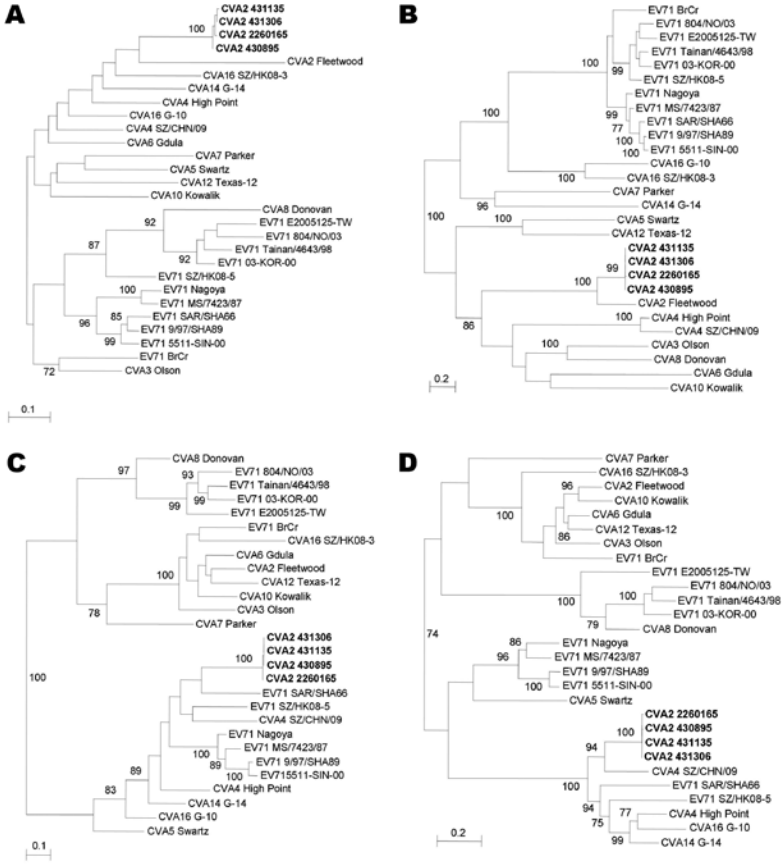

Figure 1. Phylogenetic trees of A) 5′-untranslated region (UTR), B) capsid protein (P1), C) nonstructural protein 2 (P2), and D) nonstructural protein 3 (P3) regions of 4 coxsackievirus A2 (CVA2) strains, Hong Kong, 2012 and other human enterovirus (EV) A strains with complete genome sequences. Trees were inferred from data by using the maximum-likelihood method with bootstrap values calculated from 1,000 trees. Sequences for 758-nt positions in each 5′-UTR, 2,595 nt positions in each P1 region, 1,734 nt positions in each P2 region, and 2,259 nt positions in each P3 region were included in the analysis. Only bootstrap values >70% are shown. Scale bars indicate estimated number of nucleotide substitutions per 5 (B and D) or 10 (A and C) nucleotides. CVA2 strains isolated in this study are indicated in **boldface**. Virus strains (GenBank accession nos.) used were CVA2 Fleetwood (AY421760), CVA3 Olson (AY421761), CVA4 High Point (AY421762), CVA4 SZ/CHN/09 (HQ728260), CVA5 Swartz (AY421763), CVA6 Gdula (AY421764), CVA7 Parker (AY421765), CVA8 Donovan (AY421766), CVA10 Kowalik (AY421767), CVA12 Texas-12 (AY421768), CVA14 G-14 (AY421769), CVA16 G-10 (U05876), CVA16 SZ/HK08–3 (GQ279368), EV71 BrCr (U22521), EV71 Nagoya (AB482183), EV71 MS/7423/87 (U22522), EV71 SAR/SHA66 (AM396586), EV71 9/97/SHA89 (AJ586873), EV71 5511-SIN-00 (DQ341364), EV71 804/NO/03 (DQ452074), EV71 Tainan/4643/98 (AF304458), EV71 03-KOR-00 (DQ341356), EV71 SZ/HK08–5 (GQ279369), and EV71 E2005125-TW (EF063152).

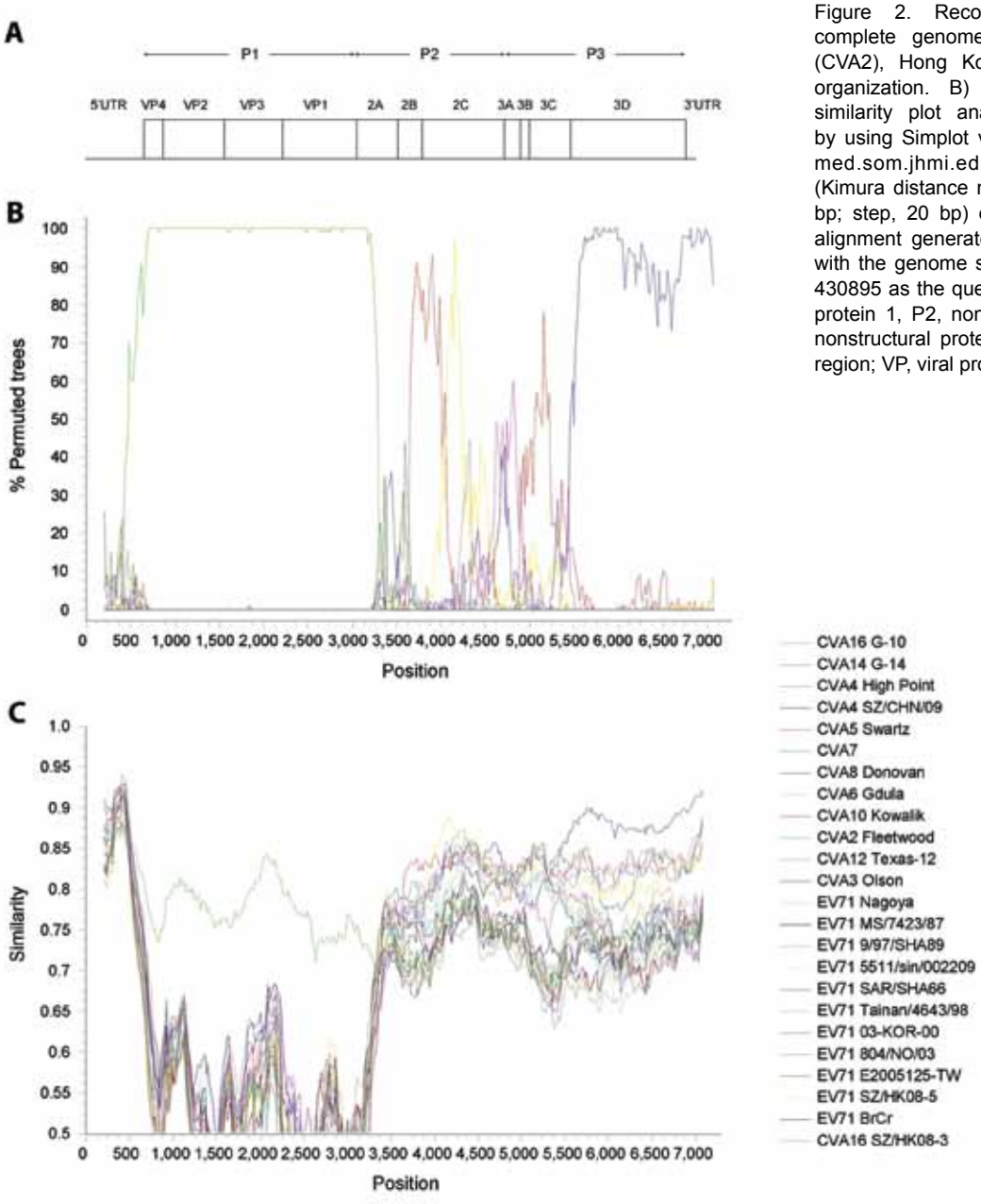

Figure 2. Recombination analysis of complete genome of coxsackievirus A2 (CVA2), Hong Kong, 2012. A) Genome organization. B) Bootscanning and C) similarity plot analyses were conducted by using Simplot version 3.5.1 (http://sray.med.som.jhmi.edu/SCRoftware/simplot/) (Kimura distance model; window size, 400 bp; step, 20 bp) on a gapless nucleotide alignment generated with ClustalX 2.0 (*8*) with the genome sequence of CVA2 strain 430895 as the query sequence. P1, capsid protein 1, P2, nonstructural protein 2; P3, nonstructural protein 3; UTR, untranslated region; VP, viral protein.

Phylogenetic trees were constructed by using nucleotide sequences of the 5′-UTR and P1, P2, and P3 regions of the 4 CVA2 strains and other human enterovirus A strains with complete genome sequences (Figure 1). Sequence alignment was performed by using ClustalX version 2.0 (*6*). The best evolutionary model (general time reversible + invariant sites) for each dataset was determined by using ModelGenerator (*7*). Maximum-likelihood phylogenetic trees were constructed by using PhyML version 3.0 (*8*), and bootstrap values were calculated from 1,000 trees. Phylogenetic analysis showed that the 4 CVA2 strains were most closely related to CVA2F in the 5′-UTR and P1 region. The 4 CVA2 strains clustered with EV71 subgenotype B3 strain SAR/SHA66 in the P2 region but with CVA4 strain SZ/CHN/09 in the P3 region.

Further analysis was performed to identify potential recombination sites (Figure 2). Multiple sequence alignment of genomes of representative CVA2 strain 430895 and other human enterovirus A strains was generated by using ClustalX version 2.0 and edited manually. Once aligned, similarity plot and bootscan analyses were conducted by using Simplot version 3.5.1 (window size 400 bp, step 20 bp) (*9*), with the genome sequence of CVA2 strain 430895 as the query sequence.

Results showed high bootstrap supports for clustering between CVA2F and the 4 CVA2 strains at nucleotide

positions 700–3400, between EV71 strain SAR/SHA66 subgenotype B3 and the 4 CVA2 strains at nt positions 3700–4030, between EV71 strain SZ/HK08–5 subgenotype C4 and the 4 CVA2 strains at nt positions 4030–4300, and between CVA4 strain SZ/CHN/09 and the 4 CVA2 strains at nt position 5700 to the 3′ end of the genome. These findings indicated that recombination events might have occurred between nt positions 3400 and 3700 (corresponding to the 2A region), at nt position 4030 in the 2B region, and between positions 5400 and 5700 (corresponding to the 3C region). Several possible recombination events were detected in other regions of the CVA2 genome but with lower bootstrap supports.

Conclusions

Because noncapsid regions of enteroviruses are not correlated by serotype (*10*), results of phylogenetic and recombination analyses were based on the highest sequence similarity between enterovirus strains. Although recombination was evident in the 4 CVA2 strains, lack of comparative sequences indicated that the timing of recombination events was unknown, and low overall similarity to comparison sequences suggested that these events could be distant in time. As in many studies of human enterovirus A strains, lack of complete genome sequences for most serotypes, with the possible exception of EV71, limits interpretation of results for recombination analysis (*10,11*). In the present study, only 2 complete genome sequences of CVA4 strains (prototype strain High Point and strain SZ/CHN/09) were included in recombination analysis. Therefore, sequencing and analysis of more complete genome sequences of human enterovirus A strains, particularly CVA strains, from a wider geographic area over a longer period will provide a clearer picture of the role of recombination in this species.

We report a novel enterovirus isolated from or detected in 4 young children with severe upper respiratory tract infections in Hong Kong, 2 of whom died. This virus was characterized by complete genome sequencing as a recombinant virus of at least 3 enteroviruses (CVA2, EV71, and CVA4), and had the capsid of CVA2. Although it could not be determined whether this virus was the cause of the deaths, this report might serve to alert other investigators of circulation of a more pathogenic enterovirus.

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Monitoring Avian Influenza A(H7N9) Virus through National Influenza-like Illness Surveillance, China

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In China during March 4–April 28, 2013, avian influenza A(H7N9) virus testing was performed on 20,739 specimens from patients with influenza-like illness in 10 provinces with confirmed human cases: 6 (0.03%) were positive, and increased numbers of unsubtypeable influenza-positive specimens were not seen. Careful monitoring and rapid characterization of influenza A(H7N9) and other influenza viruses remain critical.

As of April 28, 2013, a total of 125 cases of avian influenza A(H7N9) virus infection and 24 related deaths were confirmed in humans in 8 provinces and 2 municipalities (hereafter called affected provinces/municipalities) of mainland China (*1*). The median age of patients was 63 years; most were male and had a history of exposure to live poultry (*2*). The first confirmed case was reported on March 31. On April 3, the Chinese Center for Disease Control and Prevention (China CDC) distributed primers and probes specific for avian influenza A(H7N9) virus to all national influenza surveillance network laboratories in China. To better understand the epidemiology, geographic spread, and clinical spectrum of this virus in China, we describe the Chinese National Influenza-Like Illness Surveillance Network (CNISN) and analyze data collected since March 4, 2013.

The Study

The CNISN includes 554 sentinel hospitals conducting surveillance for influenza-like illness (ILI; hereafter called sentinel hospitals) and 408 network laboratories in all 31 provinces of China (Figure 1). On a weekly basis, sentinel hospitals report the number of outpatient visits, by age group, for ILI and the total number of outpatients. Each week, 5–15 nasopharyngeal swab samples are collected from a convenience sample of patients who visit sentinel hospitals within 3 days of ILI onset. ILI is defined as temperature ≥38°C and cough or sore throat. Demographic and epidemiologic data, including age, sex, date of illness onset, and occupation, are also collected. Patient specimens are tested by real-time reverse transcription PCR or virus isolation in the affiliated laboratories.

On April 3, 2013, to enhance surveillance for influenza A(H7N9) virus, all network laboratories were required to increase the number of specimens to a minimum of 15/week and to test all specimens collected since March 4, 2013, for influenza A(H7N9) virus by real-time reverse transcription PCR as described (*3,4*). We analyzed data collected by CNISN during March 4–April 28. Population data by age group were provided by the National Bureau of Statistics of China.

During March 4–April 28, CNISN tested 46,807 nasopharyngeal swab samples from 554 sentinel hospitals throughout mainland China. Samples included 20,739 specimens from patients with ILI at 141 sentinel hospitals in 10 affected provinces/municipalities: Anhui, Jiangsu, Zhejiang, Shandong, Henan, Fujian, Jiangxi, and Hunan Provinces and Shanghai and Beijing Municipalities (Tables 1, 2). The median number of specimens collected each week from affected provinces/municipalities was 244 (range 72–792). Of the 20,739 samples from patients with ILI, 10,035 (48.4%) were from persons 0–14 years of age, 9,319 (44.9%) were from persons 15–59 years of age, and 1,385 (6.7%) were from persons ≥60 years of age. The age distribution of ILI cases in the 10 affected provinces/municipalities was substantially different from that in the overall population; persons 25–59 years of age had a lower proportion of ILI than would be expected had ILI distribution mirrored the age distribution of the population. (online Technical Appendix Figure 1, wwwnc.cdc.gov/EID/article/19/8/13-0662-Techapp1.pdf). In the affected provinces/municipalities, the number of specimens tested increased from a mean of 2,643 during the week starting April 1 to a peak of 3,259 during the week starting April 9; the increase was highest among persons 15–24 and 25–59 years of age (online Technical Appendix Figure 2).

During April 1–28, the percentage of visits for ILI increased in 5 of the 7 affected southern provinces and 2 of 3 affected northern provinces/municipalities (Figure 2). However, during the same period, the proportion of specimens positive for influenza decreased in the affected provinces/municipalities.

Of the 10 affected provinces/municipalities, 5 reported ≥1 ILI patient with test results positive for influenza A(H7N9) virus. The percentage of specimens positive

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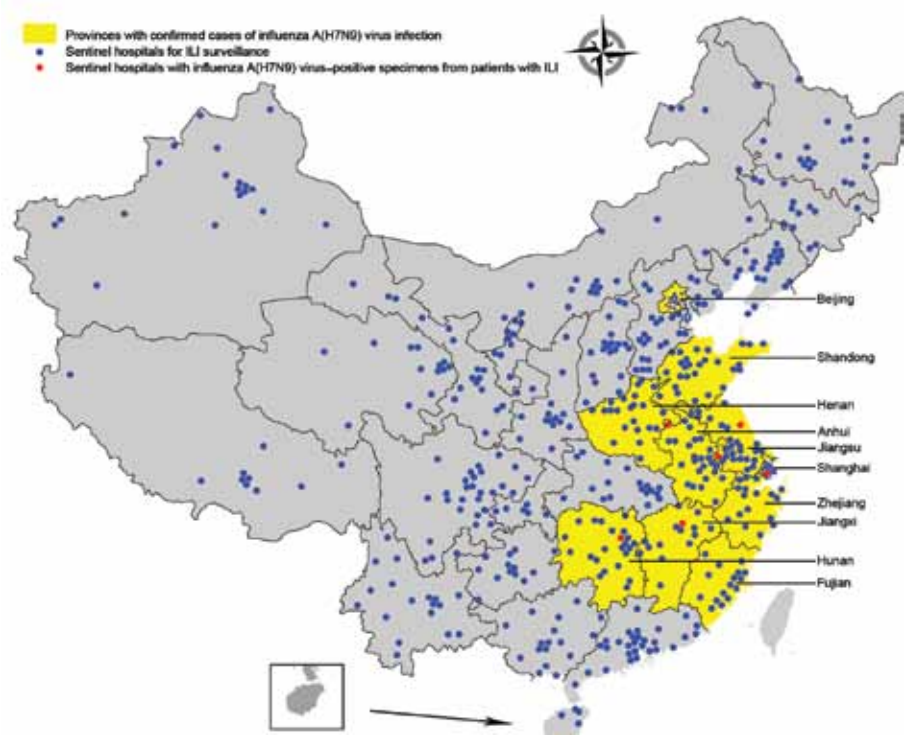

Figure 1. Geographic distribution of national influenza surveillance sentinel hospitals in Beijing and Shanghai Municipalities and 8 provinces with confirmed human cases of avian influenza A(H7N9) virus infection, China, 2013.

for influenza A(H7N9) virus, by province/municipality, ranged from 0 to 0.06% (Table 2). We detected influenza A(H7N9) virus in samples from 6 (0.03%) of the 20,739 patients with ILI; these cases were then reported as confirmed to the local CDCs and China CDC. No unsubtypeable influenza samples were reported in the affected provinces/municipalities during the study period (online Technical Appendix Table).

Epidemiologic investigations found that 2 of the 6 patients with influenza A(H7N9) infection had not been hospitalized, and the other 4 had been hospitalized for pneumonia complications. The 2 patients who were not hospitalized were 2 and 4 years of age. Of the 4 hospitalized patients, 3 were 25–59 years of age, and 1 was 69 years of age. Four of the patients had a history of contact with live chickens or visiting a live poultry market.

Conclusions

After the avian influenza A(H7N9) virus outbreak was identified in China, CNISN increased sampling and testing of ILI case-patients. CNISN has tested >46,807 specimens from all provinces, including 20,739 specimens from affected provinces/municipalities. As a result of this testing, CNISN identified 6 influenza A(H7N9) virus–positive specimens in 5 provinces that were already known to have cases. These data demonstrate that avian influenza A(H7N9) virus is an uncommon cause of ILI in any age group and in the areas reporting confirmed cases of influenza A(H7N9) infection. The confirmed case-patients included 2 children who did not require hospitalization and 4 adults with more severe disease, possibly indicating that influenza A(H7N9) virus causes milder disease in younger persons.

Although the proportion of all outpatient visits for ILI increased in affected provinces/municipalities, virologic surveillance data showed that the proportion of ILI patient specimens positive for influenza decreased, and there was no increase in unsubtypeable influenza viruses during the study period. This suggests that any increase in the percentage of consultations for ILI might be a result of increased healthcare–seeking behavior after media reports of the avian influenza A(H7N9) virus outbreak or the circulation of non-influenza respiratory viruses.

Table 1. Number of ILI patients, by age, positive for avian influenza A(H7N9) virus, China, March 4–April 28, 2013*

Patient age, y	No. positive/no. tested	
	Persons from 10 outbreak-affected areas†	Persons from 21 non-affected provinces
0–4	2/6,333	0/10,419
5–14	0/3,702	0/4,452
15–24	0/3,210	0/3,259
25–59	3/6,109	0/6,627
≥60	1/1,385	0/1,311
Total	6/20,739	0/26,068

The spectrum of illness caused by other avian influenza viruses varies tremendously and can also vary by age group. Previous human infections with avian influenza A(H7) viruses (i.e., subtypes H7N3, H7N2, and H7N7) have been generally mild, causing conjunctivitis, with the exception of very occasional cases of pneumonia and a single fatal case in the Netherlands in a highly exposed veterinarian (*5*–*10*). In contrast, avian influenza A(H5N1) virus has an overall case fatality rate of 60%, and persons with confirmed cases are usually severely ill (*11*). Recent reviews of avian influenza A(H5N1) virus seroprevalence studies found little evidence that large numbers of human infections are going undetected (*12*–*14*). Among the 82 human influenza A(H7N9) virus infections reported as of April 17, 2013, a total of 38 (46%) were in persons ≥65 years of age (*2*). We did not find evidence of widespread mild disease, suggesting that the reported cases reflect the true distribution of infection and not a surveillance artifact.

Table 2. Number of ILI patients positive for avian influenza A(H7N9) virus in areas with confirmed infections among humans, China, March 4–April 28, 2013*

Area†	No. positive/no. tested
Anhui	1/3,478
Beijing	0/1,392
Fujian	0/1,154
Henan	0/1,893
Hunan‡	1/1,912
Jiangsu	2/3,369
Jiangxi	1/1,588
Shandong	0/1,848
Shanghai	1/2,490
Zhejiang	0/1,615
Total	6/20,739

Our study had several limitations. The 554 CNISN sentinel hospitals are located in urban areas, so the surveillance system may not detect influenza A(H7N9) virus infections in rural areas. In addition, most sentinel hospitals are tertiary care hospitals, and their patient populations are not representative of the general population with ILI. The

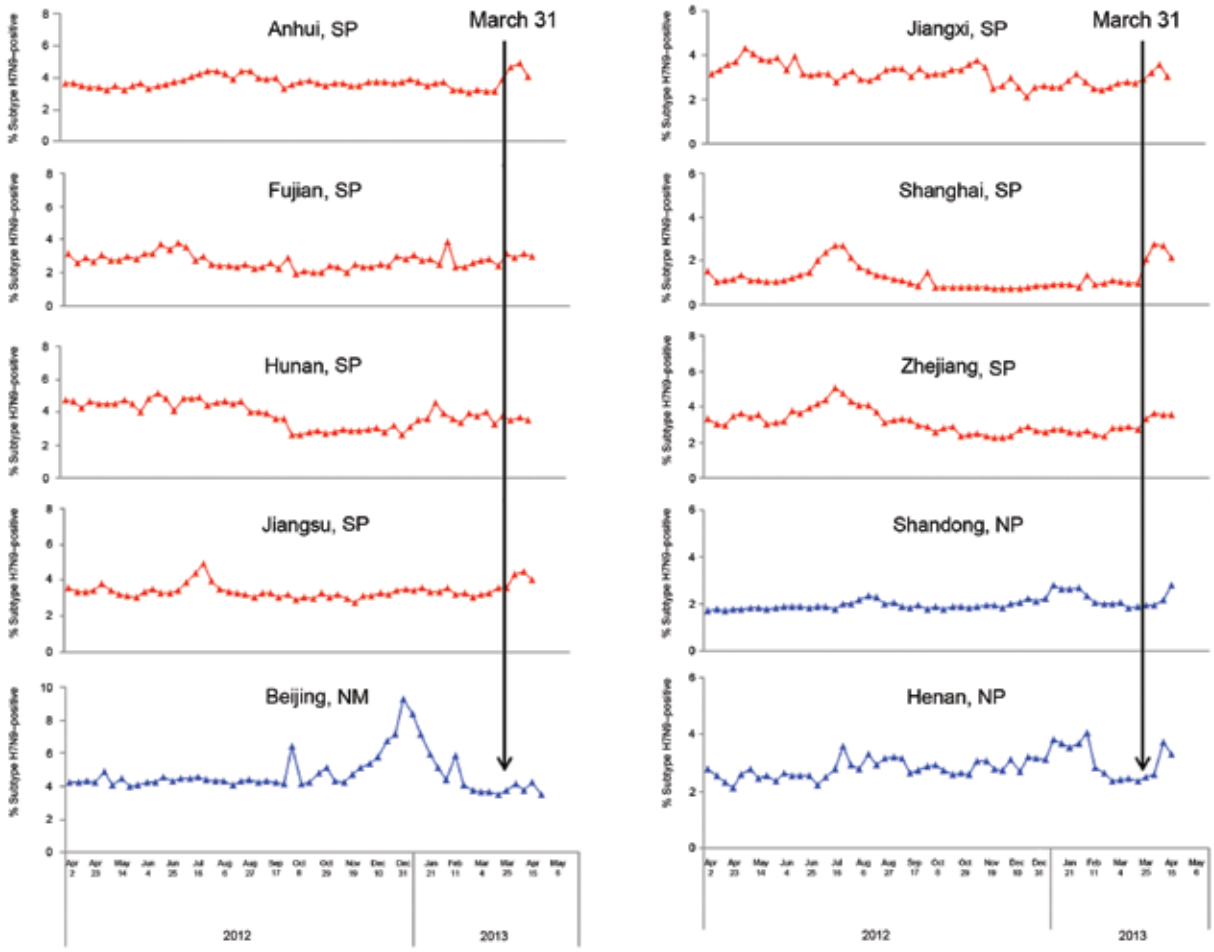

Figure 2. Percentage of hospital visits attributed to influenza-like illness, China, April 2, 2012–May 6, 2013. Hospital visits were made to sentinel surveillance hospitals in 7 southern provinces (SP) and 3 northern provinces/municipalities (NM, NP) with confirmed human cases of avian influenza A(H7N9) virus infection. Arrows indicate March 31, 2013, the date the first human case of influenza A(H7N9) virus infection was reported.

distribution of those patients who had specimens tested is not necessarily random and may not reflect the population of those with ILI. Last, our system lacks a straightforward way to calculate rates of disease because it lacks denominators.

The emergence of a reassortant between avian influenza A(H7N9) virus and seasonal influenza subtype viruses, with possible increased human transmissibility, is possible during the upcoming summer influenza season in southern China. Careful monitoring and rapid characterization of influenza A(H7N9) viruses and unsubtypeable viruses from infected humans will be critical. Enhanced surveillance studies of mild and severe respiratory disease and seroprevalence studies in focal areas are necessary to further characterize the epidemiology and clinical spectrum of this emerging virus.

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Norovirus Surveillance among Callers to Foodborne Illness Complaint Hotline, Minnesota, USA, 2011–2013

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Norovirus is the leading cause of foodborne disease in the United States. During October 2011–January 2013, we conducted surveillance for norovirus infection in Minnesota among callers to a complaint-based foodborne illness hotline who reported diarrhea or vomiting. Of 241 complainants tested, 127 (52.7%) were positive for norovirus.

Norovirus is the leading cause of foodborne disease and sporadic and outbreak-associated acute gastroenteritis in the United States (*1,2*), accounting for 21 million illnesses, 70,000 hospitalizations, and 800 deaths each year (*3*). Norovirus is not routinely tested for in clinical settings because detection requires molecular methods typically available only in public health and research laboratories. Therefore, characterization of norovirus epidemiology has been primarily through analysis of outbreak data.

Consistent with national trends (*4*), most foodborne disease outbreaks identified in Minnesota are caused by norovirus. In addition, most foodborne outbreaks in Minnesota, including virtually all norovirus outbreaks, are identified through a centralized foodborne illness complaint hotline system, operated by the Minnesota Department of Health (MDH) (*5,6*). However, most calls to the hotline represent sporadic (i.e., non–outbreak-associated) illness; only ≈7% of complaints are associated with known outbreaks (*5*). Systematic testing of hotline callers to determine illness etiology has not previously been conducted.

In this study, we conducted surveillance for norovirus among hotline callers. Our objectives were to characterize the role of norovirus as a cause of gastroenteritis in hotline callers and to describe trends in norovirus infection in this population as an indicator for norovirus activity in Minnesota.

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The Study

The MDH foodborne illness complaint system has been described in detail (*5,6*). From October 1, 2011, through January 31, 2013, eligible hotline callers (complainants) were asked to submit a self-collected fecal sample to the MDH Public Health Laboratory (PHL). Complainants were eligible to submit a stool sample on the basis of reported symptoms (≥3 loose stools in 24 hours or vomiting [symptom eligibility]) and other criteria, including timeliness of complaint (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/8/13-0462-Techapp1.pdf). If the original complainant was not eligible for or refused testing, another ill person reported in the complaint (co-complainant) was asked to submit a stool sample, if eligible. Only 1 stool sample per complaint was used in analyses. This surveillance effort was exempted from review by the MDH Institutional Review Board.

Specimen vials were refrigerated on receipt at the MDH PHL and batch tested weekly. Detection and characterization of norovirus strains were performed by using the Centers for Disease Control and Prevention CaliciNet methods (*7*). Briefly, detection of norovirus genogroups I and II was performed by duplex real-time reverse transcription PCR. Genotypes were determined by sequence analysis of the viral capsid gene and phylogenetic comparison with CaliciNet reference strains.

On the basis of the known winter seasonality of norovirus outbreaks (*8*), norovirus season was defined as October–March and the off-season as April–September. Data analysis was performed by using SAS version 9.2 software (SAS Institute Inc., Cary, NC, USA).

During October 2011–January 2013, the Minnesota foodborne illness hotline received 1,060 calls (median 60 calls/mo) (Table 1). The mean number of monthly calls to the hotline was greater during the norovirus season than during the off-season (73.6 vs. 54.0; $p = 0.025$). A total of 633 (59.7%) complainants or co-complainants met the eligibility requirements for stool sample submission; of these, 241 (38.1%) submitted a sample that was included in analyses.

Of the 241 stool samples, 127 (52.7%) were positive for norovirus: 22 (17.3%) for genogroup I, 104 (81.9%) for genogroup II, and 1 for genogroups I and II (Table 1; Figure 1). The monthly percentage of norovirus-positive samples varied from 23.1% in May 2012 to 81.3% in December 2012 (Table 1; Figure 1). Complainants who called during the norovirus season were more likely to test positive for norovirus than were those who called during the

Table 1. Demographics, signs and symptoms, and epidemiologic characteristics for callers to foodborne illness hotline, Minnesota, USA, October 2011–January 2013*

Characteristic	All symptom-eligible complainants			Complainants tested for norovirus		
	Tested, n = 241	Not tested,† n = 700	p value‡	Positive, n = 127	Negative, n = 114	p value‡
Age, y (range)	44 (0–88)	43 (1–91)	0.62	44 (1–88)	44 (0–88)	0.49
Duration, h (range)	30.80 (0.25–205.30)	29.0 (0.1–302.0)	0.63	36.0 (9.5–121.0)	18.00 (0.25–205.30)	**0.002**
Female sex	138 (57.3)	405 (58.1)	0.82	71 (55.9)	67 (58.8)	0.65
Signs and symptoms						
Diarrhea	193 (81.4)	521 (81.0)	0.30	103 (83.0)	90 (79.6)	0.50
Vomiting	183 (76.3)	513 (74.0)	0.50	110 (87.3)	73 (64.0)	**<0.001**
Bloody stools	9 (4.5)	23 (4.3)	0.94	2 (2.0)	7 (7.0)	0.09
Fever	81 (45.0)	152 (30.6)	**<0.001**	46 (52.9)	35 (37.6)	**0.04**
Onset during norovirus season§	172 (71.4)	486 (69.4)	0.57	108 (85.0)	64 (56.1)	**<0.001**
Health care visit	14 (6.5)	91 (13.5)	**0.006**	6 (5.6)	8 (7.5)	0.58
Outbreak associated¶	30 (12.4)	53 (7.6)	–	24 (18.9)	6 (5.3)	–

*Values are no. (%) except as indicated. Symptom-eligible complainants were callers who reported diarrhea (≥3 loose stools in 24 h) or vomiting. Denominators vary because of missing data. **Boldface** indicates significance.
†Includes all complainants eligible to submit a sample on the basis of symptom profile alone (symptom eligibility) as well as complainants not eligible to submit a stool sample for testing based on other eligibility criteria.
‡χ^2 test was used for categorical variables; Fisher exact test used when expected cell frequencies were <5; Wilcoxon 2-sample test was used for comparison of medians.
§Season defined as October–March.
¶One complainant per outbreak identified through the hotline was included in analyses, if otherwise eligible.

off-season (62.8% vs. 27.5%; p<0.001) (Table 2). Norovirus-positive complainants were more likely than norovirus-negative complainants to report vomiting (87.3% vs. 64.9%; p<0.001) and fever (52.9% vs. 36.2%; p = 0.049) and to have longer illness duration (median 36 vs. 18 hours; p<0.001) (Table 2).

The most common genotypes among the 122 norovirus-positive specimens that could be sequenced were GII.4 New Orleans (44, 36.1%), GII.4 Sydney (20, 16.4%), GII.1 (14, 11.5%), GI.6 (12, 9.8%), and GII.7 (10, 8.2%) (Figure 2). GII.4 New Orleans was predominant during the 2011–2012 norovirus season, and GII.4 Sydney was most common during the first 4 months of the 2012–2013 norovirus season (Figure 2).

Conclusions

This study highlights the predominant role of norovirus infections among callers to a foodborne illness complaint hotline in Minnesota. Call volume may be partially driven by norovirus activity: more calls were taken during the norovirus season, when a higher proportion of callers were norovirus positive. GII.4 norovirus strains were more prominent during peak norovirus season, and GI and less common GII genotypes were more prominent in the off-season. A review of published norovirus outbreaks found that GII outbreaks were significantly associated with winter seasonality compared with GI outbreaks (*9*). Additionally, GII.4 outbreaks have been associated with severe outcomes, such as hospitalization and death (*10*),

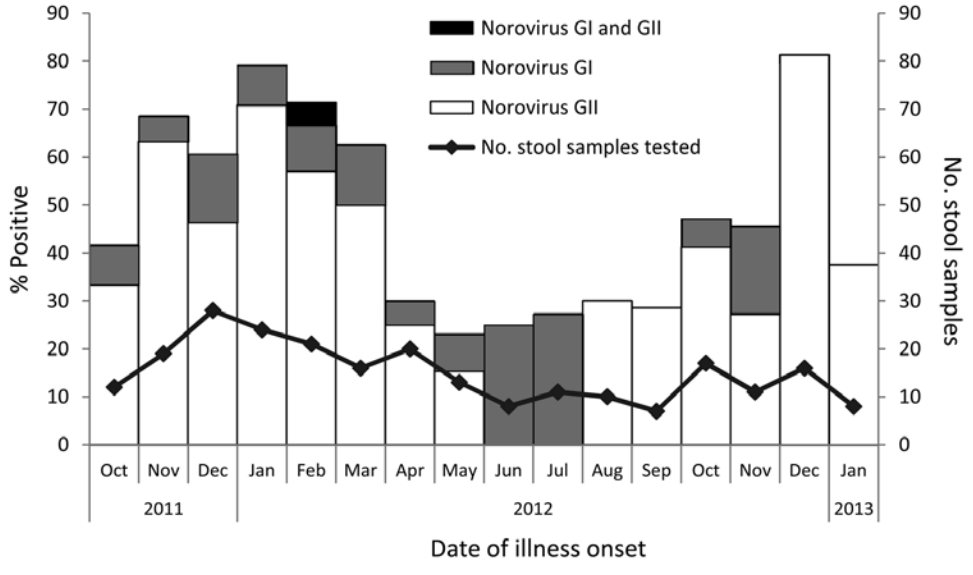

Figure 1. Percentage of stool samples submitted by callers to foodborne illness hotline that were positive for norovirus, by month of illness onset and genogroup, Minnesota, USA, October 2011–January 2013.

Table 2. Results for testing of stool samples submitted by callers to foodborne illness hotline, by month of complainant illness onset, Minnesota, October 2011–January 2013

Year and month	Total no. complainants	No. (%) eligible to submit sample*	No. (%) tested	No. (%) positive for		
				Any norovirus	Norovirus genogroup I†	Norovirus genogroup II†
2011						
Oct	48	28 (58.3)	12 (42.9)	5 (41.7)	1 (20.0)	4 (80.0)
Nov	52	37 (71.2)	19 (51.4)	13 (68.4)	1 (7.7)	12 (92.3)
Dec	117	70 (59.8)	28 (40.0)	17 (60.7)	4 (23.5)	13 (76.5)
2012						
Jan	96	62 (64.6)	24 (38.7)	19 (79.2)	2 (10.5)	17 (89.5)
Feb‡	67	46 (68.7)	21 (45.7)	15 (71.4)	2 (13.3)	12 (80.0)
Mar	79	40 (50.6)	16 (40.0)	10 (62.5)	2 (20.0)	8 (80.0)
Apr	57	34 (59.6)	20 (58.8)	6 (30.0)	1 (16.7)	5 (83.3)
May	54	38 (70.4)	13 (34.2)	3 (23.1)	1 (33.3)	2 (66.7)
Jun	51	29 (56.9)	8 (27.6)	2 (25.0)	2 (100.0)	0
Jul	54	26 (48.1)	11 (42.3)	3 (27.3)	3 (100.0)	0
Aug	62	35 (56.5)	10 (28.6)	3 (30.0)	0	3 (100.0)
Sep	46	30 (65.2)	7 (23.3)	2 (28.6)	0	2 (100.0)
Oct	80	47 (58.8)	17 (36.2)	8 (47.1)	1 (12.5)	7 (87.5)
Nov	79	46 (58.2)	11 (23.9)	5 (45.5)	2 (40.0)	3 (60.0)
Dec	76	44 (57.9)	16 (36.4)	13 (81.3)	0	13 (100.0)
2013 Jan	42	21 (50.0)	8 (38.1)	3 (37.5)	0	3 (100.0)
Total	1,060	633 (59.7)	241 (38.1)	127 (52.7)	22 (17.3)	104 (81.9)

*All samples tested at Minnesota Department of Health (MDH) Public Health Laboratory. Eligibility criteria: Minnesota residency, symptom eligibility (≥3 loose stools in 24 h or vomiting), complaint received ≤4 d after vomiting/diarrhea onset or ≤2 d after vomiting/diarrhea recovery, and complainant interviewed by MDH staff. Completed complaints forwarded to MDH by local jurisdictions were excluded.
†Of those positive for norovirus.
‡One February complainant was positive for genogroups I and II (not included in individual genogroup totals).

underscoring the importance of monitoring their emergence and effects.

The greater proportion of vomiting and fever and longer illness duration among norovirus-positive complainants suggests that a bacterial intoxication, especially with diarrheal toxin agents such as *Clostridium perfringens*, may have caused a substantial proportion of illness among norovirus-negative complainants. However, complainant samples were not routinely tested for bacterial intoxication agents in this study because of the lag time from onset to complaint. Differences in rates of fever and health care visits between eligible complainants and those tested (Table 2) limit the accuracy of extrapolated estimates if these variables affect the likelihood that a caller is norovirus positive. However, if all symptom-eligible complainants are assumed to have the same risk for norovirus infection as the subpopulation of those tested, an estimated 1 in 5 callers during the peak off-season and

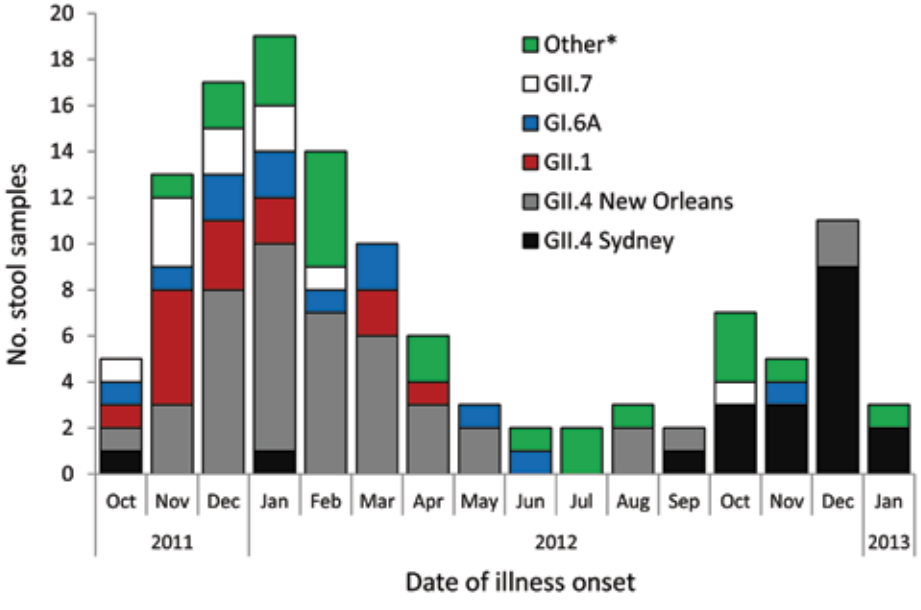

Figure 2. Norovirus genotypes identified in stool samples submitted by norovirus-positive callers to the foodborne illness hotline, Minnesota, USA, October 2011–January 2013. *Other genotypes identified: GII.4 Minerva, GI.3B, GII.3, GI.2, GI.7, GII.12, GI.4, GI.5, GII.6, GII.8

3 in 4 callers during the peak season would be infected with norovirus.

These results have limited potential for extrapolation to norovirus incidence estimates for Minnesota. The proportion of the population who would call the hotline when ill is unknown; in addition, hotline callers are not necessarily representative of the general population. However, trends observed among hotline callers, including norovirus prevalence, genotype diversity, and call volume, can serve as indicators of general norovirus activity. For example, our study demonstrates the transition in predominant circulating norovirus strain from GII.4 New Orleans to the emergent GII.4 Sydney strain, as has been observed among US outbreaks (*11*). The emergence of a new GII.4 strain has sometimes been associated with an increase in norovirus outbreak activity (*12*). However, an increase in proportion of callers positive for norovirus during the beginning of the 2012–2013 season was not observed in our study after the emergence of GII.4 Sydney. During this same period, the number of norovirus outbreaks identified by MDH was likewise not higher than in recent years (*12*; MDH, unpub. data), suggesting that GII.4 Sydney did not cause increased norovirus activity in Minnesota. Of note, a complainant with a sporadic case from October 2011 tested through this project was initially identified as being infected with GII.4 New Orleans, but GII.4 Sydney infection was retrospectively identified after CaliciNet updated its reference strains in November 2012 to include GII.4 Sydney.

In conclusion, norovirus accounted for most cases of acute gastroenteritis among hotline callers in Minnesota, particularly during the fall and winter norovirus season. Trends in positive specimens, genotype distribution, and symptom histories observed during complaint-based surveillance can be used to better understand the epidemiology of norovirus gastroenteritis.

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Travel-associated Diseases, Indian Ocean Islands, 1997–2010

Hélène Savini, Philippe Gautret, Jean Gaudart, Vanessa Field, Francesco Castelli, Rogelio López-Vélez, Poh Lian Lim, Marc Shaw, Frank von Sonnenburg, Louis Loutan, and Fabrice Simon, for the GeoSentinel Surveillance Network[1]

Data collected by the GeoSentinel Surveillance Network for 1,415 ill travelers returning from Indian Ocean islands during 1997–2010 were analyzed. Malaria (from Comoros and Madagascar), acute nonparasitic diarrhea, and parasitoses were the most frequently diagnosed infectious diseases. An increase in arboviral diseases reflected the 2005 outbreak of chikungunya fever.

The outbreak of chikungunya fever in Indian Ocean islands (IOI) provides new insights on emerging infections in this geographic region (*1*). We present data collected over 14 years from travelers to IOI who visited GeoSentinel clinics.

The Study

GeoSentinel sites are specialized travel clinics providing surveillance data for ill travelers. Detailed methods for recruitment of patients for the GeoSentinel database are described elsewhere (*2*). Demographics, travel characteristics, and individual medical data were obtained from travelers to Comoros (including Mayotte), Madagascar, Maldives, Mauritius, Réunion Island, and Seychelles during March 1, 1997–December 31, 2010. Statistical significance was determined by using Fisher exact test for categorical variables and Kruskal-Wallis test for quantitative variables. A 2-sided significance level of $p<0.05$ was considered significant.

This study comprised 1,415 ill patients (Table 1). Demographic data varied according to the visited island. Median age was 36 years, and the male to female ratio was 1.1:1.0. The most common reason for travel was tourism (44.5%), followed by visiting friends and relatives (VFR) (30.8%). Only 43.0% of travelers had a pre-travel encounter with a travel medicine specialist or general practitioner.

Illness patterns varied by place of exposure (Figure 1). Malaria, the most frequently diagnosed illness (388 [27.4%] travelers), accounted for 74.1% of diagnoses for VFR but only 6.6% for non-VFR travelers ($p<0.01$). *Plasmodium falciparum* malaria represented 88.0% of cases, including 12 cases of severe malaria, mostly from Comoros or Madagascar. One case of *P. ovale* malaria was reported from Mauritius in a person who had previously traveled to Cameroon.

Arboviral disease diagnoses included 40 cases of chikungunya and 24 cases of dengue. Overall, arboviral diseases accounted for 4.5% of the total diagnoses. Arboviral diseases accounted for 36.0% of diseases acquired by travelers to Réunion Island (vs. 3.6% in non–Réunion Island travelers, $p<0.01$) and were more frequent in tourists than in nontourists (6.5% vs. 2.9%, $p<0.01$). Numbers of arboviral diseases showed a sustained increase and peaked in 2006. Dengue was noted only after 2001. Chikungunya cases dramatically increased in 2006 and were sustained at a lower level during 2007–2010, suggesting local transformation from epidemic to endemic phases or better notification of the diagnosis (Figure 2).

Parasitic infections other than malaria accounted for 131 (9.3%) diagnoses. A higher proportion of parasitoses occurred in travelers to Madagascar than in persons who had not traveled there (21.3% vs. 2.6%, $p<0.01$) and in missionary than non-missionary travelers (18.7% vs. 7.9%, $p<0.01$). Intestinal helminths or protozoans were the most commonly identified parasites. Schistosomiasis (21 cases) was reported from Madagascar only.

Acute nonparasitic diarrhea accounted for 162 (11.5%) final diagnoses. Higher proportions of such diarrhea occurred in travelers to Madagascar than in persons who had not traveled there (15.7% vs. 9.1%, $p<0.01$) and in travelers to Maldives than in persons who had not traveled there (18.4% vs. 10.5%, $p<0.01$). In 23 (14.2%) cases, a pathogen was identified. Acute nonparasitic diarrhea and skin infections were more frequently reported in tourists than in nontourists (17.3% and 12.4% vs. 6.8% and 3.8%, respectively [$p<0.01$]). The proportion of respiratory infections

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[1]Additional members of the GeoSentinel Surveillance Network who contributed data are listed at the end of this article.

Table 1. Characteristics of 1,415 ill travelers returning from Indian Ocean islands, 1997–2010*

Characteristic	All islands, n = 1,415	Madagascar, n = 502	Comoros, n = 444	Maldives, n = 174	Mauritius, n = 153	Seychelles, n = 81	Réunion Island, n = 39	>1 Island, n = 22
Female sex, %†	47.8	47.2	42.3	51.7	51.6	63	53.8	50
Median age, y (95% CI)†	36 (19–65)	33 (20–66)	39 (18–64)	34 (6–62)	37 (15–69)	39 (24–69)	33 (14–65)	32 (23–62)
Median travel duration, d (95% CI)†	29 (7–341)	30 (8–665)	41 (12–176)	14 (5–366)	14 (6–109)	14 (7–112)	19 (2–3,270)	35 (9–416)
Reason for travel, %†								
Tourism	44.5	53.8	5.6	62.6	81.0	85.2	48.7	59.1
VFR	30.8	5.2	89.4	0	5.2	1.2	10.3	0.0
Missionary/ volunteer/student/ military	12.9	24.7	1.4	21.9	3.3	1.2	5.1	27.3
Business	10.9	15.9	1.8	15.5	10.5	11.1	30.8	9.1
Other	0.1	0.4	1.8	0	0	1.2	5.1	4.5
Pre-travel health advice, %†	43.3	55.2	32.2	47.1	35.9	37	25.6	72.7
Inpatient care, %	30.0	7.2	79.3	8.0	9.2	3.7	12.8	4.5

*VFR, visiting friends and relatives.
†p<0.01 for the comparison among islands.

was higher in persons traveling for business than in persons traveling for other reasons (11.2% vs. 5.1%, p<0.01).

Mosquito bites, food and water consumption, and direct contact with skin were the most frequent modes of disease transmission (Table 2). The proportion of mosquito-transmitted diseases was higher among travelers to Comoros than among other travelers (80.2% vs. 10.0%, p = 0.006). The proportion of foodborne diseases was higher

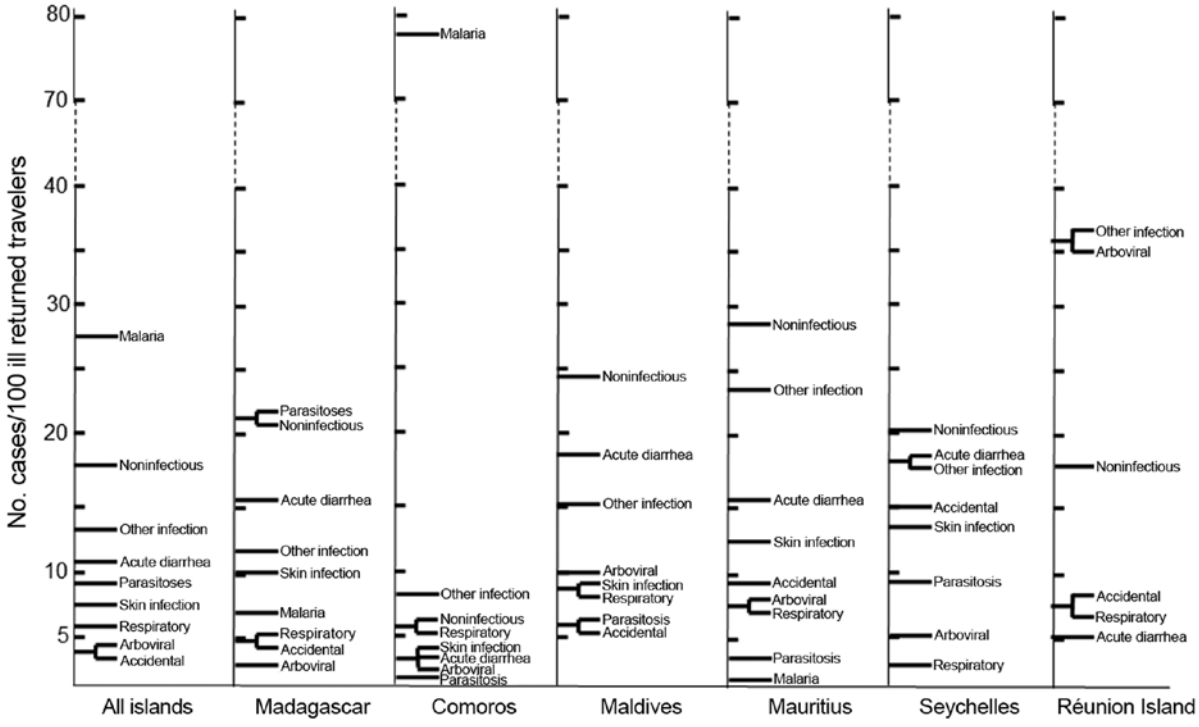

Figure 1. Relative proportion of different diagnoses among 1,415 ill travelers returning from Indian Ocean islands, 1997–2010. The numbers are shown for each diagnosis for all ill travelers returning from each island. Some patients had >1 diagnosis. Malaria: *Plasmodium falciparum* infection (341 cases, including 12 severe cases), *P. vivax* infection (24), *P. ovale* infection (11), *P. malariae* infection (10). Acute diarrheal infections: campylobacteriosis (12), salmonellosis (6), shigellosis (5). Parasitic infections: gardiasis (33), schistosomiasis (21), strongyloidiasis (13), miyases (13), amoebiasis (9), cutaneous larva migrans (9), trichuriasis (7), ascariasis (5), hookworm infection (5), enterobiasis (2), neurocysticercosis (2), filariasis (1), blastocystosis (1). Respiratory infections: upper respiratory tract infections (26), influenza (6), tuberculosis (4). Arboviral infections: chikungunya (40), dengue (24). Other infections: urinary tract infections (22), leptospirosis (2), rickettsial infections (3), Q fever (1). Among accidental diseases: insect bites (28), rabies postexposure treatments (6), marine envenomization (5).

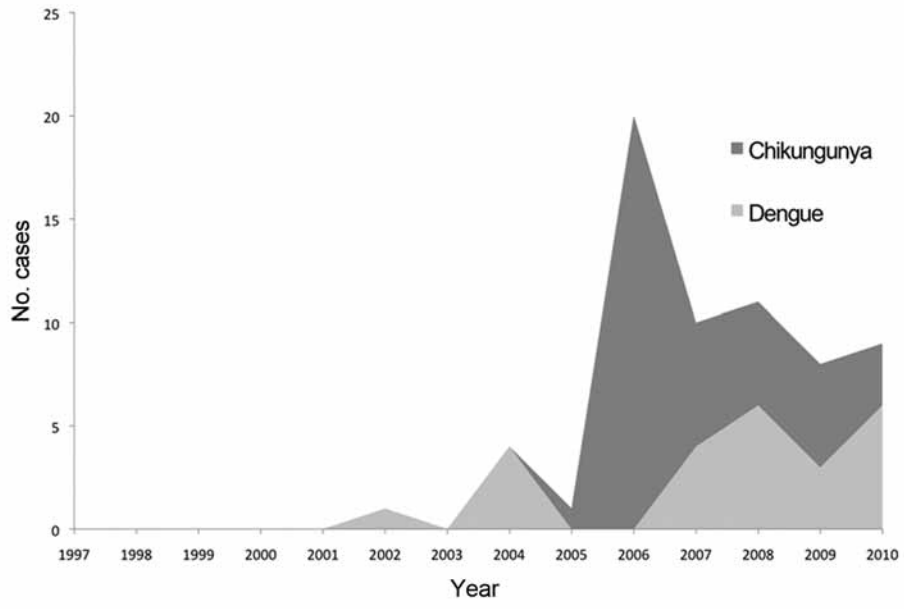

Figure 2. Annual occurrence of arboviral disease cases (dengue and chikungunya) among 1,415 travelers returning from Indian Ocean islands and seen at GeoSentinel sites, 1997–2010.

among travelers to Madagascar than in travelers to other areas (27.5% vs. 10.9%, p<0.001) and to Maldives than to other areas (23.0% vs. 15.8%, p = 0.03). Diseases transmitted through skin contact accounted for a higher proportion of diagnoses in travelers returning from Madagascar than from other areas (18.1% vs. 7.6%, p<0.001). Compared with nonbusiness travelers, business travelers had a higher proportion of respiratory-transmitted diseases (1.9% vs. 12.3%, p<0.001) and sexually and blood-transmitted diseases (0.3% vs. 6.6%, p = 0.03).

Conclusions

This large study addresses travel-associated diseases in travelers returning from IOI. *P. falciparum* infection was the most common reason for seeking post-travel care, notably when returning from Comoros, a well-known malaria-endemic archipelago (*3*). Imported malaria is frequently described in France, particularly in Marseille, which is the preferred residence city for migrants from Comoros and their descendants (*4*). Previous reports have shown that VFR sought pre-travel advice less frequently than did other travelers, possibly because of economic concerns, language barriers, or cultural beliefs (*5–7*). We observed a lower proportion of malaria in persons who had traveled to Madagascar, where both *P. falciparum* and *P. vivax* are endemic, and only 1 case in a traveler to Mauritius, where few cases are reported (*3*). No malaria cases were identified from Réunion Island, Seychelles, or Maldives, which is consistent with travel medicine guidelines that do not recommend chemoprophylaxis for travelers visiting these islands (*8*).

The reports of dengue and chikungunya fever from all islands reflect the wide distribution of the vector, *Aedes* spp. mosquitoes. Our results parallel those of the chikungunya fever outbreak that spread throughout IOI during 2005–2006 (*9*), facilitated by an adaptive virus mutation that led to increased infectivity, replication, and transmission by *A. albopictus* mosquitoes (*10*). The outbreak affected hundreds of travelers to IOI (*11*). Concern about the possible spread of chikungunya fever increased with the autochthonous outbreak of chikungunya fever in Italy in 2007 that developed from a patient returning from India (*12*). This sporadic case confirmed the ability of the virus to settle in countries colonized by *Aedes* sp. mosquitoes as a result of increasing intercontinental exchanges. Surveillance of travelers with a view toward early diagnosis is a key element in controlling outbreaks of imported arboviral diseases.

Parasitic infections, including schistosomiasis, accounted for a major proportion of final diagnoses in travelers to Madagascar, where these infections represent a public health concern (*13*). Testing for such diseases should be considered in ill travelers returning from this island.

Nonparasitic diarrhea was reported mainly in tourists returning from Madagascar and the Maldives. Few pathogens were documented, reflecting the practice of empiric antimicrobial treatment before laboratory testing (*14*). The higher incidence of diarrheal illness among tourists could be explained by an immature mucosal immunity (*15*) and easier access to medical care.

Business travelers had a higher proportion of respiratory diseases, independent of the island visited. This finding may relate to longer stays in air conditioned hotels and close human-to-human contact in this population.

These data have at least 4 limitations. First, we included only returning travelers who were ill and receiving

Table 2. Modes of disease transmission for 1,415 ill travelers returning from Indian Ocean islands, 1997–2010

Mode of transmission	Total no. (%), n = 1,415	Island visited, no. (%) travelers						
		Madagascar, n = 502	Comoros, n = 444	Maldives, n = 174	Mauritius, n = 153	Seychelles, n = 81	Réunion Island, n = 39	>1 Island, n = 22
Mosquito bite	452 (31.9)	48 (9.6)	356 (80.2)	17 (9.8)	13 (8.5)	4 (4.9)	14 (35.9)	0 (0.0)
Food/water consumption	236 (16.7)	138 (27.5)	10 (2.3)	40 (23.0)	27 (17.6)	20 (24.7)	3 (7.7)	2 (9.1)
Direct skin contact	159 (11.2)	91 (18.1)	9 (2.0)	20 (11.5)	24 (15.7)	17 (21.0)	0	2 (9.1)
Respiratory droplet	102 (7.2)	33 (6.6)	25 (5.6)	20 (11.5)	15 (9.8)	7 (8.6)	8 (20.5)	4 (18.2)
Animal contact	44 (3.1)	15 (3.0)	0	7 (4.0)	10 (6.5)	12 (14.8)	1 (2.6)	1 (4.5)
Fresh water contact	23 (1.6)	21 (4.2)	0	0	1 (0.7)	0	1 (2.6)	0
Sex/blood	7 (0.5)	3 (0.6)	1 (0.2)	1 (0.6)	0	1 (1.2)	1 (2.6)	0
Tick bite	3 (0.2)	1 (0.2)	1 (0.2)	0	1 (0.7)	0	0	0

care at GeoSentinel sites. Second, self-limited diseases or diseases of short duration may be underrepresented. Third, the lack of a denominator does not permit calculation of prevalence. Fourth, diseases with very short or very long incubation periods might not, with certainty, be attributed to any particular destination. Nevertheless, our study describes the spectrum of diseases among travelers returning from each IOI based on robust numbers of ill travelers.

Ill travelers returning from IOI are heterogeneous in their demographic and travel characteristics and display specific diseases that depend on the island and the travel reason. These findings reflect the different economic, ecologic, and public health situations found across this region (online Technical Appendix Table, wwwnc.cdc.gov/EID/article/19/8/12-1739-Techapp1.pdf). More than two thirds of diseases in travelers to IOI were, theoretically, preventable by reinforcing food and hand hygiene and by avoiding insect bites or direct contact with soil and fresh water. Most travelers in our survey traveled to a single island; thus, targeted destination-specific pre-travel advice and post-travel medical management of ill persons should be provided on a country-level basis rather than addressed nonspecifically.

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Whole Genome Sequencing of an Unusual Serotype of Shiga Toxin–producing *Escherichia coli*

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Shiga toxin–producing *Escherichia coli* serotype O117:K1:H7 is a cause of persistent diarrhea in travelers to tropical locations. Whole genome sequencing identified genetic mechanisms involved in the pathoadaptive phenotype. Sequencing also identified toxin and putative adherence genes flanked by sequences indicating horizontal gene transfer from *Shigella dysenteriae* and *Salmonella* spp., respectively.

There are >400 serotypes of Shiga toxin–producing *Escherichia coli* (STEC), and >100 of these are known to be associated with severe disease in humans (*1*). STEC are defined by the presence of 1 or both phage-encoded Shiga toxin genes *stx1* and *stx2*. However, those serotypes associated with more severe disease generally harbor additional virulence genes, such as *eae* (intimin), which is encoded on the locus of enterocyte effacement, or virulence regulation genes, such as *agg*R, which is located on the aggregative adherence plasmid. Both of these genes mediate attachment of the bacteria to the host gut mucosa (*2*). The *stx*1 gene is also found in *Shigella dysenteriae* serotype 1.

A range of molecular typing methods show that the shigellae belong within the *Escherichia coli* species (*3*). Peng et al. (*4*) described an evolutionary path of *Shigella* spp. from *E. coli* involving gene acquisition (virulence plasmid and pathogenicity islands) and gene loss (pathoadaptivity). Gene loss, or loss of gene function, may result from changes to bacterial biosynthesis pathways driven by the abundance of resources in the host or because the genes may encode proteins adverse to bacterial virulence.

Olesen et al. (*5*) described a strain of STEC serotype O117:K1:H7 found in travelers from Denmark who returned from tropical locations. The strain was unusual because it was negative for the production of lysine decarboxylase and b-galactosidase (ortho-nitrophenol test) and positive only for *stx*1.

Since 2004, 19 isolates of STEC O117:K1:H7 have been submitted to the Gastrointestinal Bacteria Reference Unit at the Health Protection Agency in London, UK, from frontline diagnostic microbiology laboratories in England and Wales for confirmation of identification and typing (Table). All isolates were originally misidentified by the submitting laboratory as *Shigella sonnei* or *Shigella* spp., probably because of the unusual biochemical phenotype exhibited by this strain. The purpose of this study was to use whole genome sequencing to investigate the evolutionary origins, putative virulence genes, and pathoadaptive mechanisms of this unusual STEC serotype.

The Study

DNA from 5 isolates (151/06, 371/08, 290/10, 754/10, and 229/11) was prepared for sequencing by using the Nextera sample preparation method and sequenced with a standard 2 × 151 base protocol on a MiSeq instrument (Illumina, San Diego, CA, USA) (*6*). Sequences were analyzed as described (*7*). In brief, Velvet version 1.1.04 (www.ebi.ac.uk/~zerbino/velvet/) was used to produce an average of 489 contigs with an average N50 length of 38722. Illumina reads were mapped to the reference strain (GenBank accession no. CU928145) by using Bowtie2 2.0.0 β-5 (http://bowtie-bio.sourceforge.net/bowtie2/) and a variant call format file was created from each of the binary alignment maps, which were further parsed to extract only single nucleotide polymorphism (SNP) positions that were of high quality in all genomes.

Concatenated SNPs generated against the reference strain 55989 were used to produce a maximum-likelihood phylogeny of 5 strains in the Gastrointestinal Bacteria Reference Unit archive and 36 other publically available *E. coli* genomes and *Shigella* spp. (Figure). Despite temporal and spatial diversity of the 5 sequenced isolates, they clustered on the same branch, but they were distant from other publically available sequences of STEC strains.

A phylogenetic tree based on a diverse range of *E. coli* showed that the 5 strains of STEC O117 have 130 polymorphic positions, and the closest 2 strains (299/11 and 754/10) are 26 SNPs apart (Table; Figure). Furthermore, on the basis of a diverse range of *E. coli*, genome sequences of EDL933 and Sakai, 2 well-described strains of STEC O157, are ≈35 SNPs apart. The multilocus sequence type ST504 was assigned in accordance with the *E. coli* multilocus sequence type databases at the Environment Research Institute, University College (Cork, Ireland).

Conclusions

Alignment of the genome of strain 229/11 with STEC O157 (EDL933) and *Shigella dystenteriae* serotype 1

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Table. Shiga toxin–producing *Escherichia coli* O117:K1:H7 strains submitted to GBRU from frontline diagnostic microbiology laboratories, United Kingdom, 2004–2012*

Strains	Year isolated (no.)	Clinical signs and symptoms of patient	Country or region visited by patient
Strains sequenced			
151/06	2006	Not reported	India
371/08	2008	Not reported	Egypt
290/10	2010	Bloody diarrhea	Cuba
754/10	2010	Pyrexia	South America
229/11	2011	Diarrhea, abdominal pain	Kenya and India
Additional strains	2004 (2); 2008 and 2009 (3); 2010 (4); 2011 (2); 2012 (2)	Diarrhea, bloody diarrhea, fever, persistent nausea, abdominal pain	Afghanistan, Bali, Egypt, Ecuador, Ghana, India, Jordan, Libya, Tanzania, Uganda

*GBRU, Gastrointestinal Bacteria Reference Unit.

(Sd197) indicated gene acquisition, loss, and rearrangement in 229/11. The *stx*1 gene is adjacent to the *yjh*S gene in 229/11 and Sd197, and in 229/11 this fragment is flanked by phage-like sequences that are closely related to Stx2-converting phage sequences but not to other Stx1-converting phages. This unusual gene arrangement was described by Sato et al. (*8*). In Sd197, this region is flanked by integrases and insertion sequences. Other open reading frames homologous to those of *Shigella* spp. in *stx*-flanking regions in *E. coli* have been described, and it is likely that *E. coli* and the shigellae have exchanged *stx* many times in their evolutionary past but only certain strains, such as 229/11, have the appropriate genomic background to retain and stably express Stx (*9*).

Strain 229/11 also contains a 10-kb pathogenicity island (PAI) harboring the *rat*A, *Siv*l, and *Siv*H genes and shares homology with PAI CS54 found in *Salmonella* spp. (*10*) and a PAI found in avian pathogenic *E. coli* (*11*). *Siv*H has been described as similar to the intimin gene (*10*). *Siv*H may facilitate attachment to the host gut mucosa and could explain the long persistence of STEC O117:K1:H7 in infected patients (*5*). In vitro inactivation of *sivH* in *S. enterica* serovar Typhimurium resulted in a reduced ability to colonize Peyer's patches (*10*). In *S. enterica* serovar Typhimurium, CS54 is 25-kb and encodes *shd*A, *rat*A, *rat*B, *siv*l, and *siv*H, whereas in *S. enterica* subsp. II, *S. bongori* serotypes and 229/11, *rat*B, and *shd*A are absent (*10*).

Cadaverine has an inhibitory effect on enterotoxin activity by preventing full expression of the virulent phenotype, and it has been suggested that there is evolutionary pressure to mutate or delete the *cadA* gene (*12*). This gene is missing from *S. flexneri* (Sf301) and *S. boydii* (Sb227) because of inversion-associated deletions, and in Sd197 and *S. sonnei* (Ss046) it is inactivated by a frameshift mutation and an insertion sequence, respectively (*12*). In 229/11, loss of *cad*A (lysine decarboxylase) activity is caused by repositioning of the of the *cad*A activator gene, *Cad*C, upstream of the *cad*A gene and a 90-bp deletion at the 5′ end of *cad*C. The *cad*A gene and truncated *cad*C gene are separated by a large fragment of DNA inserted into the *cad*C gene. This fragment contains several open reading frames, including genes encoding aerobactin siderophore biosynthesis proteins.

Lactose fermentation is a biochemical property commonly used for distinguishing *Shigella* spp. from *E. coli* because shigellae are non- or late-lactose fermenters. In Sd197 and Ss046 (late lactose–fermenting strains), the key gene, *lac*Z (encoding b-D-galactosidase) is intact, although *lac*Y (encoding galactose permease) is a pseudogene (*12*). Like Sf301 and Sb227, *lac*Z and *lac*Y are deleted in strain 229/11. The lack of a functional *lac* operon has been associated with pathogenicity mechanisms in *S. enterica* (*13*).

E. coli as a species contains a large diversity of adaptive paths. This diversity is the result of a highly dynamic genome, with a constant and frequent flux of insertions and deletions (*3*). Pathogenicity in STEC O117:K1:H7 is most

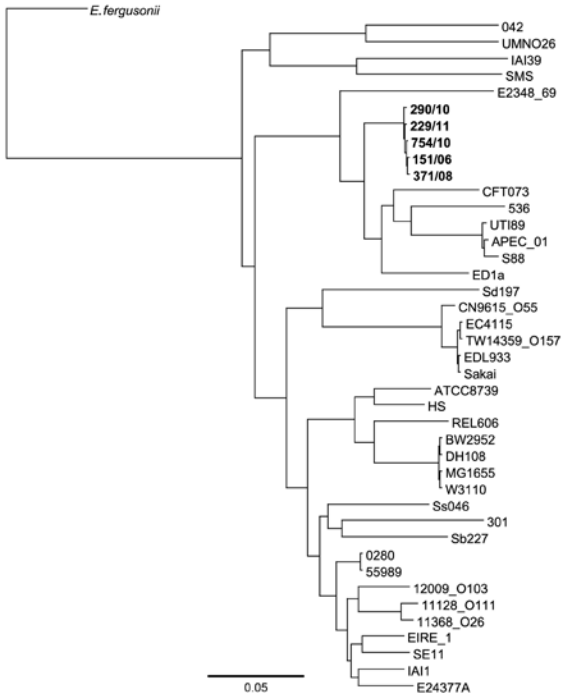

Figure. Maximum-likelihood dendrogram for 5 strains of Shiga toxin–producing *Escherichia coli* serotype O117 in the Gastrointestinal Bacteria Reference Unit (Health Protection Agency, London, UK) archive (**boldface**), 32 other *E. coli* genomes, and 4 *Shigella* spp. genomes. *E. fergusonii* was used as an outgroup. Scale bar indicates nucleotide substitutions per site.

likely multifactorial and results from a novel combination of lack of *cad*A and *lac*Z expression and the presence of *stx*1 and the intimin-like *siv*H genes, demonstrating pathoadaptivity and horizontal gene transfer.

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Acute Gastroenteritis Surveillance through the National Outbreak Reporting System, United States

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Implemented in 2009, the National Outbreak Reporting System provides surveillance for acute gastroenteritis outbreaks in the United States resulting from any transmission mode. Data from the first 2 years of surveillance highlight the predominant role of norovirus. The pathogen-specific transmission pathways and exposure settings identified can help inform prevention efforts.

Acute gastroenteritis (AGE; defined as diarrhea or vomiting) is a major cause of illness in the United States; an estimated 179 million episodes occur annually (*1*). AGE is caused by a variety of viral, bacterial, and parasitic pathogens and by toxins, chemicals, and other noninfectious causes. Noroviruses are the leading cause of epidemic gastroenteritis, detected in ≈50% of AGE outbreaks across Europe and the United States (*2*,*3*). However, until 2009, national surveillance for AGE outbreaks in the United States had been limited to foodborne or waterborne disease outbreaks because no national surveillance existed for AGE outbreaks spread by other transmission modes.

To better understand and guide appropriate interventions to prevent epidemic gastroenteritis, the Centers for Disease Control and Prevention (CDC) launched a novel national surveillance system in 2009—the National Outbreak Reporting System (NORS). This system enhanced and expanded upon 2 existing surveillance systems, the Foodborne Disease Outbreak Surveillance System and the Waterborne Disease and Outbreak Surveillance System. NORS is an Internet-based system for local, state, and territorial health departments to report all outbreaks of foodborne and waterborne disease; AGE outbreaks caused by contact with infected persons, animals, or environmental sources; and AGE outbreaks caused by other or unknown modes of transmission (*4*). As such, NORS provides a national surveillance system for all pathways of AGE outbreaks in the United States. To assess the roles of specific pathogens, temporal trends, and exposure pathways, we summarized AGE outbreak data submitted through NORS during the first 2 years after implementation of the system.

The Study

In the United States, outbreaks (defined as ≥2 cases of a similar illness epidemiologically linked to a common exposure, e.g., setting or food) can be reported through NORS by all 50 US states, the District of Columbia, US territories (American Samoa, Guam, Commonwealth of the Northern Mariana Islands, Puerto Rico, and the United States Virgin Islands), and Freely Associated States (Federated States of Micronesia, Republic of Marshall Islands, and Republic of Palau). NORS was launched in February 2009, but sites were encouraged to report outbreaks that occurred since January 1, 2009.

For analysis, we extracted data reported through NORS for AGE outbreaks in which the symptom onset date for the first reported illness was during January 1, 2009–December 31, 2010. Outbreaks of diseases that do not typically cause AGE (e.g., listeriosis, legionellosis, hepatitis A) were excluded from analysis (*1*). We analyzed various outbreak characteristics: date of first illness onset, primary transmission mode, confirmed or suspected etiology (*5*), exposure setting, and number of outbreak-associated illnesses, hospitalizations, and deaths. Primary mode of transmission is determined by each reporting site on the basis of the local public health investigation and CDC guidance documents (*6*).

Of 4,455 outbreaks reported through NORS during 2009–2010, a total of 4,376 (98%) were AGE outbreaks (1,883 in 2009, 2,493 in 2010) (Table 1), associated with 122,488 reported illnesses, 2,952 hospitalizations, and 168 deaths. A single suspected or confirmed etiology was implicated in 2,819 (64%) outbreaks, associated with 88,958 (73%) illnesses, 2,381 (81%) hospitalizations, and 146 (87%) deaths. Norovirus, the leading cause of single-etiology outbreaks, was responsible for 1,908 (68%) outbreaks, associated with 69,145 (78%) illnesses, 1,093 (46%) hospitalizations, and 125 (86%) deaths. *Salmonella* spp., *Shigella* spp., and Shiga toxin–producing *Escherichia coli* (STEC), the next most frequently reported etiologic agents, were responsible for 355 (13%), 109 (4%), and 101 (4%) outbreaks, respectively. *Salmonella* spp. were the second most frequent cause of outbreak-associated hospitalizations (773 [32%]), and STEC was the second most frequent cause of outbreak-associated deaths (9 [6%]).

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Table 1. Numbers of acute gastroenteritis outbreaks and outbreak-associated outcomes caused by various etiologic agents, National Outbreak Reporting System, United States, 2009–2010*

Outbreak etiology	No. (%) outbreaks			No. (%) outbreak-associated outcomes		
	Confirmed	Suspected	Total	Illnesses	Hospitalizations	Deaths
Single agent†						
Norovirus‡	1,355 (64.2)	553 (78.1)	1,908 (67.7)	69,145 (77.7)	1,093 (45.9)	125 (85.6)
Salmonella spp.	344 (16.3)	11 (1.6)	355 (12.6)	8,590 (9.7)	773 (32.5)	6 (4.1)
Shigella spp.§	99 (4.7)	10 (1.4)	109 (3.9)	2,135 (2.4)	115 (4.8)	1 (0.7)
STEC	88 (4.2)	13 (1.8)	101 (3.6)	1,091 (1.2)	250 (10.5)	9 (6.2)
Campylobacter spp.¶	56 (2.7)	13 (1.8)	69 (2.4)	1,550 (1.7)	52 (2.2)	0
Clostridium spp.#	41 (1.9)	21 (3.0)	62 (2.2)	3,242 (3.6)	16 (0.7)	3 (2.1)
Cryptosporidium spp.**	17 (0.8)	30 (4.2)	47 (1.7)	598 (0.7)	21 (0.9)	1 (0.7)
Bacillus spp.††	13 (0.6)	12 (1.7)	25 (0.9)	522 (0.6)	3 (0.1)	0
Staphylococcus aureus	11 (0.5)	11 (1.6)	22 (0.8)	263 (0.3)	0	0
Giardia intestinalis	13 (0.6)	6 (0.8)	19 (0.7)	121 (0.1)	5 (0.2)	0
Scombroid toxin/histamine	18 (0.9)	0	18 (0.6)	76 (0.1)	0	0
Ciguatoxin	14 (0.7)	0	14 (0.5)	59 (0.1)	6 (0.3)	0
Rotavirus	9 (0.4)	5 (0.7)	14 (0.5)	372 (0.4)	9 (0.4)	0
Other‡‡	33 (1.6)	23 (3.2)	56 (2.0)	1,194 (1.3)	38 (1.6)	1 (0.7)
All single-agent etiologies	2,111 (98.9)	708 (31.6)	2,819 (64.4)	88,958 (72.6)	2,381 (80.7)	146 (86.9)
Multiple agents	24 (1.1)	9 (0.4)	33 (0.8)	1,236 (1.0)	61 (2.1)	2 (1.2)
Unknown agent	0	1,524 (68.0)	1,524 (34.8)	32,294 (26.4)	510 (17.3)	20 (11.9)
All outbreaks	2,135 (100.0)	2,241 (100.0)	4,376 (100.0)	122,488 (100.0)	2,952 (100.0)	168 (100.0)

*STEC, Shiga toxin–producing *Escherichia coli*.
†Percentages for specific single agents are those among all single-agent etiology outbreaks (N = 2,819).
‡A norovirus genogroup was provided for 1,160 outbreaks: 150 GI, 1,003 GII, and 7 GI/GII.
§*S. sonnei* (95 confirmed and 8 suspected outbreaks), *S. flexneri* (5 confirmed outbreaks), *Shigella* sp. not known (1 confirmed outbreak).
¶*C. jejuni* (55 confirmed and 4 suspected outbreaks), *Campylobacter* sp. not known (8 confirmed and 2 suspected outbreaks).
#*C. perfringens* (37 confirmed and 20 suspected outbreaks), *Clostridium* sp. not known (4 confirmed and 1 suspected outbreak).
**C. parvum* (10 confirmed and 1 suspected outbreak), *C. hominis* (6 confirmed outbreaks), *Cryptosporidium* sp. not known (30 confirmed outbreaks).
††*B. cereus* (13 confirmed and 11 suspected outbreaks), *Bacilllus* sp. not known (1 suspected outbreak).
‡‡Includes *Vibrio* sp. (8 outbreaks), cyanobacterial toxins (6 outbreaks), enterotoxigenic and enteropathogenic *E. coli* (4 outbreaks), *Enterococcus* spp. (3 outbreaks), mycotoxins (3 outbreaks), *Cyclospora* spp. (2 outbreaks), pesticides (2 outbreaks), sapovirus (2 outbreaks), paralytic shellfish poison (1 outbreak), *Pseudomonas* sp. (1 outbreak), sodium hydroxide (1 outbreak), *Yersinia* sp. (1 outbreak), and other unspecified etiologies (22 outbreaks).

AGE outbreaks were reported by the District of Columbia, Puerto Rico, and all states except Delaware (Figure 1). A median of 42 outbreaks (range 2–331) was reported by each site, and the median rate was 7.3 outbreaks/1 million person-years (range 0.9–44.8). Overall, AGE outbreaks exhibited winter seasonality: 2,972 (68%) of the 4,376 outbreaks occurred during November–April (Figure 2). This trend was driven largely by outbreaks caused by norovirus and by unknown etiologies, of which 1,530 (80% of 1,908 total) and 1,086 (71% of 1,524 total), respectively, occurred during November–April. In contrast, 62% of outbreaks caused by other etiologies, primarily bacteria, occurred during May–October.

The primary reported mode of transmission in most AGE outbreaks was person to person (2,271 [52%]), followed by foodborne (1,513 [35%]), waterborne (65 [2%]), animal contact (44, 1%), and environmental contamination (9, 0.2%); the transmission mode was unknown in 474 (10%) outbreaks (Table 2). Person-to-person transmission was implicated in most outbreaks caused by norovirus (1,261 [66%]) and *Shigella* spp. (86 [79%]), whereas foodborne transmission was implicated in most outbreaks caused by *Salmonella* spp. (254 [72%]) and STEC (64 [63%]). Among the 3,052 (70%) AGE outbreaks for which a single exposure setting was reported, health care facilities, primarily nursing homes, were the most frequent settings (1,499 [49%]), followed by restaurants or banquet facilities (657 [22%]), schools or day-care facilities (290 [10%]), and private residences (227 [7%]). Most norovirus outbreaks (64%) occurred in health care facilities, whereas shigellosis outbreaks (74%) occurred predominantly in schools or day-care facilities. Private residences and restaurants/banquet facilities were the most frequent exposure settings for outbreaks caused by *Salmonella* spp. (32% and 36%, respectively) and STEC (46% and 20%, respectively).

Conclusions

As the national surveillance system for US AGE outbreaks, NORS provides valuable insights into the epidemiology of the pathogens most often involved. Building upon previous surveillance systems and analyses focused on specific transmission modes (*7*–*10*), NORS provides a more complete characterization of AGE outbreaks, particularly the relative importance of specific transmission modes and settings for the key pathogens. This analysis highlights norovirus as not only the leading cause of reported AGE outbreaks but also the leading cause of AGE outbreak–associated hospitalizations and deaths. Although norovirus usually causes self-limiting disease, it can cause severe outcomes when outbreaks occur among vulnerable populations, such as nursing-home residents (*11*). *Salmonella*

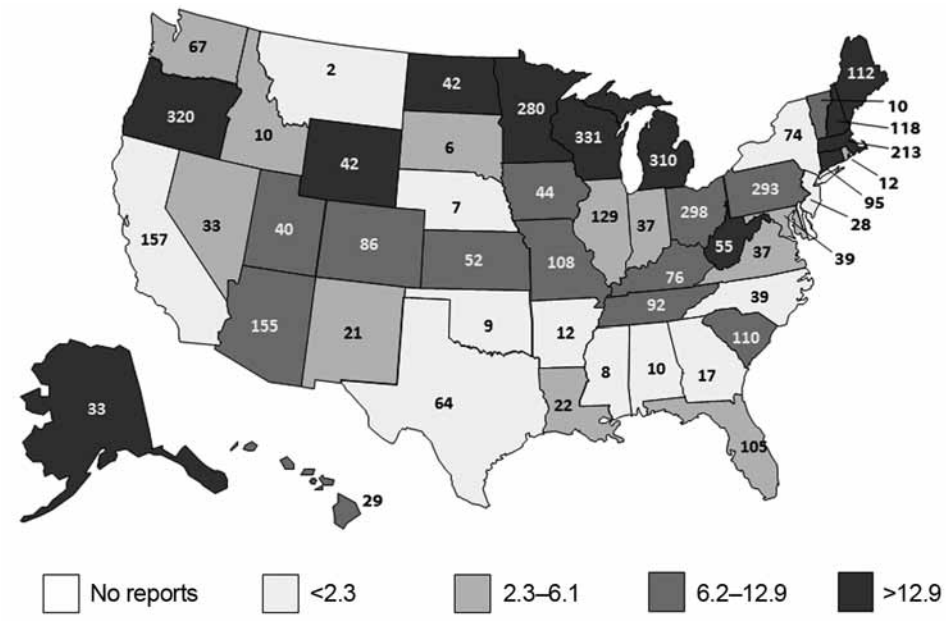

Figure 1. Total number and annual rate of reported acute gastroenteritis outbreaks per 1 million population by reporting state, National Outbreak Reporting System, United States, 2009–2010. The number given in each state indicates the total number of outbreaks over the 2-year study period; the shading denoted by the legend indicates the reporting rate by quartiles. Multistate outbreaks (n = 48) and those reported by Puerto Rico (n = 15) and the District of Columbia (n = 24) are not shown.

spp., *Shigella* spp., and STEC are also key contributors to AGE outbreaks. Expanded surveillance through NORS revealed that 28%, 91%, and 31%, respectively, of outbreaks caused by these 3 bacteria result from routes other than contaminated food or water. In addition, NORS provides information on non-AGE outbreaks transmitted by food or water. For example, ≈25% of waterborne disease outbreaks are caused by *Legionella* spp. (*8*,*9*), and among foodborne disease outbreaks, listeriosis is a major cause of outbreak-related hospitalizations and deaths (*10*).

As a passive reporting system, NORS is subject to variability in reporting practices between states and among outbreaks associated with different transmission modes and exposure settings. Reporting rates and data completeness may be improved through ongoing NORS enhancements, including direct data upload functionality and all-mode collection of setting information. For 35% of outbreaks, no suspected or confirmed etiology was identified, primarily because diagnostic specimens were not collected. However, outbreaks of unknown etiology exhibited

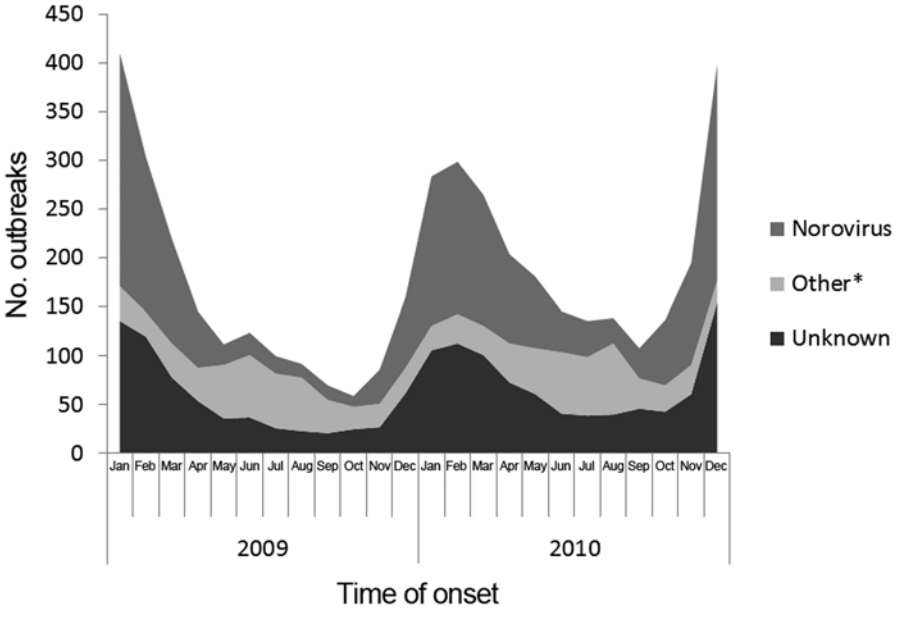

Figure 2. Number of reported acute gastroenteritis outbreaks by month of first illness onset and etiology, National Outbreak Reporting System, United States, 2009–2010. *Includes outbreaks caused by a single etiologic agent other than norovirus or multiple etiologies.

Table 2. Primary transmission mode and exposure setting of acute gastroenteritis outbreaks, by etiologic agents, National Outbreak Reporting System, United States, 2009–2010*

Outbreak characteristic	No. (%) outbreaks						
	Norovirus, n = 1,908	*Salmonella* spp., n = 355	*Shigella* spp., n = 109	STEC, n = 101	Other, n = 379†	Unknown, n = 1,524	Total, N = 4,376
Primary transmission mode							
Person to person	1,261 (66.1)	17 (4.8)	86 (78.9)	11 (10.9)	47 (12.4)	849 (55.7)	2,271 (51.9)
Foodborne	494 (25.9)	254 (71.5)	8 (7.3)	64 (63.4)	220 (58.0)	473 (31.0)	1,513 (34.6)
Waterborne	4 (0.2)	0	2 (1.8)	6 (5.9)	51 (13.5)	2 (0.1)	65 (1.5)
Animal contact	0	26 (7.3)	0	5 (5.0)	12 (3.2)	1 (0.1)	44 (1.0)
Environmental contamination	5 (0.3)	2 (0.6)	1 (0.9)	0	0	1 (0.1)	9 (0.2)
Unknown	144 (7.5)	56 (15.8)	12 (11.0)	15 (14.9)	49 (12.9)	198 (13.0)	474 (10.8)
Exposure setting‡							
Health care facility	932 (48.8)	5 (1.4)	0	0	25 (6.6)	537 (35.2)	1,499 (34.3)
Restaurant or banquet facility	287 (15.0)	69 (19.4)	5 (4.6)	12 (11.9)	77 (20.3)	207 (13.6)	657 (15.0)
School or day-care facility	98 (5.1)	14 (3.9)	50 (45.9)	6 (5.9)	15 (4.0)	107 (7.0)	290 (6.6)
Private residence	31 (1.6)	62 (17.5)	4 (3.7)	28 (27.7)	60 (15.8)	42 (2.8)	227 (5.2)
Other single setting	114 (6.0)	42 (11.8)	9 (8.3)	15 (14.9)	101 (26.6)	98 (6.4)	379 (8.7)
Multiple	33 (1.7)	19 (5.4)	13 (11.9)	10 (9.9)	10 (2.6)	21 (1.4)	106 (2.4)
Not reported	264 (13.8)	86 (24.2)	15 (13.8)	15 (14.9)	42 (11.8)	313 (20.5)	735 (16.8)
Not collected§	149 (7.8)	58 (16.3)	13 (11.9)	15 (14.9)	49 (12.9)	199 (13.1)	483 (11.0)

*Data include both suspected and confirmed etiologies. STEC, Shiga toxin-producing *Escherichia coli*.
†Includes outbreaks caused by a single etiologic agent other than norovirus, *Salmonella* spp., *Shigella* spp., and STEC or by multiple etiologic agents, as listed in Table 1.
‡Data on specific settings are restricted to outbreaks with a single exposure setting; for foodborne outbreaks, setting refers to the setting where implicated food was consumed.
§The setting was systematically not collected for outbreaks caused by environmental contamination or unknown transmission mode.

similar temporal trends and epidemiologic characteristics as norovirus outbreaks, suggesting that many of these may have been caused by norovirus. NORS does not include AGE outbreaks on international cruise ships; however, if combined with the outbreaks reported to NORS, these cruise-ship outbreaks would represent <1% of all reported outbreaks attributed to norovirus and all-cause AGE (*12*).

Although a small minority of AGE cases in the United States are associated with reported outbreaks (≈1 in 3,000), outbreak surveillance provides unique insights that can inform prevention efforts. Norovirus control through hand hygiene, environmental disinfection, and isolation of ill persons should remain a priority and likely affords protection against other AGE agents (*13*). Ongoing surveillance through NORS will help further elucidate trends, identify gaps, and assess the effects of future interventions on reducing epidemic gastroenteritis.

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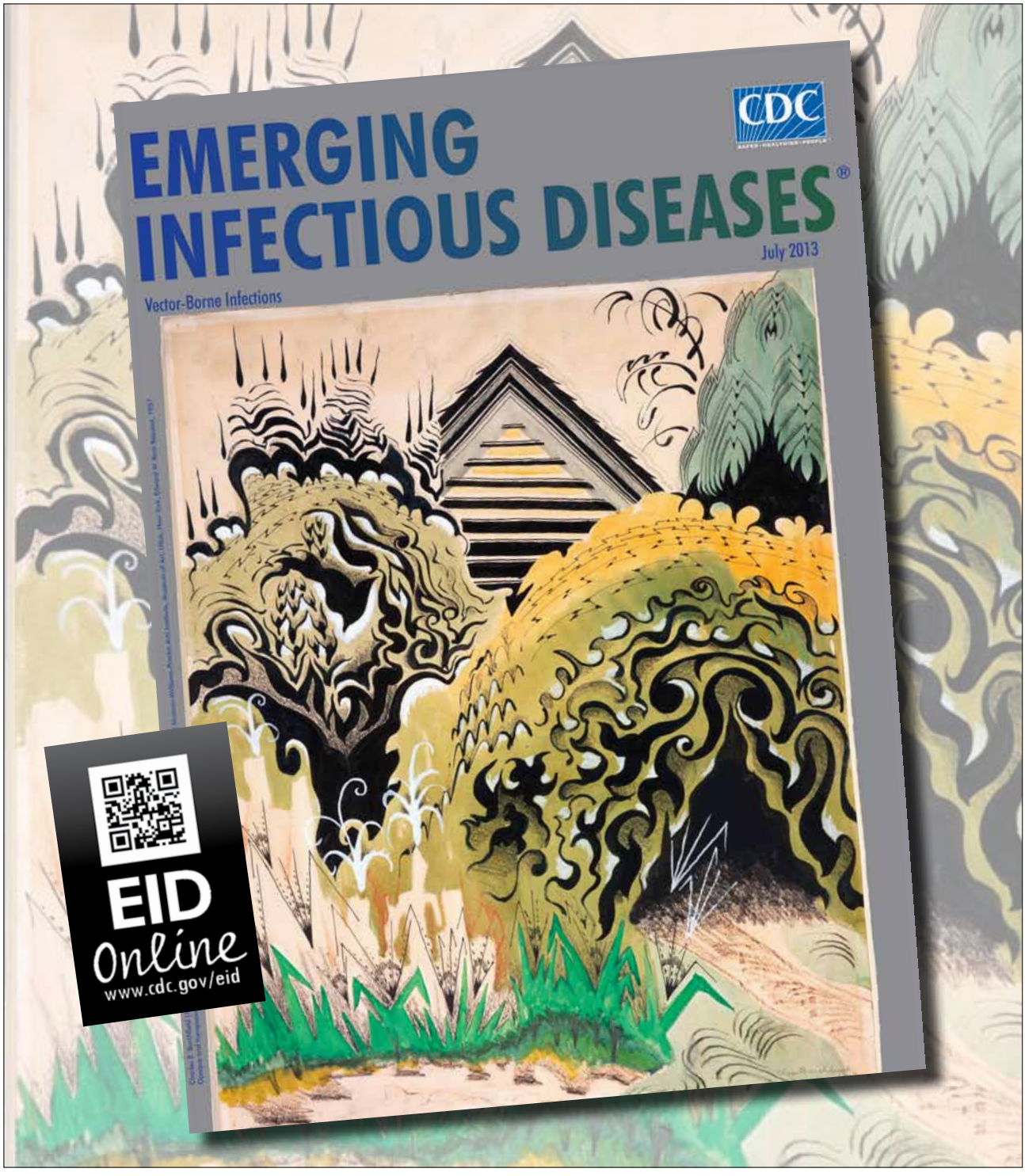

Duck Liver–associated Outbreak of *Campylobacter* Infection among Humans, United Kingdom, 2011

Muhammad Abid, Helen Wimalarathna, Janette Mills, Luisa Saldana, Winnie Pang, Judith F. Richardson, Martin C. J. Maiden, and Noel D. McCarthy

Campylobacter spp.–related gastroenteritis in diners at a catering college restaurant was associated with consumption of duck liver pâté. Population genetic analysis indicated that isolates from duck samples were typical of isolates from farmed poultry. *Campylobacter* spp. contamination of duck liver may present a hazard similar to the increasingly recognized contamination of chicken liver.

Although bacteria in the genus *Campylobacter* commonly cause gastroenteritis, identified outbreaks are relatively rare. In England and Wales, 21 identified campylobacteriosis outbreaks during 1992–1994 (*1*) and 50 during 1995–1999 (*2*) accounted for 0.2% and 0.4% of reported outbreaks of gastroenteritis, respectively. Water and milk were the main sources of *Campylobacter* spp. outbreaks in the United Kingdom and the United States, although becoming less so (*2*,*3*). Poultry consumption and restaurant dining are the most common foodborne illness risks, although many foodstuffs are implicated (*2*,*3*). Outbreaks associated with chicken liver pâté or parfait have increased: 14 outbreaks were associated with these items in England and Wales during 2007–2009 compared with 11 during the 15 preceding years (*4*). There were also large outbreaks in Scotland (*5*,*6*). The peer-reviewed literature identifies chicken as the type of poultry liver or refers to poultry without specifying type.

Multilocus sequence typing is increasingly used to identify animal origins of human campylobacteriosis (*7*). The presence of multiple *Campylobacter* strains (*6*) in individual outbreaks linked to chicken liver is consistent with documentation that chickens harbor multiple strains (*8*), that pâté is prepared from multiple livers (*5*,*6*), or both. We describe epidemiologic evidence for a duck liver pâté–associated outbreak and compare sequence types (STs) of isolates with animal and food isolate datasets.

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The Study

The outbreak involved a group of 3 persons and a group of 29 persons who ate lunch at a catering college restaurant. A probable case-patient was defined as a restaurant diner with diarrhea onset within 7 days after eating at the restaurant on May 12, 2011. Infections were confirmed by laboratory test results.

Environmental health officers inspected the restaurant kitchen and reviewed food preparation processes on May 17. The lunches had been ordered in advance, and officers recorded the food choices made by each diner. Menu choices and occurrence of illness were verified by face-to-face interviews (22 diners), postal interviews (9 diners), and other diners for 1 diner who had died. When food consumption history differed from the diner's lunch order, which occurred mainly through sharing of food, consumption history was used. Fisher exact test p-values and odds ratios with CIs were calculated for the association of each menu option with illness. All case-patients reported exposure to pâté. Lower CIs were estimated by using the Cornfield method in Stata 11 (StataCorp LP, College Station, TX, USA). Repeat analysis was restricted to patients with laboratory-confirmed illness and those who were not ill.

Symptomatic patients were requested to provide fecal samples. In addition, a sample of duck liver, not from the batch used to prepare the meals in question, was obtained from the supplier on June 13 and tested for *Campylobacter* spp. by using 25 g of sample cultured on Campylobacter Blood-Free Selective Agar Base after enrichment in Bolton broth (Oxoid, Basingstoke, UK). Multilocus sequence typing was performed by using standard methods. STs for samples from case-patients and the liver sample were compared with those of published isolates from chickens (mainly sampled in the United Kingdom during 2001–2005) (*9*,*10*), farmed ducks (sampled in the United Kingdom, 2007) (*11*), wild ducks (sampled in the United Kingdom, 2007) (*11*), and wild geese (sampled in the United Kingdom, 2002–2004) (*12*) by using a neighbor-joining algorithm and default parameters in MEGA (www.megasoftware.net) as described (*13*).

Of the 32 diners, 18 (56%) reported diarrhea: 8 had laboratory-confirmed campylobacteriosis, 6 had samples that were negative for *Campylobacter* infection, and 4 were not tested (Figure 1). Median duration of illness was 4 days; 1 case-patient died. Five case-patients described severe diarrhea (profuse, explosive, uncontrollable, or watery), 5

reported fever or shivering, and 2 reported abdominal pain. Consumption of duck liver pâté was strongly associated with illness. No other positive associations were identified (Table). When analysis was restricted to confirmed cases, campylobacteriosis was strongly associated with pâté (lower CI of odds ratio 5.5; p = 0.001).

Through review of cooking processes, we found that ≈1 kg of duck livers was seared and flambéed in batches without ensuring that adequate internal cooking temperatures were achieved. The seared livers were blended with other ingredients and chilled. No other high-risk ingredients or processes were identified. No illness among staff members was recorded on or immediately preceding May 12. A catering student who made and tasted the pâté became ill on May 16. No food samples remained.

Campylobacter isolates were available from 6 of 8 confirmed case-patients and the duck liver. One isolate was positive for *C. coli* and 5 for *C. jejuni*. The *C. jejuni* STs were ST356 (3 cases), ST50, and ST607. These STs are genetically diverse (Figure 2), but each clustered with chicken and farmed duck rather than wild waterfowl isolates. The duck liver isolate, ST5097, clustered with wild waterfowl isolates (Figure 2).

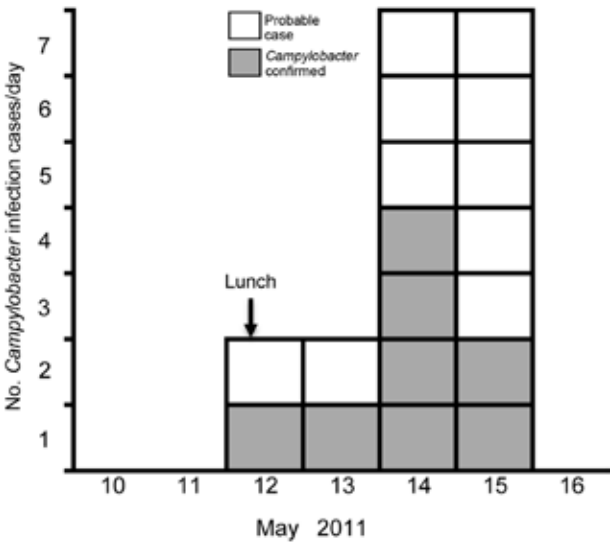

Figure 1. Onset dates of diarrheal illness related to a duck liver–associated outbreak of campylobacteriosis among humans, United Kingdom, 2011. Symptoms recorded with or without laboratory confirmation of *Campylobacter* infection, among persons eating lunch at a catering college restaurant on May 12, 2011. Vertical arrow indicates exposure date.

Conclusions

The attack rate of 86% among persons who ate duck liver pâté was similar to rates for outbreaks associated with chicken liver pâté (*5*,*6*). Pâté consumption was strongly associated with illness and laboratory-confirmed infection. Diners who did not eat this dish were unaffected. Pan frying of chicken livers is effective for killing internal *Campylobacter* spp. if the internal temperature reaches 70°C and is sustained for at least 2 minutes and if total cooking time is at least 5 minutes (*14*). The cooking process for the pâté, as reviewed by environmental health officers, was insufficient to kill bacteria inside the livers. This finding corroborates the epidemiologic evidence.

Aseptic testing of 30 chicken livers showed internal infection in 90% (*14*); testing of 50 chicken and 50 duck livers identified *Campylobacter* spp. contamination in 20 and 18, respectively (*15*). The high level of internal and external contamination in chicken liver in these studies and failure of insufficient cooking to destroy the bacteria in the current outbreak suggest that internal contamination of duck liver also occurs. Undercooked duck liver may therefore present a hazard similar to that presented by undercooked chicken liver. Cooking time should be sufficient to destroy bacteria throughout the liver. Deliberate undercooking was identified in 68% of 25 poultry liver–associated campylobacteriosis outbreaks that occurred during 1992–2009

Table. Association between food consumed and campylobacteriosis among diners at a catering college restaurant, United Kingdom, 2011

Food item	Foods diners ate		Foods diners did not eat		Attack rate, %	Odds ratio*	p value†
	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic			
Starters							
Duck liver pâté	18	3	0	11	86	– (12.7–∞)	<0.001
Vegetable broth	2	11	16	3	15	0.030 (0.005–0.200)	<0.001
Main courses							
Pot roasted breast of lamb	12	9	6	5	57	1.1 (0.3–4.8)	1.00
Poached plaice in white wine sauce	5	5	13	9	50	0.7 (0.2–3.1)	0.71
Vegetarian polenta romaine	1	0	17	14	100	– (0.0–∞)	1.00
Desserts							
Vanilla gateaux chantilly	12	9	6	5	57	1.1 (0.3–4.8)	1.00
Chocolate pudding soufflé	5	5	13	9	50	0.7 (0.2–3.1)	0.71
Cheese	1	0	17	14	100	– (0.0–∞)	1.00

*95% Cornfield CIs are in parentheses. Where odds ratio is undefined, lower CI is presented.
†By Fisher exact test.

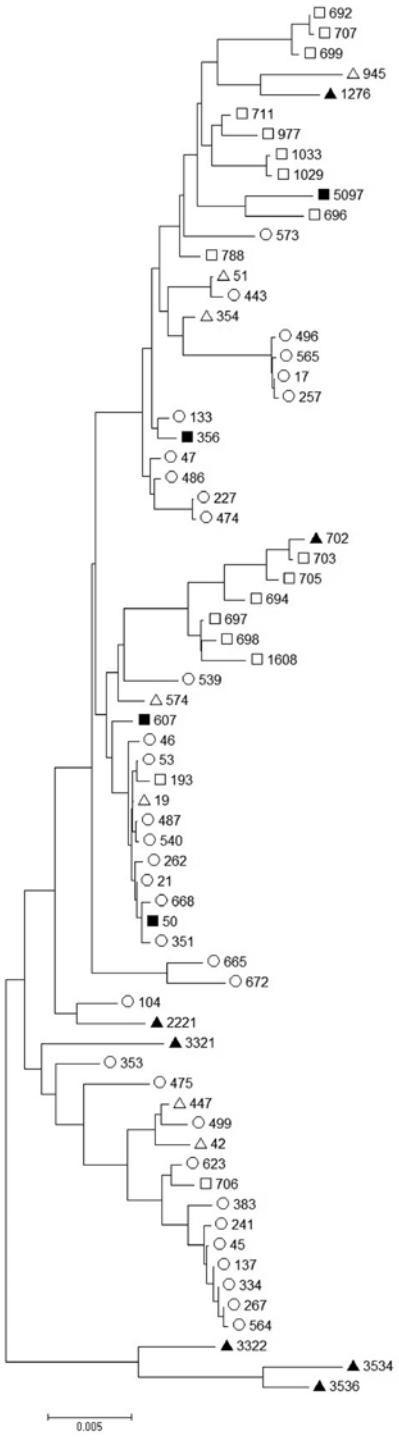

Figure 2. Comparison of *Campylobacter jejuni* sequence types (STs) from a duck liver–associated outbreak of campylobacteriosis among humans in the United Kingdom during 2011 (solid squares) with published sequence types of isolates from chicken (hollow circles) (*9,10*), domesticated duck (hollow triangles) (*11*), wild duck (solid triangles) (*11*), and wild geese (hollow squares) (*12*). ST5097 was isolated from a duck liver sample, ST356 from 3 case-patients, and ST50 and ST607 each from 1 case-patient. Scale bar indicates nucleotide substitutions per site.

(*4*). Outbreaks associated with chicken and duck liver pâté and parfait are being increasingly identified in the United Kingdom and are likely to occur in other countries because the cooking procedures described in the United Kingdom outbreaks are not based on recipes restricted to the United Kingdom. Sporadic cases associated with similar home cooking of poultry liver products are also likely to occur, but such cases will be difficult to identify unless specifically sought.

The diversity of isolates in this outbreak resembles that in an outbreak of campylobacteriosis related to chicken liver pâté (*6*). As with that outbreak, the diversity in the outbreak in this study could reflect individual livers coinfected with >1 *Campylobacter* strain, >1 infected liver in the food item, or both. This diversity suggests that bacterial invasion of chicken and duck livers is possible for a wide range of fairly distantly related *Campylobacter* spp. strains, including those of *C. jejuni* and *C. coli*. The clustering of *C. jejuni* isolates from this outbreak with STs associated with farmed duck and farmed chicken and the genetic separation from wild duck and wild goose isolates (Figure 2) suggests that the farm environment may favor some *Campylobacter* spp. subtypes sufficiently to overcome natural host associations. An alternative hypothesis is that among a wide range of subtypes infecting ducks, those that are found in other farm animals are more effective at causing human disease. The single *Campylobacter* isolate from a later, non–outbreak-associated batch of duck liver clustered with isolates from wild waterfowl rather than the outbreak isolates or other isolates from farmed ducks. The limited data on *Campylobacter* populations in poultry other than chickens restrict our ability to interpret this discrepancy. Further work to characterize the *Campylobacter* populations of wild and farmed ducks may facilitate more reliable inference.

Acknowledgments

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etymologia

Campylobacter [kam″pə-lo-bak′tər]

From the Greek *kampylos* (curved) and *baktron* (rod), a genus of gram-negative curved or spiral rods that is among the most common causes of foodborne diarrheal illness worldwide. Illness caused by *Campylobacter* spp. was first described by Theodor Escherich in 1886, but they were not successfully isolated from human fecal samples until 1972. For many years, they were classified among the vibrios, but Sebald and Véron proposed the genus *Campylobacter* in 1963 for these "slender, curved bacilli" that differ from the classical cholera and halophilic vibrios.

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Diarrhetic Shellfish Poisoning, Washington, USA, 2011

Jennifer K. Lloyd, Jeffrey S. Duchin, Jerry Borchert, Harold Flores Quintana, and Alison Robertson

Diarrhetic shellfish poisoning is a gastrointestinal illness caused by consumption of bivalves contaminated with dinophysistoxins. We report an illness cluster in the United States in which toxins were confirmed in shellfish from a commercial harvest area, leading to product recall. Ongoing surveillance is needed to prevent similar illness outbreaks.

Diarrhetic shellfish poisoning (DSP) is an acute gastrointestinal illness caused by consumption of bivalve mollusks that have accumulated okadaic acid (OA) or related dinophysistoxins through filter feeding. DSP toxins are produced by several species of marine dinoflagellates from the genera *Dinophysis* and *Prorocentrum* (*1*–*4*). Symptoms of DSP include nausea, abdominal pain, vomiting, diarrhea, headache, chills, and fever (*5*). Onset occurs 0.5–4 hours after consumption of contaminated food, and symptoms last up to 72 hours; treatment is supportive. To date, no sequelae have been reported, but speculation has suggested that chronic exposure may increase risk for gastrointestinal cancers (*6,7*).

The earliest clinical reports of DSP were from the Netherlands in 1961; however, DSP toxins were structurally elucidated >15 years later in Japan (*2*,*8*,*9*). DSP illnesses have since been documented worldwide. In the United States, sporadic DSP-like illnesses have been recorded on the East Coast since 1980, coinciding with detection of toxin-producing dinoflagellates in shellfish beds (*2*,*4*). In 2002, shellfish beds in Chesapeake Bay, Virginia, were briefly closed because *Dinophysis* spp. dinoflagellates were detected, although hazardous DSP toxin levels were not detected and no illnesses were reported (*10*). More recently, in Texas, harvest areas were closed for >1 month following a large *Dinophysis* bloom that contaminated oyster beds with OA in excess of the Food and Drug Administration (FDA) regulatory guidance level; no illnesses were reported (*11*,*12*).

In the Pacific Northwest, *Dinophysis* spp. dinoflagellates, predominantly *D. acuminata,* have been observed for many years (*13*). During 2010, the Washington Department of Health (WDOH) and FDA Gulf Coast Seafood Laboratory (FDA-GCSL, Dauphin Island, AL) initiated a pilot program to gather baseline monitoring data on *Dinophysis* species abundance and associated DSP toxins in shellfish from 18 growing areas (Figure 1). During this pilot study, shellfish were collected for toxin analysis when *Dinophysis* light microscopy counts exceeded 3,000 cells/L for ≥2 consecutive weeks. During 2010, *Dinophysis* counts were reported above threshold at 15 sites, and >50 shellfish samples were analyzed for DSP toxins. All were below the FDA guidance level for total OA equivalents (free OA, DTX-1, DTX-2, and acyl esters) of 16 μg/100 g shellfish tissue. On the basis of these data, monitoring was scaled down to 5 field sites for ongoing cell monitoring and toxin analysis in 2011.

We describe a cluster of DSP illnesses that occurred in 2011 in which shellfish from the implicated harvest area exceeded the FDA regulatory guidance level for DSP toxins. This cluster resulted in closure of harvest areas and a recall of commercial shellfish product.

The Study

In July 2011, Public Health–Seattle & King County received a report from WDOH of a family who experienced illness after consuming recreationally harvested mussels. Interviews were conducted to characterize the illness, determine the location of harvest, and describe preparation of the shellfish meal. Three family members, ages 2, 5, and 45 years, experienced symptoms beginning 4, 7, and 14 hours after consumption of cooked mussels, respectively. A fourth adult family member who consumed mussels did not become ill. Signs and symptoms included vomiting, diarrhea, body aches, fever, and chills; no neurologic symptoms were described. The average duration of vomiting and diarrhea was 3 and 52 hours, respectively. All ill persons recovered within 96 hours; no medications were taken, and medical care was not sought.

The family collected mussels from a public dock at Sequim Bay State Park, Sequim, Washington, on June 29, 2011. The mussels were stored in seawater until they were cooked 2 hours later. The mussels were boiled in water, wine, herbs, and butter for 10 minutes until the shells opened, then consumed immediately. The case-patients each consumed 8–15 mussels; the family member who did not become ill consumed 4 mussels.

Although a meal remnant was not available, the dock from which the family collected mussels was a 2011 DSP monitoring site. Eleven composite samples of mussels

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(*Mytilus trossulus*) representing a minimum of 20 individuals had been collected before and after the collection date of the outbreak. Samples were received by FDA-GCSL on August 2, 2011, and hydrolyzed extracts were analyzed by using liquid chromatography tandem mass spectrometry (*14*). Analyses were performed under acidic chromatographic conditions and performed in negative-ion mode by multiple-reaction monitoring. Matrix dilution experiments were performed to ensure that sample matrix did not suppress or enhance ionization during the analyses. Total OA equivalents (free OA, DTX-1, DTX-2, and acyl esters of OA, DTX-1, and DTX-2) were quantified by external standard calibration with OA. Certified reference standards (OA, DTX-1, and DTX-2) from the National Research Council (Halifax, Nova Scotia, Canada) were used for verification of retention time and equimolar response. Nine mussel samples exhibited toxin levels above the FDA guidance threshold, ranging from 37.6 μg–160.3 μg total OA equivalents per 100 g. In all cases, DTX-1 was identified as the principal DSP toxin. These elevated DSP toxin levels followed observed *Dinophysis* blooms (Figure 2).

On the basis of the analytical results, a recall was initiated for clams and oysters harvested after August 1, 2011, from the commercial growing area adjacent to Sequim Bay State Park; no mussels were harvested commercially. The park and commercial site remained closed until 2 consecutive shellfish samples collected 7–10 days apart demonstrated total DSP toxin levels <16 μg/100 g. Clams and oysters were cleared for commercial harvest on September 2, 2011; recreational mussel harvest was allowed starting in late October. During these closures, WDOH issued a press release and posted information online describing the dangers of DSP. Warning signs instructing visitors not to collect shellfish for consumption were posted at affected beaches. Similar warnings were issued on the WDOH biotoxin hotline and online maps of shellfish harvest areas (www.doh.wa.gov/shellfishsafety.htm). Surveillance for DSP illnesses is ongoing; at the time this article was written, no additional reports had been received.

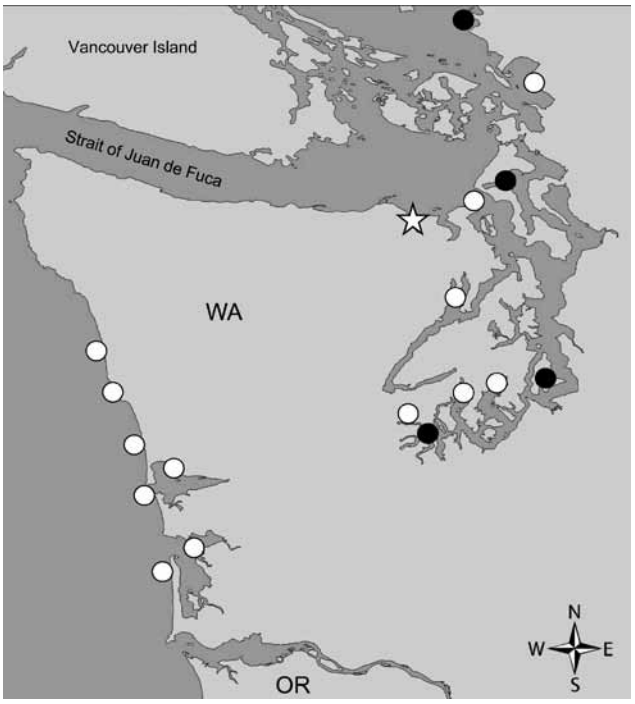

Figure 1. Diarrhetic shellfish poisoning toxin monitoring sites, Washington, USA, 2010–2011. Eighteen sites were monitored during the 2010 pilot study (open circles); 5 pilot sites were selected for continued monitoring during 2011 (solid circles). Sequim Bay State Park (star), the site implicated in the human illnesses described in this article, was among the sites monitored in 2011.

Conclusion

We describe a cluster of DSP illnesses in the US Pacific Northwest with confirmation of DSP toxins in shellfish from the implicated harvest area. Mussels contained levels of DSP toxin 2–10 times the guidance level, resulting in closure of recreational and commercial areas. Coincidentally, roughly 60 DSP illnesses occurred in July–August 2011 in British Columbia, Canada, and were traced to Pacific Coast mussels (*15*).

Although *Dinophysis* spp. dinoflagellates have been found in Pacific Coast waters for many years, illnesses consistent with DSP have not been reported. Research is ongoing to determine why elevated toxin levels are being observed in the region now. Underreporting of DSP is possible because of the nonspecific nature of the illness. To detect cases of DSP and other shellfish-related illnesses, clinicians should inquire about shellfish consumption preceding onset of symptoms. Ill patients with a history of shellfish consumption should be reported to public health authorities immediately to prevent further illnesses.

Liquid chromatography tandem mass spectrometry is a preferred method for FDA regulatory testing of marine biotoxins because it provides quantification and unambiguous identification of toxin congeners. WDOH is now equipped with this instrumentation and received FDA training in chemical methods for DSP toxins, which allows local monitoring of ≥40 shellfish growing areas on a regular basis. Such surveillance efforts are critical for early warning of toxicity and prompt response to this emerging public health issue. Relationships with and frequent communication between public health and shellfish program staff regarding human illness, increases in bloom frequency, and hazardous levels of DSP toxins in locally harvested shellfish will facilitate preventions of additional illnesses.

Acknowledgments

We thank Kathryn MacDonald for supporting this investigation. We also thank collaborators on the 2010 DSP Pilot

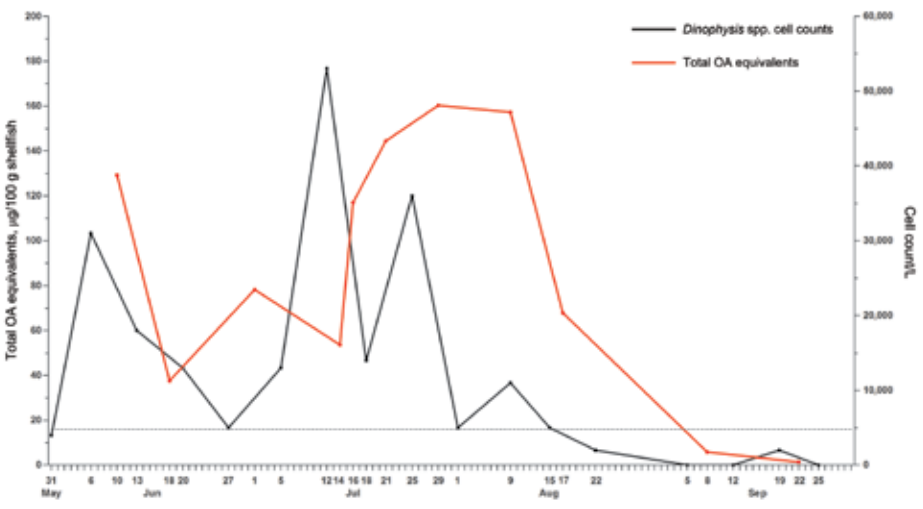

Figure 2. Timeline comparing blooms of *Dinophysis* spp. dinoflagellates and diarrhetic shellfish poisoning toxin levels detected in mussels collected during 2011 from Sequim Bay State Park, Sequim, Washington, USA. *Dinophysis* spp. cell counts per liter (black line) were determined by using light microscopy. Total okadaic acid (OA) equivalents (red line), in micrograms per 100 g shellfish tissue, were determined by using liquid chromatography mass spectrometry analysis (*14*). Dashed line indicates US Food and Drug Administration guidance level of 16 µg total OA equivalents per 100 g shellfish tissue. Dates shown are collection dates for each tested sample.

Project, including the Quinault Nation, the Olympic Region Harmful Algal Bloom partnership, Washington State Department of Fish and Wildlife, the Pacific Shellfish Institute, the Port Townsend Marine Science Center, the Jamestown S'Klallam Tribe, Coast Shellfish, Taylor Shellfish, and citizen volunteers in the SoundToxins program.

Ms Lloyd is an epidemiologist in the Communicable Disease Epidemiology and Immunization Section of Public Health–Seattle & King County. Her interests include foodborne disease epidemiology and the practical application of public health surveillance data.

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Genotype GI.6 Norovirus, United States, 2010–2012

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We report an increase in the proportion of genotype GI.6 norovirus outbreaks in the United States from 1.4% in 2010 to 7.7% in 2012 (p<0.001). Compared with non-GI.6 outbreaks, GI.6 outbreaks were characterized by summer seasonality, foodborne transmission, and non–health care settings.

Noroviruses are the leading cause of epidemic gastroenteritis, including foodborne outbreaks, and a major cause of sporadic gastroenteritis in the United States (*1*–*3*). Hospitalizations and deaths associated with norovirus infection occur most frequently among elderly persons, young children, and immunocompromised persons (*2*). Noroviruses can be divided into at least 5 genogroups (GI–GV) and at least 35 genotypes. Human disease is primarily caused by GI and GII noroviruses, and most norovirus outbreaks are caused by genotype GII.4 viruses (*5*). During the past decade, new GII.4 strains have emerged every 2–3 years, replacing previously predominant GII.4 strains (*6*–*8*). GI noroviruses are relatively uncommon, and systematic descriptions of GI outbreak epidemiology and characteristics are scarce (*9*). Before 2010, genotype GI.6 noroviruses were rarely reported in the United States; ≤5 GI.6 outbreaks were reported each year to the Centers for Disease Control and Prevention (J. Vinjé, pers. comm.). We report the emergence of GI.6 norovirus as a cause of outbreaks in the United States and discuss its effect on public health.

The Study

Since 2009, the Centers for Disease Control and Prevention has operated 2 surveillance systems for norovirus outbreaks in the United States: CaliciNet and the National Outbreak Reporting System (NORS). CaliciNet is an electronic laboratory surveillance network that collects information on genetic sequences of noroviruses implicated in outbreaks (*5*). As of 2011, public health laboratories in all 50 states are either certified members of CaliciNet or submit norovirus-positive specimens to 1 of 5 regional CaliciNet Outbreak Support Centers. NORS is an electronic surveillance system for reporting all enteric disease outbreaks, regardless of etiology or mode of transmission (*3*). Data reported in NORS include outbreak characteristics, demographics, symptoms, implicated exposures, clinical outcomes, and etiologies

We identified GI.6 outbreaks with a first illness onset date during January 1, 2010–December 31, 2012, from CaliciNet and linked them to NORS by using unique outbreak identification numbers. Supplemental information derived from NORS included mode of transmission, outbreak setting, and patients' demographic features and clinical outcomes. State health departments were queried about outbreaks that could not be linked to NORS and requested to provide such supplemental information directly. Annual variation in GI.6 outbreaks was assessed by χ^2 test for trend, and GI.6 seasonality was identified on the basis of visual examination of trends over time and compared with non-GI.6 seasonality by using Mid-P exact test. Norovirus-positive specimens were typed by using region D sequence analysis (*5*) (Figure 1).

A total of 141 GI.6 outbreaks in 27 states were identified over the 3-year study period. During 2010 and 2011, causitive strains for 12 (1.4%) of 879 and 30 (3.9%) of 760 outbreaks, respectively, reported through CaliciNet were typed as GI.6. During 2012, 99 (7.7%) of the 1,279 norovirus outbreaks reported through CaliciNet tested positive for GI.6, indicating a significant increase in genotype GI.6 outbreaks over the 3-year period (Figure 2; p<0.001). During 2010–2012, a total of 66 (46.8%) of 141 GI.6 outbreaks occurred during April–July, compared with 382 (13.8%) 2,777 non-GI.6 outbreaks (p<0.001).

The most commonly identified mode of transmission was person-to-person, which occurred in 81 (57.4%) GI.6 outbreaks (Table 1). Foodborne transmission was more frequent among GI.6 than among non-GI.6 outbreaks reported to CaliciNet during the same period (rate ratio [RR] 1.77, 95% CI 1.25–2.51). Waterborne transmission also was more common in GI.6 outbreaks; the 2 waterborne GI.6 outbreaks occurred during June and July.

The most commonly reported outbreak setting was long-term–care facilities, representing 51 (36.2%) outbreaks. GI.6 outbreaks were reported less frequently in health care–related settings (hospitals and long-term–care facilities) than were non-GI.6 outbreaks (36.9% vs. 65.7%; RR 0.56, 95% CI 0.45–0.70).

GI.6 outbreaks accounted for 4,375 reported illnesses, with a median of 22 (range 2–178) reported illnesses per outbreak. Supplementary demographic and clinical outcome information was available for 66 (46.8%) outbreaks, comprising 2,220 reported illnesses. Data on hospitalization and death were provided for most (>79.0%) of these illnesses; other patient characteristics were reported less frequently (Table 2). Most (52.2%) patients were male, and

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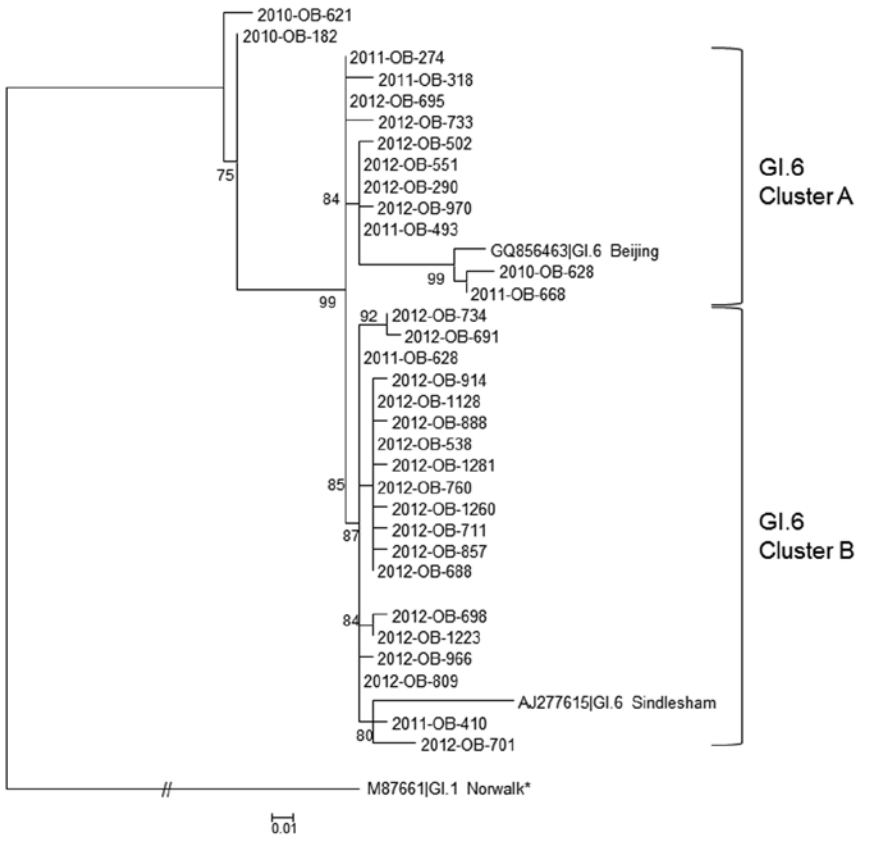

Figure 1. Phylogenetic typing results for GI.6 noroviruses, United States, 2010–2012. Representative outbreak nucleotide sequences were genotyped by region D (*5*). Sequences were downloaded, trimmed, and analyzed as described (*5*). In brief, a 3-parameter model, TPM1, with equal frequencies and invariable sites was run in PhyML 3.0 (www.atgc-montpellier.fr/phyml/binaries.php) as determined by jModel test by using the corrected Akaike information criterion. The best of 5 random trees was used to start the analysis, and the approximate likelihood ratio test was used for branch support. GI.6 reference sequences (GenBank accession nos. GQ856463| GI.6 Beijing and AJ277615| GI.6 Sindlesham) were included. Two clusters of genetically related outbreaks (cluster A and cluster B) are marked by brackets. *The distance of GI.1 Norwalk to the nearest GI.6 cluster is 2.29 substitutions per site. Scale bar indicates nucleotide substitutions per site for the phylogenetic tree.

22.2% were >75 years of age; 1.4% of GI.6 outbreak patients were hospitalized, and 0.2% died.

Molecular typing data demonstrated that GI.6 viruses can be grouped into 2 clusters (Figure 1), with earlier outbreaks occurring deeper in the tree. One of the earliest occurring outbreaks in cluster A (2011-OB-274) occurred in Tennessee in February 2011 and involved a conference with 8,000 attendees and 143 reported cases in persons from 12 states.

Conclusions

We detected an increase in GI.6 outbreaks in the United States since 2010, with peak activity during summer 2012. Summer seasonality, foodborne transmission, and non–health care settings characterized GI.6 outbreaks, compared with non-GI.6 outbreaks reported through CaliciNet. Noroviruses are the most common cause of gastroenteritis outbreaks, and although GI.6 noroviruses remain responsible for a relatively small proportion of all reported norovirus outbreaks, they have unique characteristics and public health implications that differ from those of more common genotypes.

During 2010–2012, genotype GII.4 consistently represented most (70%) of the norovirus outbreaks reported through CaliciNet (J. Vinjé, pers. comm.). Therefore,

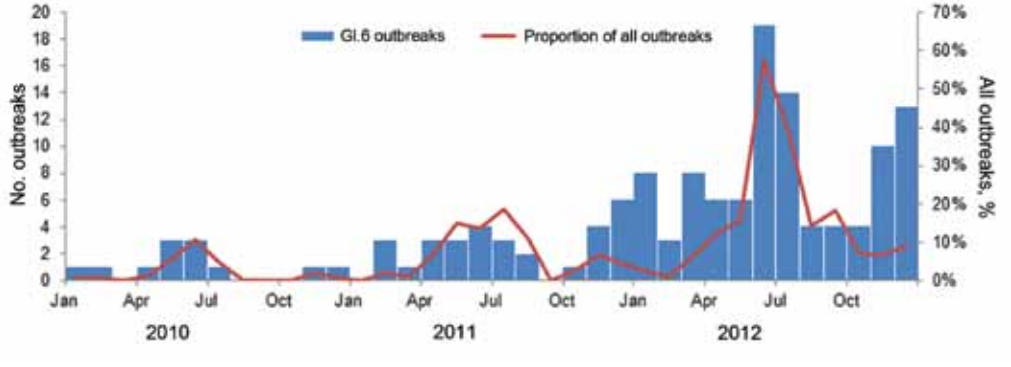

Figure 2. GI.6 norovirus outbreaks reported by CaliciNet, United States, 2010–2012.

Table 1. Mode of transmission and setting for Norovirus outbreaks reported through CaliciNet, United States, 2010–2012

Characteristic	GI.6 outbreaks, no. (%) n = 141	Non-GI.6 outbreaks, no. (%), n = 2,777	Rate ratio (95% CI)
Mode of transmission			
Person-to-person	81 (57.4)	1,701 (61.2)	0.94 (0.81–1.08)
Foodborne	28 (19.9)	311 (11.2)	1.77 (1.25–2.51)
Waterborne	2 (1.4)	2 (0.1)	19.70 (2.80–138.80)
Other	0	15 (0.5)	Not applicable
Unknown	30 (21.3)	748 (26.9)	0.79 (0.57–1.09)
Setting			
Long-term–care facilities	51 (36.2)	1,715 (61.8)	0.59 (0.47–0.73)
Schools or daycare centers	23 (16.3)	198 (7.1)	2.29 (1.54–3.40)
Restaurants	20 (14.2)	258 (9.3)	1.53 (1.00–2.33)
Parties and events	17 (12.1)	153 (5.5)	2.19 (1.37–3.51)
Cruise ships	4 (2.8)	71 (2.6)	1.11 (0.41–3.00)
Hospitals	1 (0.7)	109 (3.9)	0.18 (0.03–1.29)
Other non–health care settings	20 (14.2)	202 (7.3)	1.95 (1.27–2.99)
Unknown	5 (3.5)	71 (2.6)	1.39 (0.57–3.38)

the unique characteristics of GI.6 outbreaks described here primarily reflect differences between GI.6 and GII.4 noroviruses.

The absolute number of outbreaks and the proportion of outbreaks caused by GI.6 noroviruses peaked during April–July. This summer seasonal pattern contrasts with the overall winter seasonality of norovirus outbreaks driven primarily by winter surges in GII.4 norovirus activity (*3*,*6*–*8*). A study of GI norovirus outbreaks in Australia demonstrated peak outbreak activity during their summer months, compared with a late winter peak for GII norovirus outbreaks (*9*). In a previous study in the United States, the highest number of GI outbreaks occurred during April–May, but no apparent seasonality was noted (*7*).

Comparisons of hospitalization and death rates reported during GI.6 outbreaks with those observed in recent outbreaks caused by the emergent GII.4 Sydney strain (*8*) indicated slightly lower rates of hospitalization (1.4% vs. 2.2%; RR 0.63, 95% CI 0.39–1.02) and death (0.2% vs. 0.4%; RR 0.44, 95% CI 0.12–1.62). This observation may reflect a previously described association of GII.4 outbreaks with severe outcomes (*10*).

Region D typing data presented in this study indicates 2 clusters of GI.6 noroviruses in the United States. In February 2011, an outbreak among persons from multiple states occurred at a conference in Tennessee; this outbreak might have been a dissemination event for GI.6 activity because outbreaks of genetically related GI.6 noroviruses belonging to the same cluster occurred later in several of the states in which the conference attendees resided. However, more sequence information from the complete open reading frame (ORF2) or the hypervariable region of the protruding domain (P2) is needed to confirm possible links among the outbreaks (*5*).

Our study has several limitations. These include incomplete linkage of outbreaks reported in CaliciNet to outbreak reports in NORS and the resulting gaps in data on transmission mode and setting, as well as low rates of reporting of demographic characteristics, symptoms, and clinical outcomes. These limitations preclude direct comparison of GI.6 outbreak characteristics with characteristics of outbreaks linked to other genotypes. Efforts to improve reporting rates and integration between CaliciNet and NORS are under way (*8*).

Noroviruses are a diverse group of pathogens with varied characteristics. Continued surveillance for norovirus outbreaks through CaliciNet and NORS will enable further assessment of the public health implications and significance of emergence of relatively rare noroviruses, such as GI.6. Proper hand hygiene, environmental disinfection, and isolation of ill persons remain the mainstays of norovirus prevention and control (*11*).

Table 2. Characteristics of case-patients in outbreaks of acute gastroenteritis caused by GI.6 norovirus, United States, 2010–2012*

Characteristic	No. affected/total (%)
Sex	
M	465/890 (52.2)
F	425/890 (47.8)
Age, y	
<5	8/802 (1.0)
5–9	30/802 (3.7)
10–19	345/802 (43.0)
20–49	166/802 (20.7)
50–74	75/802 (9.4)
≥75	178/802 (22.2)
Outcome	
Outpatient visit	50/946 (5.3)
Emergency department visit	14/966 (1.4)
Hospitalization	24/1,753 (1.4)
Death	3/1,762 (0.2)

*Includes 66 GI.6 outbreaks (2,220 ill persons) for which at least partial supplementary data were available through the National Outbreak Reporting System or directly from state health departments.

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Detection of Novel Rotavirus Strain by Vaccine Postlicensure Surveillance

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Surveillance for rotavirus-associated diarrhea after implementation of rotavirus vaccination can assess vaccine effectiveness and identify disease-associated genotypes. During active vaccine postlicensure surveillance in the United States, we found a novel rotavirus genotype, G14P[24], in a stool sample from a child who had diarrhea. Unusual rotavirus strains may become more prevalent after vaccine implementation.

Active vaccine postlicensure surveillance for rotavirus-associated diarrhea is informative for determination of vaccine effectiveness and for characterization of disease-associated rotavirus genotypes (*1*–*5*). Most rotaviruses circulating in the United States belong to a limited number of strains, routinely characterized by serologic or genetic identification of the outer capsid protein antigens viral protein (VP) 7, which defines G types, and VP4, which defines P types (*6*,*7*). Of circulating strains in the United States, 85% contain a G or P antigen that is included in both US-licensed rotavirus vaccines (*2*,*8*). However, >70 G and P antigen combinations have been reported, and uncommon strains may suddenly appear in a new geographic area (*1*,*2*,*5*,*9*). Ongoing active surveillance is conducted through the Centers for Disease Control and Prevention's New Vaccine Surveillance Network, a prospective, population-based surveillance program for acute gastroenteritis among children <5 years old, the details of which have been published (*3*–*5*). This surveillance has detected the emergence of G12P[8] and G9P[8] rotavirus genotypes, as well as 3 reported instances of US children infected with G8P[4] rotavirus (*3*–*5*,*10*). During the 2009 winter season (December 2008–June 2009) in Rochester, New York, 54 (30%) of 183 enrolled children with acute gastroenteritis had rotavirus infection. Fifty (94%) of 51 rotavirus strains were typical US strains, with G or P antigens contained in the licensed rotavirus vaccines; 3 were G8P[4] (*10*). One strain, however, appeared to be an unusual reassortant not previously reported in human infection. We describe this novel rotavirus genotype, G14P[24], found along with enteric adenovirus in a stool sample from a child with diarrhea.

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The Study

A 36-month-old girl was brought to the emergency department of the Golisano Children's Hospital at University of Rochester Medical Center with a 4-day history of emesis (2 times/day) and low-grade fever (37.7°C). During the previous 2 days, she also had experienced diarrhea (8 loose stools/day) and lethargy. Physical examination was only remarkable for mild dehydration; there were no other abnormalities. She was previously healthy, born after a full-term gestation, and breast-fed for the first 7–12 months of life. The child had not been vaccinated against rotavirus. She lived in the Rochester metropolitan area and had no unusual dietary or travel exposures. She had contact with pet dogs and cats at home and at a childcare setting. Approximately 1 month before her illness, she had visited a petting zoo at which farm animals but no nonhuman primates were present; her mother could not remember whether horses or cows were present but recalled the child petting sheep.

After oral rehydration, the child's activity increased, and she was discharged to home. She continued to experience intermittent emesis and diarrhea for 1 month, although she maintained her weight. No other family members (1 sibling, 2 parents) became ill.

The child was enrolled, with parental informed consent, into the New Vaccine Surveillance Network. A stool sample taken during the hospital visit was positive for rotavirus antigen by enzyme immunoassay (Premier Rotaclone; Meridian Bioscience, Inc., Cincinnati, OH, USA). The specimen was analyzed at the Centers for Disease Control and Prevention by transmission electron microscopy, reverse transcription PCR genotyping, and nucleotide sequencing, as described (*11*). Electron microscopy showed 2 types of virions, 1 characteristic of rotavirus and 1 of enteric adenovirus (Figure 1).

Analyses of VP7 and VP4 sequences using RotaC 2.0 (*12*) identified the rotavirus strain as genotype G14P[24] (*6*,*7*) (Figure 2). Phylogenetic analyses indicated monophyly of the VP7 gene with an equine rotavirus strain from Argentina and clustering of the VP4 gene with the simian rotavirus strain TUCH (Figure 2). The novel strain was designated as RVA/Human-wt/USA/2009727118/2009/G14P[24], in accordance with guidelines from the

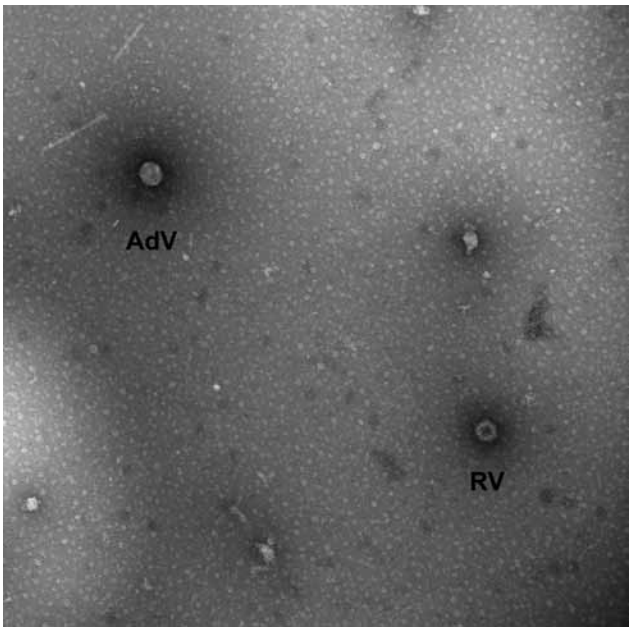

Figure 1. Transmission electron micrograph image of stool sample from 36-month-old child with diarrhea, showing viral particles characteristic of rotavirus (RV) and enteric adenovirus (AdV Magnification ×92,300. Image courtesy of Charles Humphrey.

Rotavirus Classification Working Group (*6*,*7*). Full genome sequencing is in progress; the preliminary 11-gene genotype is G14-P[24]-I9-R2-C3-M3-A9-N3-T3-E3-H6, which indicates that this novel strain may be a reassortant containing genes from equine, simian, human, and bovine rotaviruses. In particular, the VP7 gene seems to be most related to equine G14 strains; the VP4 gene, to simian P[24]; the VP1 gene, to bovine R2; and the nonstructural protein 3 gene, to human T3 strains.

Conclusions

Human rotavirus infection is commonly associated with ≈6 of the >70 known human G and P antigen combinations reported among >160 known rotavirus strains (*1*,*6*). The G14P[24] strain we found had not been reported in human infection, but interspecies transmission of both reassorted and nonreassorted animal viruses has been described (*9*). The emergence of unusual reassortant animal strains raises questions about the effectiveness of current rotavirus vaccines, which might share neither G nor P types with such viruses. However, immunity to rotavirus is believed to be polygenic and probably involves antigens in addition to G and P antigens (*14*).

In summary, we identified infection with a novel G14P[24] rotavirus strain in a 36-month-old child with diarrhea. Whether this strain was responsible, entirely or in part, for the child's symptoms is not certain, because enteric adenovirus was also identified. Co-infection with rotavirus and enteric adenovirus has been described, but it is unclear whether such co-infection is associated with more severe gastroenteritis (*15*). Nevertheless, the rotavirus strain we identified appears to be an unusual reassortant containing equine, human, simian, and bovine rotavirus genes. Further study of this and other unusual reassortant rotaviruses may lead to insight on rotavirus evolution. Continued surveillance is critical for assessing whether unusual genotypes of rotavirus become more prevalent after the implementation of rotavirus vaccination.

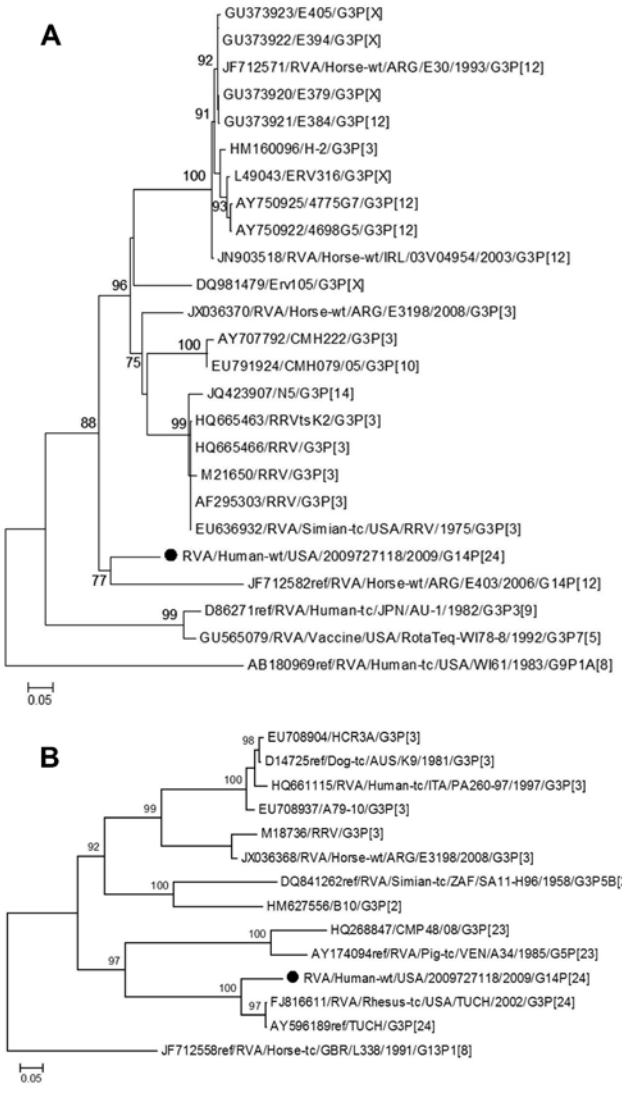

Figure 2. Genetic relationships of partial viral protein 7 (A) and viral protein 4 (B) nucleotide sequences for novel rotavirus strain (black dot) isolated from 36-month-old child with diarrhea compared with representatives of known equine, simian, and human rotavirus genotypes. Evolutionary relationships and distances were inferred by using the maximum-likelihood method in PhyML 3.0 (*13*). Numbers next to nodes are approximate likelihood-ratio test values calculated by PhyML. Rotavirus strain designations, and G and P genotypes are shown. Scale bars indicate number of nucleotide substitutions per site.

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Novel G10P[14] Rotavirus Strain, Northern Territory, Australia

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We identified a genotype G10P[14] rotavirus strain in 5 children and 1 adult with acute gastroenteritis from the Northern Territory, Australia. Full genome sequence analysis identified an artiodactyl-like (bovine, ovine, and camelid) G10-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genome constellation. This finding suggests artiodactyl-to-human transmission and strengthens the need to continue rotavirus strain surveillance.

Group A rotavirus infection is the major cause of acute gastroenteritis in children worldwide. The rotavirus genome consists of 11 segments of double-stranded RNA encoding 6 structural viral proteins (VP1–4, VP6, VP7) and 6 nonstructural proteins (NSP 1–5/6) (*1*). Genotypes are assigned on the basis of 2 outer capsid proteins into G (VP7) and P (VP4) genotypes; these proteins also elicit type-specific and cross-reactive neutralizing antibody responses (*1*). Strains that include genotypes G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] cause most rotavirus disease in humans (*1*). Since 2008, rotaviruses have been classified by using the open reading frame of each gene. The nomenclature Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx represents the genotypes of the gene segments encoding VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 (*2*). To date, 27 G, 35 P, 16 I, 9 R, 9 C, 8 M, 16 A, 9 N, 12 T, 14 E, and 11 H genotypes have been described (*2*).

Two live oral vaccines are available globally: Rotarix (GlaxoSmithKline Biologicals, Melbourne, Victoria, Australia) and RotaTeq (Merck, Whitehouse Station, NJ, USA). Rotarix is a monovalent vaccine that contains a single human G1P[8] strain (*3*). RotaTeq is a pentavalent vaccine comprised of 5 human–bovine reassortant virus strains (*3*). Both vaccines were introduced into the Australian National Childhood Immunization Program in July 2007. The strategy of a rotavirus vaccination program is to target the most frequently circulating rotavirus strain(s) and provide homotypic and heterotypic protection.

G10P[14] rotavirus strains are rarely reported as the source of infection in humans. Of 7 previously reported G10P[4] rotavirus infections, 1 each was in the United Kingdom and Thailand and 5 were in Slovenia (*4*). During 2011, the Australian Rotavirus Surveillance Program identified 6 G10P[14] strains in the Northern Territory (NT). We report the characterization of G10P[14] strains detected in Australia.

The Study

Six rotavirus-positive specimens collected from NT were genetically untypeable by reverse transcription PCR (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/8/12-1653-Techapp1.pdf). Sequence analysis of the VP7 and VP4 genes of these strains demonstrated highest nucleotide identity with G10 and P[14] rotaviruses, respectively. The G10P[14] strains were from specimens collected from 5 children and 1 adult (84 years of age) during August and September 2011 (Table 1). Of the 6 G10P[14] case-patients, 5 were from Tennant Creek, NT, ≈1,000 km south of Darwin in northern Australia; the residence of the other case-patient is unknown. All strains were detected in indigenous Australians. Specimens V585, V582, and WDP280 were collected from case-patients who had received 2 doses of Rotarix, and specimen SA179 was collected from a case-patient who had received 1 dose. No vaccination data were available for the case-patient from whom specimen SA175 was collected.

Sanger sequencing was used to generate the complete genome of specimen V585 (online Technical Appendix). For the other 5 G10P[14] strains, the complete open reading frames of VP7, VP4, NSP4, and NSP5 and partial reading frames of VP1, VP2, VP3, VP6, NSP1, and NSP2 were sequenced (Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/8/12-1653-T2.htm). These 5 strains demonstrated >99.5% sequence identity to V585, confirming that V585 was representative of all 6 strains. The genotype of each segment of V585 was determined by using RotaC version 2.0 (http://rotac.regatools.be), a web-based genotyping tool for group A rotaviruses; a G10-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 constellation was identified. Maximum-likelihood phylogenetic analyses were performed by using full-length open reading frame nucleotide sequences of V585 and other group A rotavirus strains (online Technical Appendix). The nucleotide sequences of the 11 gene segments of V585 and the VP7 and VP4 genes of the other 5 G10P[14] strains were deposited in GenBank (accession nos. JX567748–JX567768).

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[1]These authors contributed equally to this article.

Table 1. Cohort and vaccination status for novel G10P[14] rotavirus strain, Northern Territory, Australia, 2011*

Case-patient specimen ID	Case-patient age	Date specimen collected	Location (postal code) of specimen collection†	Rotavirus vaccine (no. doses)‡
V582	8 mo	Aug 13	0860	Rotarix (2)
WDP280	10 mo	Aug 19	0872	Rotarix (2)
V585	2 y	Aug 19	0860	Rotarix (2)
SA175	3 mo	Sep 2	Unknown	Unknown
SA179	4 mo	Sep 6	0872	Rotarix (1)
D355	84 y	Sep 11	0860	Not applicable

*ID, identification.
†Postal code zone 0860 encompasses Tennant Creek, Northern Territory; samples WDP280 and SA179 were collected in post code zone 0872, in communities near code zone 0860.
‡Rotarix, GlaxoSmithKline Biologicals, Melbourne, Victoria, Australia.

Phylogenetic analysis of the VP7 gene identified 10 lineages (Figure 1, panel A). The 6 G10P[14] strains from NT (lineage IX) were distinct from human G10P[14] rotaviruses RVA/human-tc-GBR/A64/1987/G10P14 (lineage II) and RVA/human-tc/THA/Mc35/1987–1989/G10P[14] (lineage V), and they were most closely related to bovine strains identified predominantly in Ireland, China, and Australia (lineage IV). V585 had the highest level of nucleotide

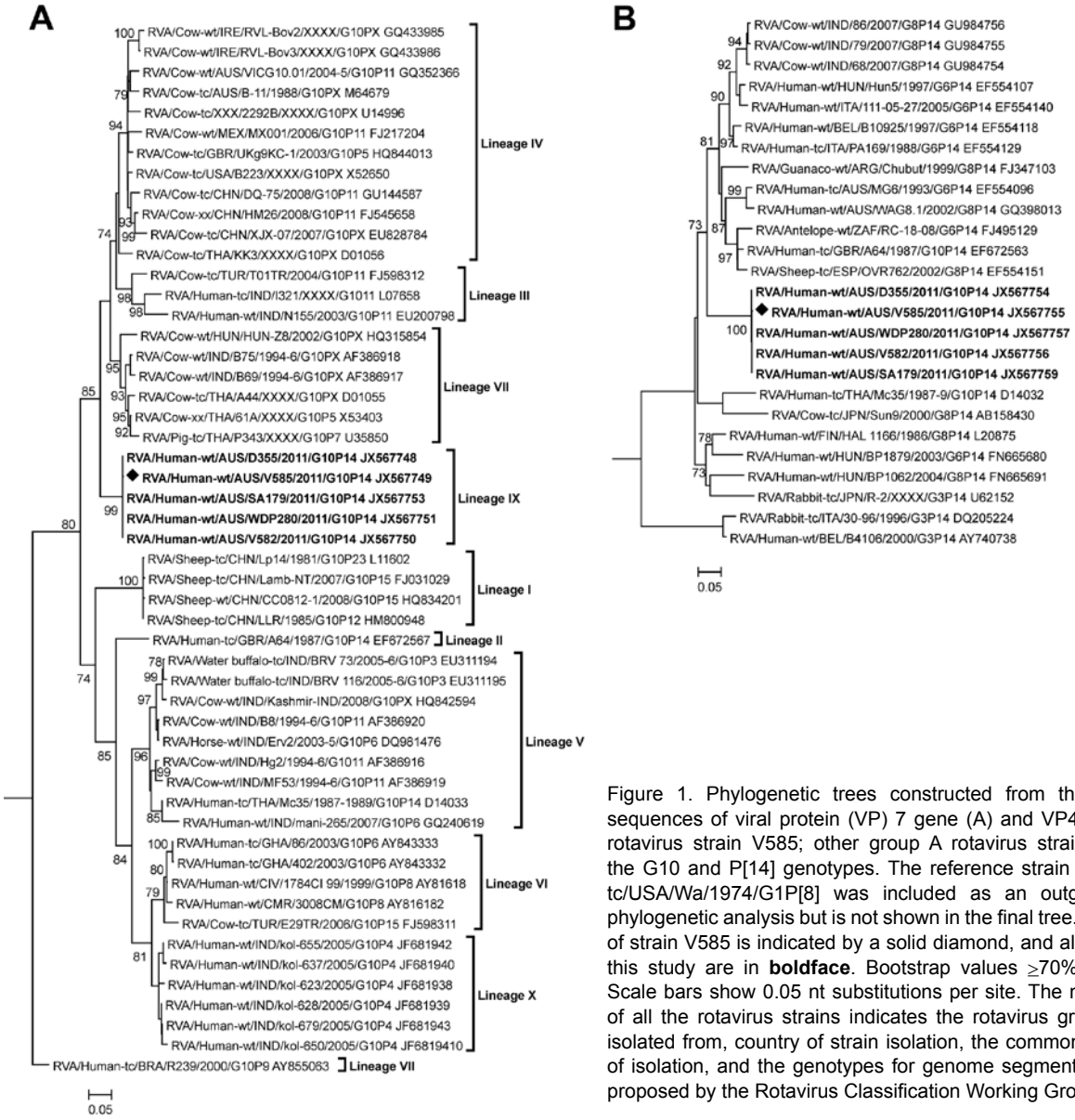

Figure 1. Phylogenetic trees constructed from the nucleotide sequences of viral protein (VP) 7 gene (A) and VP4 gene (B) of rotavirus strain V585; other group A rotavirus strains represent the G10 and P[14] genotypes. The reference strain RVA/human-tc/USA/Wa/1974/G1P[8] was included as an outgroup in the phylogenetic analysis but is not shown in the final tree. The position of strain V585 is indicated by a solid diamond, and all strains from this study are in **boldface**. Bootstrap values ≥70% are shown. Scale bars show 0.05 nt substitutions per site. The nomenclature of all the rotavirus strains indicates the rotavirus group, species isolated from, country of strain isolation, the common name, year of isolation, and the genotypes for genome segment 9 and 4, as proposed by the Rotavirus Classification Working Group (*2*).

Table 3. Nucleotide identity of 11 genome segments of the G10P[14] rotavirus strain V585, Northern Territory, Australia*

Gene encoding	Genotype of V585	Cutoff value†	Identity of V585 against indicated strains	
			Genotype reference strain	GenBank strains‡
VP1	R2	83	85.6 (DS-1)	96.9 (GirRV)
VP2	C2	84	84.9 (DS-1)	89.5 (B12)
VP3	M2	81	85.1 (DS-1)	88.9 (MG6)
VP4	P[14]	80	87.4 (A64)	88.9 (B10925)
VP6	I2	85	86.6 (DS-1)	93.8 (RotaTeq BrB-9/SC2-9/W17-9)
VP7	G10	80	86.8 (A64)	92.5 (RVL-Bov3)
NSP1	A11	79	79.9 (Hun5)	80.2 (BP1879)
NSP2	N2	85	87.4 (DS-1)	94.3 (B12)
NSP3	T6	85	92.4 (WC3)	94.0 (GirRV/A64)
NSP4	E2	85	88.7 (DS-1)	90.8 (Azuk-1)
NSP5	H3	91	92.9 (AU-1)	94.1 (RUBV81/Egy3399)

*VP, viral structural protein; NSP, nonstructural protein.
†Numeric values are given as percentage nucleotide identity. Percentage nucleotide cutoff values, genotype designation, and genotype reference strains proposed in (*2*).
‡Strains that shared the highest nucleotide identity with the Australian G10P[14] strain V585.

identity (92.5%) to the bovine strain RVA/cow-wt/IRE/RVL-Bov3/XXXX/G10P[X] (Table 3). Nucleotide identity was lower to Australian bovine G10 strains RVA/cow-wt/AUS/VICG10.01/2004-5/G10P[11] (91.1%) and RVA/cow-tc/AUS/B-11/1099/G10P[X] (90.6%).

The VP4 genes of the G10P[14] strains from NT formed a cluster distinct from other characterized P[14] sequences identified globally from humans and animals (Figure 1, panel B). V585 had the highest level of nucleotide identity (88.9%) to the human strain RVA/human-wt/BEL/B10925/1997/G6P[14] (Table 3). Nucleotide identity was lower to other Australian P[14] sequences, RVA/human-tc/AUS/MG6/1993/G6P[14] (87.5%) and RVA/human-wt/AUS/WAG8.1/2002/G8P[14] (87.2%).

Phylogenetic analysis of VP1, VP2, NSP2, and NSP3 demonstrated that V585 clustered with genes of rotaviruses identified in the mammalian order Artiodactyla (bovine, ovine, and camelid) and human strains derived from zoonotic infections (Figure 2, Appendix, panels A, B, F, G, wwwnc.cdc.gov/EID/article/19/8/12-1653-F2.htm). Similarly, VP3, which clustered with the RVA/human-tc/AUS/MG6/1993/G6P[14], was thought to be the result of zoonotic transmission (*5*) (Figure 2, Appendix, panel C). NSP1, NSP4, and NSP5 clustered with sequences from artiodactyl hosts, however branching was not supported by significant bootstrap values (Figure 2, Appendix, panels E, H, I). The NSP1 and NSP5 genes were divergent from sequences that define their respective genogroups (Table 3). Overall, the 11 genome segments of the G10P[14] strains from NT had relatively low nucleotide identity (80.2%–96.9%) to other strains in each of the respective genogroups, demonstrating that this G10P[14] strain identified in Australia was divergent from other strains identified globally (Table 3).

Conclusions

The V585 strain possessed a G10-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genome constellation. With the exception of the VP7 gene, the constellation is consistent with G6P[14] and G8P[14] strains identified globally: G6/G8-P[14]-I2-(R2/R5)-C2-M2-(A3/A11)-N2-T6-(E2/E12)-H3 (*6*). Human P[14] strains are related to rotavirus strains isolated from even-toed ungulates belonging to the mammalian order Artiodactyla (*6*). Consistent with this observation, each individual genome segment of V585 was most closely related to artiodactyl-derived strains or human zoonotic rotavirus strains characterized to be derived from artiodactyl hosts. In Australia, G10P[11] strains have been isolated from calves, and G8P[14] strains and G6P[14] strains have been isolated from children (*7,8*). However, the V585 strain demonstrated modest nucleotide identity with these 3 strains identified in Australia. These data suggest that V585 is novel and probably derived from a strain circulating in an artiodactyl host and transmitted to humans. A large feral animal population, including goats, rabbits, and camels, exists in the region where these specimens were collected, thereby supporting the possibility of an interspecies transmission event (*9*).

Vaccination with the monovalent G1P[8] Rotarix is available in NT, where 2-dose vaccine coverage is 74% for indigenous Australian infants (*10*). Rotarix vaccination status was available for 4 of the 5 children in this study: 3 were fully vaccinated, and 1 had received the primary dose. The heterotypic G10P[14] strain identified in these vaccinated children suggests a lack of protective immunity, although it cannot be excluded that vaccination provided protection against severe disease from other genotypes. Vaccine effectiveness against gastroenteritis leading to hospitalization has been variable in NT; vaccine was estimated to be 77.7% effective during a 2007 G9P[8] outbreak (*11*) and 19% effective against a fully heterotypic G2P[4] strain in 2009 (*12*). Rotarix has been effective for decreasing rotavirus infection notification rates in Darwin, NT (*10*), and New South Wales (*13*). However, in 1 location in central NT, reported rotavirus infection rates have remained similar in the vaccine era to

those in the prevaccine era (*10*), suggesting low vaccine uptake, low vaccine take, or waning immunity. The living conditions of the indigenous Australian population in central NT are typically crowded, with inadequate facilities for sanitation and food preparation (*14*). The number of diarrheal disease cases is high: admissions coded for enteric infections in NT indigenous Australian infants occur at a rate 10-fold higher than among nonindigenous Australian infants (*15*). The concurrent medical conditions present in the NT indigenous Australian population, combined with diversity of circulating rotavirus types, may have contributed to a lack of immunity. Detection of these unusual G10P[14] strains emphasizes the need for continued rotavirus surveillance to help guide current and future vaccination strategies.

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Primary and Secondary Human Bocavirus 1 Infections in a Family, Finland

Alma Jula, Matti Waris, Kalle Kantola, Ville Peltola, Maria Söderlund-Venermo, Klaus Hedman, and Olli Ruuskanen

Human bocavirus 1 (HBoV1) was detected in a young child hospitalized for pneumonia and subsequently in his twin brother and other family members. The mother's nasopharyngeal samples intermittently showed HBoV1 DNA; the grandmother had HBoV1 reinfection. Findings in this family lead to consideration of HBoV virulence, latency, and reactivation.

Human bocavirus 1 (HBoV1) is a frequent cause of common cold, bronchiolitis, acute wheezing, and pneumonia in children worldwide. The causative role of HBoV1 has been questioned because of HBoV1 DNA presence after primary infection and common co-infection with other respiratory viruses (*1–5*). We report life-threatening HBoV1 pneumonia in a child and transmission within his family.

The Cases

The index patient was a male twin who was born prematurely at 26 weeks' gestation. He weighed 1,540 g at birth and had severe bronchopulmonary dysplasia. He was not administered pneumococcal vaccine. At the age of 16 months, he was admitted to the hospital for evaluation and treatment of wheezing. Rhinorrhea and cough had been present for 12 days. No fever was recorded at admission. The child had a heart rate of 170 beats/min (reference range 75–130 beats/min) and a respiratory rate of 50 breaths/min (reference range 25–30 breaths/min). He experienced severe respiratory distress and was transferred to the intensive care unit. A chest radiograph showed bilateral pulmonary infiltrations and atelectasis of the upper right lobe (Figure). His serum C-reactive protein level was 1 mg/L, and his leukocyte count was 19.6 $\times 10^9$/L. He was treated with intravenous cefuroxime, clarithromycin, and methylprednisolone. Tracheal intubation and bronchoalveolar lavage (BAL) were performed. Cultures of blood and BAL sample did not grow bacteria.

After a week, ventilator-associated pneumonia developed in the child. His serum C-reactive protein level rose to 68 mg/L; intravenous vancomycin and meropenem treatment were initiated. *Pseudomonas aeruginosa* was identified in a second BAL culture. The child was extubated after 3 weeks, and after 5 weeks of hospitalization, he recovered completely.

The patient's twin brother had recovered from a common cold, which had started 2 weeks before the patient was hospitalized. Two days after the patient was admitted, his brother contracted another mild respiratory infection lasting 4 days. The 34-year-old mother remained asymptomatic, but the 58-year-old grandmother, who stayed in the household daily for many hours, had rhinitis. All family members were tested for respiratory viruses.

Nasopharyngeal aspirate (NPA) collected from the index patient was negative by PCR for influenza A and B, parainfluenza type 1–3 viruses, adenovirus, human metapneumovirus, respiratory syncytial virus, enteroviruses, and coronavirus types 229E, OC 43, NL63, and HKU1. PCR revealed a low load of human rhinovirus (HRV) RNA and a much higher load of HBoV1 DNA (Table). The index patient had an HBoV1 infection proven by detection of HBoV1 DNA in high copy numbers in NPA and BAL samples, as well as in serum. The DNA levels gradually decreased until they were not detectable (Table). PCR showed the index patient's serum samples to be HBoV1-positive for 4 weeks and nasal mucus samples to be positive for at least 3 months. All samples were negative for HBoV2–4 DNA. The child had an HBoV1-specific IgM response and a >4-fold increase in HBoV1 IgG. The avidity of the IgG was initially low and then matured, but IgM declined and were undetectable by the end of the 3-month follow-up period.

The twin brother's NPA was positive for HBoV1 DNA for 6 weeks. Two months later, his test results were HBoV1 IgM–negative but exhibited HBoV1 IgG at a high level and of high avidity. The children's mother, who was asymptomatic, lacked HBoV1–4 DNA in serum but had intermittent low HBoV1 DNA loads in NPA. She had neither HBoV1-specific IgM nor IgG but exhibited a stable level of HBoV2-specific IgG of high avidity. NPA collected from the grandmother was intermittently HBoV1 DNA–positive twice during the first 9 days and twice thereafter. On day 13 of her grandson's hospitalization, her serum was HBoV1 DNA–positive; on follow-up, she had an increase of high-avidity HBoV1 IgG but no IgM response.

The index patient was tested for HRV RNA 8 times during hospitalization. His results were positive (1.5×10^5 copies/mL) once, on day 4 after admission. His mother's

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test result was positive (1.9×10^4 copies/mL) on day 5. The twin brother's test results were positive (5.4×10^7 – 1,500 copies/mL) on days 5, 14, and 29, indicating he had an acute HRV infection. Three months after this episode of HBoV1 infection, all 4 family members had symptomatic parainfluenza type 1 virus infections, and clinical samples for the twin brother were again positive for HBoV1 DNA. Four months later, both brothers had an adenovirus infection, and the twin brother's test result was again positive for HBoV1.

For each family member tested, quantitative PCR for HBoV1 in NPA was performed as described (*9*). HBoV1–4 DNA in serum was measured by multiplex and singleplex PCRs (*6*). For other respiratory viruses, qualitative multiplex PCR (SeeplexRV12, Seegene, Seoul, South Korea) and quantitative PCR methods were used (*10*). Biotinylated HBoV1–3 VP2 virus-like particles were applied as antigens in enzyme immunoassays for measurement of HBoV-specific IgM, IgG, and IgG avidity (*8,11*). For removal of cross-reacting heterologous HBoV antibodies, virus-like particle–based competition assays were used (*7*).

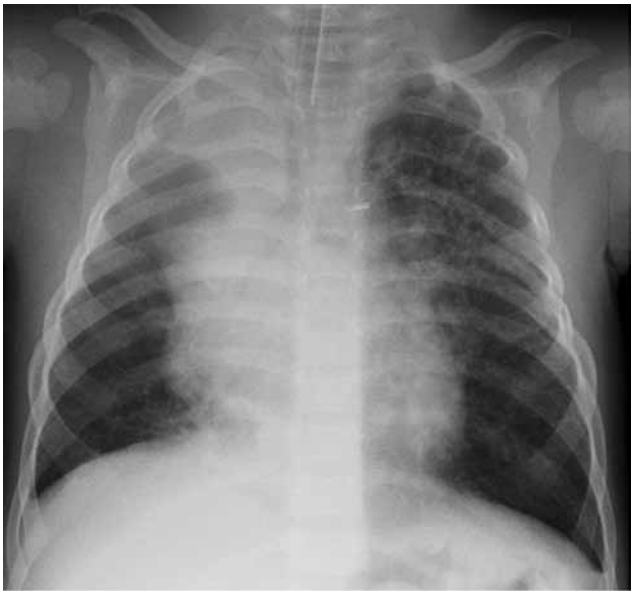

Figure. Chest radiograph of the index patient, a 16-month-old boy in Finland with human bocavirus 1 pneumonia, on day 2 of hospitalization. Bilateral pulmonary infiltrations and atelectasis of the upper right lobe can be seen.

Conclusions

The index patient had life-threatening pneumonia associated with acute HBoV1 infection and was at high risk for complications from the infection. Cases of severe lower respiratory tract HBoV1 infection in 3 other young children have been reported (*12–14*). Their illnesses were characterized by severe respiratory distress associated with pneumothorax and pneumomediastinum; 2 of them required mechanical ventilation, and 1 required extracorporeal membrane oxygenation. The children fully recovered.

This article adds to evidence of HBoV1 virulence by describing probable transmission of HBoV1 infection within a family. The index patient had a severe HBoV1 infection, and his twin brother had a mild infection with low viral load. This finding is supported by serologic studies 2–3 months later when the index patient had HBoV1 IgG of stable level and high avidity with slight maturation. The mother seems not to have acquired an HBoV1 infection. She was nonviremic, yet once showed borderline HBoV1 DNA positivity in NPA, reflecting either replicative infection or noninfective mucosal contamination. HBoV2 IgG in the mother appears to have preceded her exposure to HBoV1, which failed to raise specific HBoV1 IgG, possibly because of a phenomenon known as original antigenic sin (*15*). The lack of symptoms in the mother further suggests that her preexisting HBoV2 IgG may have been cross-protective. Of note, despite the presence of preexisting HBoV1 IgG, the grandmother had a symptomatic HBoV1 infection, which was probably caused by reinfection characterized by low-load viremia and simultaneous increases in HBoV1 and HBoV2 IgG.

During the acute phase of HBoV1 infection in the index patient, HRV RNA was identified in the NPA of 3 family members, an observation similar to that of many studies (*1*). The effect of HRV on the symptoms in this family cannot be reliably judged, but it is possible that the high-risk index patient's severe illness was partly caused by the simultaneous or preceding HRV infection. During follow up, all family members experienced parainfluenza type 1 virus infections, and the children also had adenovirus infections. In both instances, a recurrence of HBoV1 DNA was observed in NPA from the twin brother. These observations suggest that other respiratory viruses might reactivate HBoV1 from latency, as has been seen with several other DNA viruses, or that they alter the intranasopharyngeal environment and release persisting HBoV.

Our observations raise several questions that warrant further study. First, can the presence of HBoV1 in the NPA represent only passive, nonreplicating mucosal contamination from a family member? Second, can HBoV1 establish true latency, and if so, can other respiratory viruses reactivate the virus? Third, how often does HBoV1 cause symptomatic reinfections? Last, does immunity to HBoV2 or HBoV3 cross-protect against HBoV1 and vice versa?

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Table. HBoV1 DNA, other respiratory viruses, and HBoV antibodies in family members during 7 months, Finland*

Patient and no. days after hospital admission of index patient	Symptoms of acute infection	HBoV1 DNA in NPA, copies/mL	HBoV1 DNA in serum, copies/mL†	HBoV1 IgM‡	HBoV IgG avidity	HBoV1 IgG‡	HBoV2 IgG‡	HBoV3 IgG‡§	Other viruses detected in NPA by PCR
Index									
1	Yes	1.6×10^{10}	NT	NT	NT	NT	NT	NT	None
4	Yes	4.1×10^{8}	3,000	2.148	2.0	1.034	0	0	HRV
9	Yes	3.0×10^{5}	1,400	2.089	2.0	3.127	0	0.034	HRV negative
29	Yes	300	440	1.613	9.7	3.028	0.060	0	HRV negative
64	No	NT	Negative	0.154	17.1	3.426	0.050	0	NT
74	No	5,400	NT	NT	NT	NT	NT	NT	None
109	Yes	600	Negative	0.040	31.3	3.221	0	0	PIV1
185	ND	Negative	NT	NT	NT	NT	NT	NT	NT
232	Yes	Negative	NT	NT	NT	NT	NT	NT	AdV
Twin brother									
5	Yes	6,300	NT	NT	NT	NT	NT	NT	HRV
14	ND	7,800	NT	NT	NT	NT	NT	NT	HRV
29	ND	3,900	NT	NT	NT	NT	NT	NT	HRV
64	No	NT	Negative	0.022	26.9	3.305	0	0	None
74	No	Negative	NT	NT	NT	NT	NT	NT	None
109	Yes	99,000	Negative	0.011	38.2	3.242	0	0	PIV1
232	Yes	5,700	NT	NT	NT	NT	NT	NT	AdV
Mother									
5	No	Negative	NT	NT	NT	NT	NT	NT	HRV
13	ND	Negative	NT	0.013	50.2	0	0.509	0.307	HRV negative
30	No	Negative	NT	0.019	45.0	0.001	0.542	0.360	HRV negative
64	No	Negative	NT	0.019	48.1	0.012	0.480	0.259	NT
109	No	300	NT	0.032	50.2	0	0.861	0.585	PIV1
Grandmother									
5	Yes	5,100	NT	NT	NT	NT	NT	NT	None
13	ND	22,000	960	0.011	58.0	0.395	0.542	0.016	HRV negative
64	ND	NT	Negative	0.012	25.4	2.731	1.470	0	None
74	No	3,600	NT	NT	NT	NT	NT	NT	HRV negative
109	Yes	300	Negative	0.023	33.5	2.674	1.834	0	PIV1, HRV

*HBoV1, human bocavirus 1; NPA, nasopharyngeal aspirate; NT, not tested; HRV, human rhinovirus; HRV negative, tested by monoplex PCR only; PIV1, parainfluenzavirus type 1; ND, no data available; AdV, adenovirus; none, negative by multiplex PCR.
†All serum samples were negative for HBoV2–4 DNA (*6*).
‡HBoV antibodies after heterologous antigen competition are shown as absorbance values at 492 nm, cutoff 0.13 (*7*).
§IgG avidity was calculated as the ratio of 2 IgG end-point titers (urea positive/urea negative) as described (*8*). Results obtained with unblocked enzyme immunoassay and confirmed with specific competition enzyme immunoassays.

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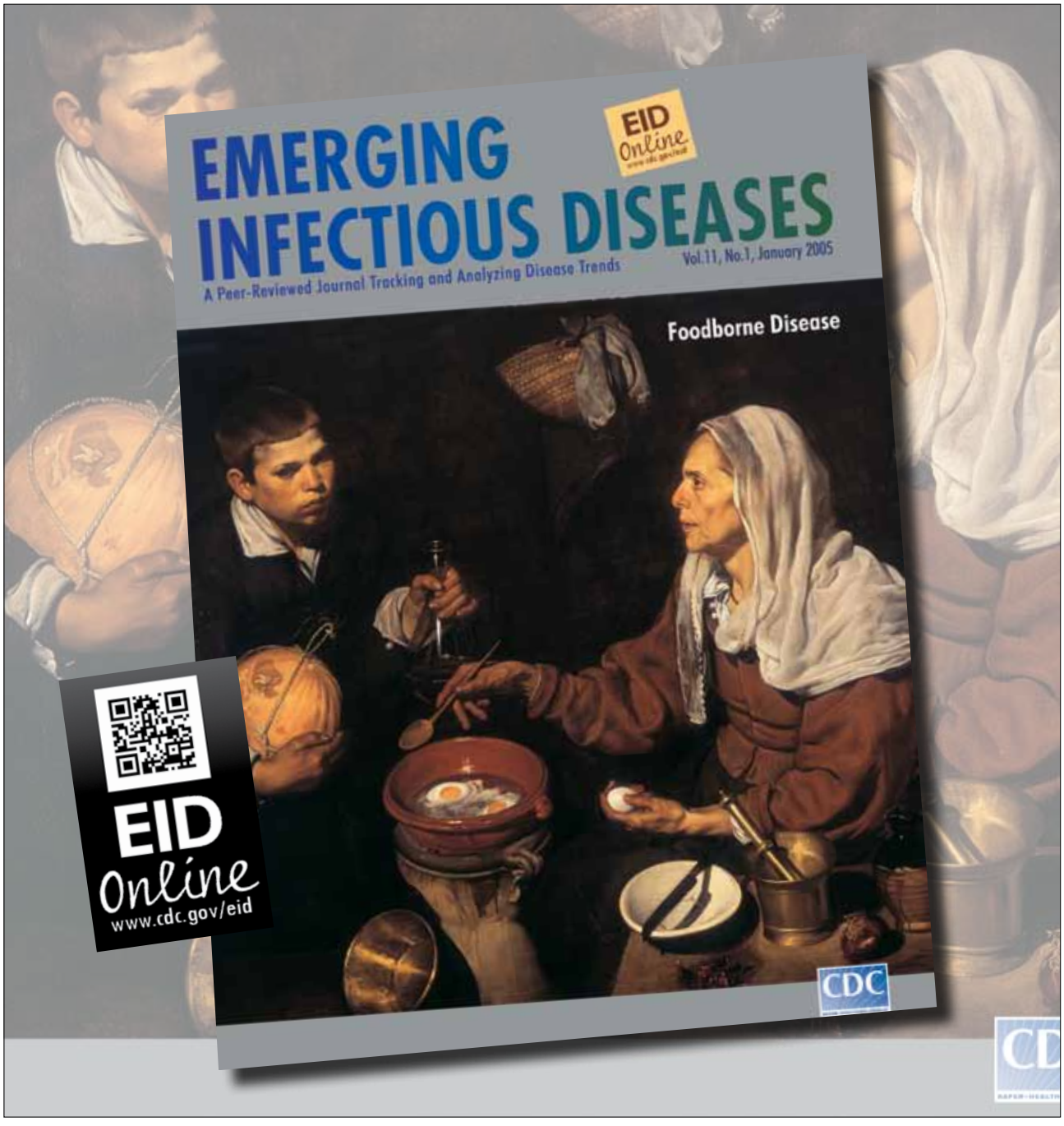

Rotavirus G9P[4] in 3 Countries in Latin America, 2009–2010

To the Editor: Group A rotaviruses are the most common viral cause of acute gastroenteritis in young children. The most frequently detected group A rotavirus genotype combinations include G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8]. The G9 genotype has been associated with multiple P types, including P[8], P[6], and P[4], although genotype G9P[8] is predominant (*1*).

In Latin America, a large number of unusual G-P combinations have been reported, and among these is the rare G9P[4] genotype, which was identified in Brazil in the 1990s (*2*), and later reported infrequently elsewhere in Latin America (*3*). In 2010, cases of group A rotavirus gastroenteritis associated with genotype G9P[4] were reported in Mexico (*4*). Increases in the incidence of group A rotavirus gastroenteritis were reported in 2010 in Mexico and Guatemala and in 2009 in Honduras (http://new.paho.org/hq/dmdocuments/2010/Epi_Alerts_2010_mar_5_rotavirus.pdf).

In response to these reports of increased group A rotavirus disease, fecal samples collected in Chiapas State, Mexico (in 2010, 30% of the cases in Mexico were from Chiapas), Guatemala, and Honduras in 2009–2010 that were positive by enzyme immunoassay were sent to the US Centers for Disease Control and Prevention (Atlanta, GA, USA) for characterization. Viral protein 4 (VP4) (P) and VP7 (G) genotyping, nucleotide sequencing, and genotype identification were performed by using consensus and genotype-specific oligonucleotide primers (*5*), and sequences were subjected to phylogenetic analyses. VP6 and nonstructural protein 4 (NSP4) genes of selected samples were also sequenced.

For 26 samples from Mexico, G9 accounted for ≈90% of all the G types; all samples had mixed P types. Approximately 80% of samples were genotype G9P[4,8]; genotypes G3P[4,8], G3,9P[4,8], and G9P[4,9] accounted for the remaining samples. We hypothesize that the G9P[4,8] genotype was the result of mixed G9P[4] and G9P[8] infections by strains with homologous G9 VP7 genes. For 41 samples from Guatemala, G9P[4] accounted for 66%, followed by G9P[8] (32%), and G3,9P[4,8] (2%). For 50 samples from Honduras, 50% were G1P[8] and 36% were G9P[4]. G3P[8], G1,3P[8], and G4P[6] comprised the remaining samples.

Results showed an increase in prevalence of the rare G9P[4] strain, which was the predominant strain in Guatemala and Mexico, and the second most predominant strain in Honduras, after G1P[8]. Group A rotavirus genotypes G1P[8] and G3P[8] have been reported to be predominant in Mexico (*4*). G1P[8] and G2P[4] were associated with most group A rotavirus infections in Guatemala (*6,7*) and G2P[4] predominated in Honduras (*7*). G9P[4] has not been reported in Guatemala or Honduras. In Mexico, the outbreak might have originated from a common source, such as untreated drinking water (http://new.paho.org/hq/dmdocuments/2010/Epi_Alerts_2010_mar_5_rotavirus.pdf).

Phylogenetic analysis of G9 gene sequences from the 3 countries showed that they clustered in a sublineage and were closest to G9P[8] strains circulating globally (Figure, Appendix, panel A, wwwnc.cdc.gov/EID/article/19/8/13-0288-F1.htm). VP4 genes from the 3 countries also clustered within a sublineage of a clade containing global strains (Figure, Appendix, panel B). VP6 sequences clustered within a sublineage of the I2 genotype clade (Figure, Appendix, panel C). NSP4 gene sequences clustered within a sublineage of the E6 genotype clade, which they shared with group A rotavirus strains from India and Bangladesh (Figure, Appendix, panel D).

The high degree of genetic similarity among these strains in all 4 genes (99.6%–100%), as demonstrated in this study, suggests that strains from all 3 countries had a common origin. In regions of overlapping sequence, VP4 gene sequences from this study shared 98.3%–100% identity (408 bases) with G9P[4] strains from Mexico (GenBank accession nos. JN180414–JN180451), and VP7 gene sequences shared 97.9%–98.9% identity (97 bases) (GenBank accession nos. JN180376–JN180413).

Rahman et al. have hypothesized that the G9P[4] genotype combination was formed by reassortment between more frequently occurring strains (e.g., G2P[4] and G9P[6] strains) (*8*). Potential parental strains have been circulating at high levels in Latin America for ≈30 years. During this period, G9 and P[4] accounted for 15% and 22% of all G and P types, respectively, in Latin America and the Caribbean (*3*). Only 0.4% of strains were G9P[4] during this period, which suggests that the markedly increased prevalence of this genotype in 2009–2010 was the result of a dramatic event, such as genetic reassortment.

Previous studies of G9P[4] strains examined only VP4 and VP7 genes and had not characterized VP6 and NSP4 genes of these strains. The presence of an NSP4 genotype E6 gene within these viruses was surprising. The NSP4 E6 genotype has been described in only 5 strains, all of which were from human cases of infection in Bangladesh or India (*9,10*) and were associated with VP4 genotype P[6] and VP7 genotypes G8 or G12. The complete global distribution of this NSP4 genotype remains to be determined.

Although many factors account for increased reports of group A rotavirus gastroenteritis observed in

Mexico, Guatemala, and Honduras in 2009–2010, our data suggest emergence of the previously rare G9P[4] group A rotavirus genotype in these countries. Whether the G9P[4] genotype becomes the common strain in Latin America or elsewhere remains to be determined.

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Recently Identified Novel Human Astroviruses in Children with Diarrhea, China

To the Editor: Human astroviruses (HAstVs), first identified in 1975, are now considered an important cause of viral gastroenteritis, predominately infecting children ≤2 years of age (*1*,*2*). HAstVs are classified into 8 serotypes. A unique astrovirus, MLB1 (AstV-MLB1), recently was discovered in a fecal sample from a child with diarrhea in Australia (*3*); subsequently, at least 6 novel astroviruses have been discovered from fecal samples, including AstV-MLB2, AstV-MLB3, HMO AstV-A/VA2, HMO AstV-C/VA1, HMO AstV-B/VA3, and AstV-VA4 (*4*–7). The prevalence of novel astroviruses in China remains unclear.

Fecal specimens were collected during July 2010–June 2011 from 723 children <5 years of age who had acute gastroenteritis. Samples were from all of 295 eligible children brought for care to First Hospital of Lanzhou University (Lanzhou, China) and every fifth eligible child (n = 428) brought for care on 2 days of the week (Tuesday and Thursday) at Nanjing Children's Hospital (Nanjing, China). The children's parents provided informed consent. The ethics committees of both hospitals approved the study.

Nucleic acids were extracted from specimens by using the Viral Nucleic Acid Extraction Kit II (Geneaid, Taipei, Taiwan). Adenovirus and caliciviruses were detected by PCR and reverse transcription PCR, respectively (*8*). Rotavirus was detected from fecal samples by ELISA (Oxoid, Cambridge, UK). Primers Mon269/Mon270 detected a region of the capsid gene (449 bp) from classic HAstV-1–8 by reverse transcription PCR (*8*). Additional astrovirus types were detected by using primers SF0073/SF0076, amplifying a

409-bp fragment of the astrovirus gene open reading frame 1b (*5*). All amplification products were sequenced and analyzed by using the software package DNAStar (DNAStar, Madison, WI, USA). Phylogenetic trees were constructed by using the neighbor-joining method and the software program MEGA4 (www.megasoftware.net). Statistical analyses were performed by using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA).

A total of 320 (44.3%) samples were positive for rotavirus and 102 (14.1%), 27 (3.7%), and 32 (4.4%) for caliciviruses, adenoviruses, and astroviruses, respectively. A total of 17 positive samples were detected with Mon269/Mon270, and an additional 15 samples were found with primers SF0073/SF0076. Phylogenetic analysis revealed that 21 of the 32 astrovirus-positive isolates were classic HAstV, dominated by HAstV-1 (12 samples); 7 samples were AstV-MLB1(GenBank accession nos. JQ673575–JQ673581), and 4 were AstV-MLB2 or HMOAstV-A (2 isolates each) (GenBank accession nos. JQ673582–JQ673585). Primers SF0073/SF0076 detected 4 classic astroviruses that were not detected by Mon269/Mon270. We found no statistically significant difference (χ^2 = 1.547, p = 0.214) between the detection rates of novel astroviruses in Lanzhou and Nanjing. The prevailing astrovirus genotypes (classic and novel) in both regions were similar. Furthermore, the prevalence and genotype distribution of classic HAstV were similar to those in a previous study in China (*8*).

Rotaviruses were a co-pathogen in 14 (43.8%) astrovirus-positive fecal samples. Three samples were AstV-MLB1 positive; the remaining 11 had classic HAstV. Differences were noted between seasonality; classic astrovirus infections (66.7%) occurred during October and December, and novel astrovirus infections were observed in March, April, May, July, and November. However no statistically significant differences in mean age (p = 0.209, Student *t* test), rate of fever and vomiting (p = 0.712 and p = 0.472, respectively, Fisher exact test), or mean duration and frequency of diarrhea (p = 0.231 and p = 0.177, respectively, Student *t* test) were observed between the classic and novel astrovirus groups.

Nucleotide sequence analysis showed that the AstV-MLB1 isolates in this study had 98.64% homology, with 99.65% identity at the amino acid level in open reading frame 1b region. Further phylogenetic analysis indicated that AstV-MLB1 viruses were closely related to AstV-MLB1 HK05, with 95%–98% genomic identity, whereas AstV-MLB2 was closely clustered with

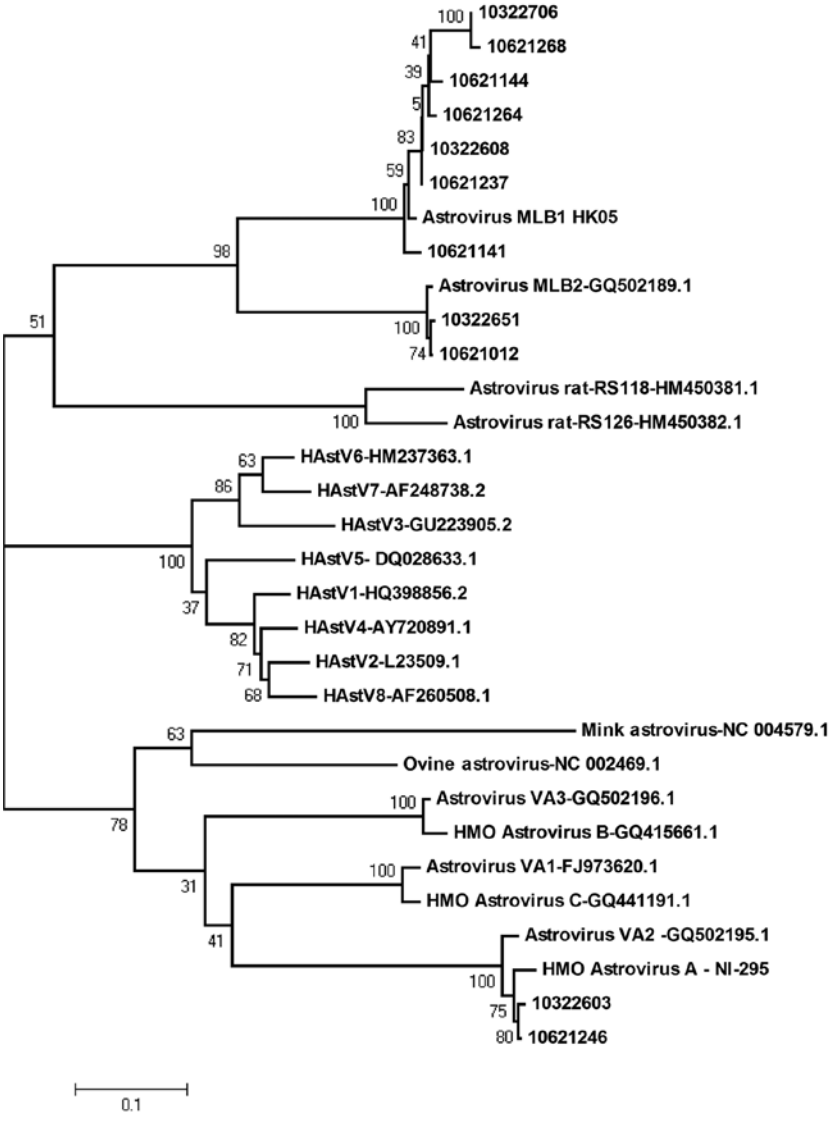

Figure. Phylogenetic analyses of human astroviruses, China. Construction of phylogenetic trees was based on alignment of a region of the open reading frame 1b nucleic acid sequence (409 bp), generated by the neighbor-joining method with 1,000 bootstrap replicates. Each strain from this study is indicated by the patient number (10621012, 10621141, 10621144, 10621237, 10621246, 10621264, 10621268, 10322603, 10322608, 10322651, 10322706) or GenBank accession number (JQ673575–JQ673585) as indicated. AstV, astrovirus; AstV-MLB, human astrovirus MLB; HAstV, human astrovirus; HMO-A, B, C, human-, mink-, and ovine-like astrovirus species A, B and C; AstV-VA, human astrovirus VA.

the strain CRI41435, sharing 99% sequence identity. AstV-MLB1 and AstV-MLB2 are phylogenetically related to the rat astroviruses RS118 and RS126. The remaining novel astroviruses, 10322603 and 10621246, clustered closely with human, mink, and ovine astrovirus strain NI-295 (Figure).

This study documented that multiple novel astroviruses circulated simultaneously with common human astrovirus types in China. The detection rates of novel astroviruses, especially Ast-MLB1, were higher than in 2 previous reports (*3*,*4*), although lower than in a study from Egypt (*9*). These results indicate that multiple novel astroviruses are spread worldwide. The differences in prevalence may have been caused by the geographic and/or study cohort differences. The phylogeny of astroviruses determined in our study basically agrees with previous analyses (*5*), supporting the idea that the novel astroviruses are related to other animal astroviruses. Additional studies using full-genome sequencing should be done to clarify the origin of the novel astroviruses.

One limitation of this study was that no asymptomatic control was included. A recent case–control study has suggested that AstV-MLB1 was not associated with diarrhea (*10*). However, other novel astroviruses were not assessed. Further study, especially with a large case–control cohort, should be initiated to determine the correlation of unique astroviruses with gastrointestinal and extraintestinal diseases.

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Call to Action for Dengue Vaccine Failure

To the Editor: Dengue is one of the most widespread infectious diseases globally; transmission now occurs in 128 countries. Although dengue virus (DENV) control strategies have targeted vector control and disease surveillance, the development of an effective vaccine is the holy grail of prevention.

Dengue vaccine development has spanned many decades. A candidate vaccine (Sanofi Pasteur, Swiftwater, PA, USA) containing all 4 DENV serotypes is in advanced clinical testing. However, when given to school children in Thailand, this live-attenuated, tetravalent, dengue–yellow fever 17D chimeric virus vaccine showed major but incomplete efficacy against 3 of the 4 DENV serotypes (DENV 1 [61.2%], DENV-3 [81.3%], and DENV-4 [89.9%]) in the intention-to-treat group but no protection against DENV 2, the most pathogenic of the DENV serotypes (*1*).

Two observations from the efficacy trial in Thailand provide insights into protective immunity that could greatly improve second-generation vaccines. The first observation was that a single dose of 4 live-attenuated chimeric DENVs given subcutaneously at a single site failed to raise type-specific protective immunity against the 4 DENV serotypes, and 2) doses 2 and 3 of the Sanofi Pasteur vaccine given to children over a 1-year period failed to improve efficacy outcomes. These results were

obtained even though 91% of the children had circulating dengue or Japanese encephalitis antibodies before vaccination, neutralizing antibodies developed to all 4 DENVs, and neutralizing antibody titers increased 2–3 fold after 3 doses of vaccine in 80%–90% of vaccinated children.

The inability of a mixture of 4 dengue chimeric viruses to elicit an initial primary neutralizing antibody response in nonhuman primates and susceptible humans was recognized during preclinical testing and explained by the phenomenon of interference (*2*). Although protective immunity was raised in susceptible rhesus monkeys inoculated with all 4 DENVs at a single site, inoculation of 4 chimeric dengue viruses at 1 or 2 sites did not result in neutralizing antibody responses to all 4 DENV (*3*). Studies on human primary immune responses to dengue infection have identified critical attachment sites on the virion for neutralizing antibodies (*4*). Serum samples from children given ≥1 doses of the dengue chimeric vaccine can now be tested for primary neutralizing antibody responses to each of the 4 DENVs. As an alternative, antibody-secreting cells may be isolated and their products identified by using methods as described for dengue-infected children in Nicaragua (*5*).

Infections with 2 different DENVs can protect against severe disease during subsequent infections (*6*). It has therefore been assumed that persons with DENV neutralizing antibodies are protected against infection. In clinical testing of the Sanofi Pasteur vaccine, failure of multiple booster doses to show protection was unexpected because the children were already substantially immune from prior exposure to DENV or Japanese encephalitis vaccine. When the tetravalent dengue chimeric vaccine was given to partially dengue–immune children and adults in the Philippines, a broad neutralizing antibody response was observed after administration of only 2 vaccine doses (*7*).

We believe that the unanticipated results of the dengue vaccine efficacy trial in Thailand call for new methods of assessing dengue immunity. Myeloid cells are major targets of dengue infection in humans. We and others have described the unique biologic responses when dengue virus–antibody complexes are presented to myeloid cells (*8*). There is evidence that DENV neutralization titers differ when the same antibodies are assayed in epithelial and Fc-receptor–bearing cells (*8*). Recent work suggests that primary monocytes and macrophages may not respond in exactly the same fashion to infection by DENV immune complexes (*8*). Few relevant studies exist in the literature, and most focused on DENV-2. Detailed studies on innate immune responses in human myeloid cells with a variety of dengue immune complexes should proceed forthwith.

To our knowledge, only once has an in vitro test correctly predicted which children would be susceptible or have silent infections accompanying a second heterotypic dengue infection (*9*). This was determined by using serum samples collected before a second dengue infection and testing these serum samples at low dilutions for their ability to protect primary human monocytes from DENV-2 infection or antibody-dependent enhanced infection. During development of the Sanofi Pasteur tetravalent chimeric dengue vaccine, serum samples from vaccinated persons were routinely tested for neutralization of DENV in an epithelial cell line (*10*). In addition to assaying for antibodies directed at the quaternary site described by de Alwis et al. (*4*), we suggest that serum samples from vaccinated persons be tested for neutralization of all DENVs in primary human myeloid cells.

Although human Fc-receptor cell lines may be convenient for assaying DENV antibodies, decisions regarding their use should be deferred until they are shown to model primary myeloid cells. Because antibody titers often wane after vaccination, the ability of serum samples from vaccinees to protect against infection of myeloid cells with the 4 DENVs should be studied over many years. Changes to in vitro systems for measuring immune responses after dengue vaccination may provide a better surrogate of protection by realigning antibody measurement systems to our contemporary understanding of the pathogenesis of this complex disease.

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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

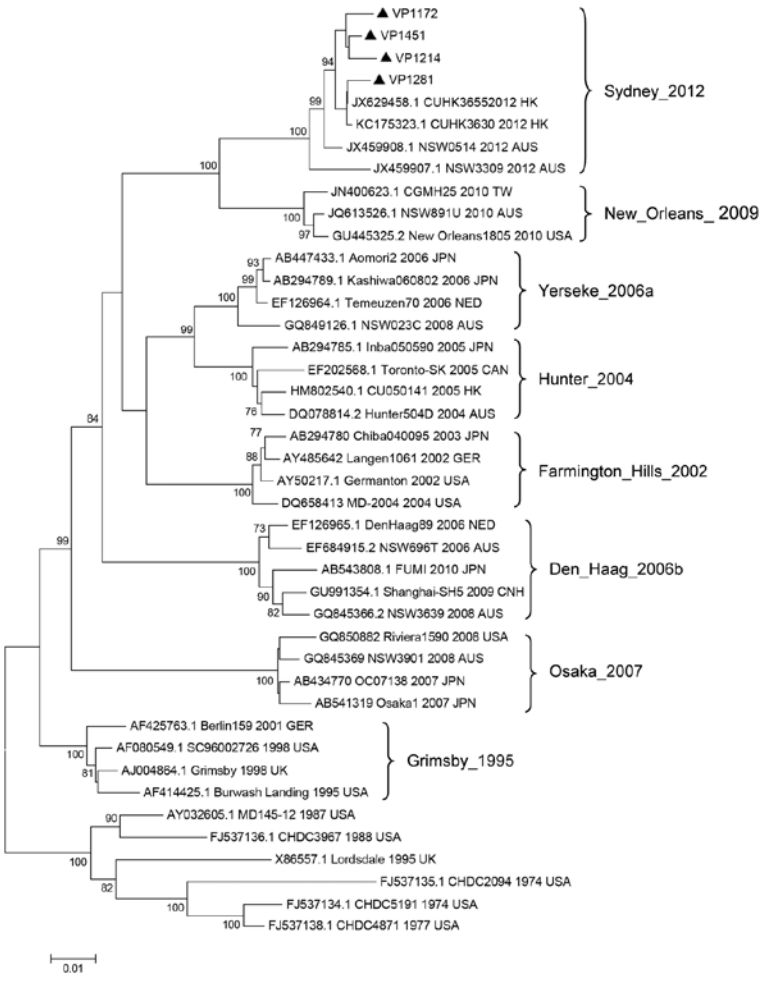

Figure. Phylogenetic tree of norovirus GII.4 capsid nucleotide sequences, Shanghai, China. The dendrogram was constructed by using the neighbor-joining method in MEGA version 5.0 (*8*). Bootstrap resampling (1,000 replications) was used, and bootstrap values ≥70% are shown. Black triangles indicate the 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*).

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

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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.

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Autochthonous Human Schistosomiasis, Malaysia

To the Editor: In Malaysia, the only histologically diagnosed autochthonous cases of human schistosomiasis were reported in the 1970s, all in rural aborigine (Orang Asli) populations (*1*–*3*) (online Technical Appendix Figure 1, wwwnc.cdc.gov/EID/article/19/8/12-1710-Techapp1.pdf). The fact that the infection had been found only among aborigines had led to the proposal of a distinct unknown schistosome with an animal reservoir causing sylvatic infections (*2*,*3*). Consequently, during the 1980s, *Schistosoma malayensis* n. sp. was described from intermediate snail (*Robertsiella* sp.) and final mammalian hosts (*Rattus muelleri* and *R. tiomanicus* [*4*]). *S. malayensis* is closely related to *S. mekongi* and differs genetically from the latter by ≈10%. Both species differ from *S. japonicum* by 25% (*5*), and adult and ova morphologies are similar (*4*). Few transmission sites for this new *S. japonicum*–complex schistosome species were identified in rural areas (*4*). We report after 30 years the histologic finding of *S. malayensis*–like eggs in the liver of a Malay man and discuss public health implications.

A 29-year-old male nonaboriginal Malay from Subang Jaya in Selangor State, Peninsular Malaysia, had died suddenly of an intoxication in 2011. According to his mother, he had reported hematuria and dysuria during adolescence. Similar symptoms had reoccurred 10 years later, accompanied by constipation. The patient had never been outside of Malaysia, and he had gone bomb fishing for many years in Sungai Lepar Utara, a river near his village (Felda Tekam Utara, Jerantut, Pahang; 3°52'30"N, 102°49'2"E). No tests on blood or feces were performed before his death. An autopsy was conducted in Sungai Buloh Hospital, and gross pathology showed a normal heart, kidneys, and brain. The lungs were edematous and congested. The liver also was congested, but no macroscopic lesions were seen. Toxicology investigations showed methadone and a derivative in his blood and urine. During a routine histologic examination, several granulomas with intensive lymphocyte, monocyte, and eosinophil infiltration surrounding clusters of ovoidal eggs were found in the liver (Figure; online Technical Appendix Figure 2). Serial sectioning showed that the eggs contained miracidia and had the overall appearance of *S. malayensis*–like ova 50 μm long × 28 μm wide. The ova were not operculated and had no bipolar plugs; the thin yellowish shell was not striated, but a knob-like structure was seen laterally. Morphologic differential diagnoses included eggs of *Capillaria hepatica* (bipolar striated ova in liver), *Dicrocoelium* (slightly smaller operculated ova typically found in feces or bile), and the similar *Eurytrema* (thick-walled operculated ova in feces).

Schistosomiasis is endemic in many developing countries and infects >207 million persons living in rural agricultural areas (*6*). In Asia, *S. japonicum*, *S. mekongi*, and *S. malayensis* cause human infection (*7*), with *S. japonicum* being the most dangerous. In Malaysia, *S. malayensis*, in addition to *S. spindale*, *S. nasale*, *S. incognitum*, *Trichobilhazia brevis*, and *Pseudobilharziella lonchurae*, is known to occur in wildlife (*8*). The first known case of human schistosomiasis in Malaysia was discovered in 1973 during an autopsy of an aborigine. *Schistosoma* eggs resembling those of *S. japonicum* were found in liver tissue (*1*). A subsequent retrospective autopsy study revealed additional cases with these *Schistosoma japonicum*–like ova in the rural aboriginal population, resulting in an overall prevalence of 3.9% (*2*). Several attempts to recover eggs from feces from the Orang Asli population in peninsular Malaysia (*3*), a biopsy-positive Orang Asli (*3*), and serologically positive persons (*9*, and others) were unsuccessful, however, which was attributed to the zoonotic nature of *S. malayensis* and thus missing adaptation to the human host. Whether hematuria, a typical sign of *S. haematobium* infection, as seen in the patient reported here also was caused by *S. malayensis* disease remains unclear because symptoms of the latter have not been reported. Serologic surveys for schistosomiasis in peninsular Malaysia showed prevalences of 4%–25% in selected rural populations (*9*). Because infected *Robertsiella* snails had been found almost exclusively in small rivers (*4*,*9*–habitats like the Sungai Lepar Utara River in our current report–we suspect that the patient most likely became infected while fishing. The travel history may not be accurate

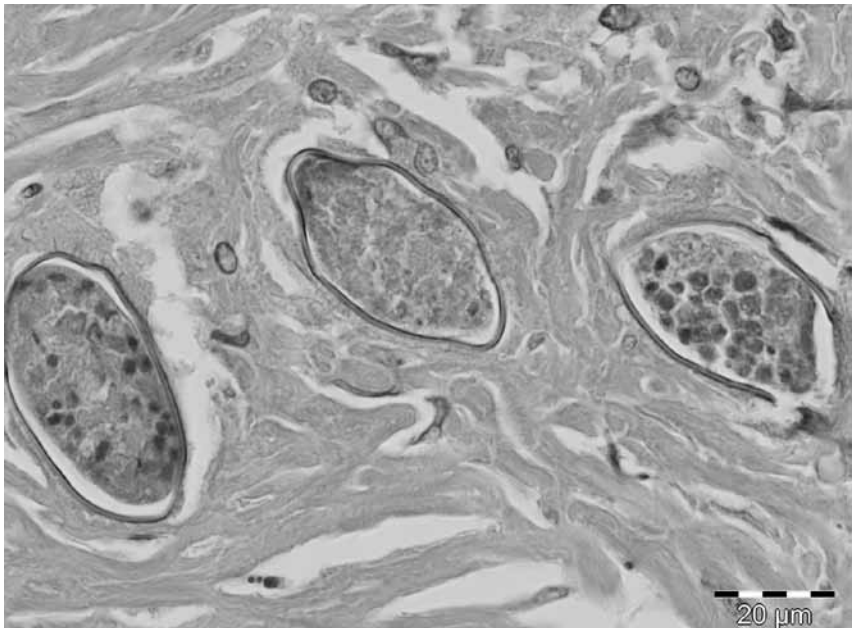

Figure. Close-up of liver granuloma with section through 3 *Schistosoma malayensis*—like ova embedded in dense fibrous tissue. The thin-walled, nonstriated helminth ova are not operculated and contain nonvital miracidial cells. Hematoxylin and eosin stain; original magnification ×100.

because it was obtained from a relative, and possible unreported drug-related travel by the patient to neighboring countries cannot be fully excluded. *R. muelleri*, the jungle rat and definitive host for *S. malayensis*, is often seen at river banks (*4*), and rodent feces could have contaminated the water with schistosome eggs.

Future field studies are needed to identify focal hot spots of sylvatic transmission by snail examination and seroprevalence studies of persons living in rural areas, especially the Orang Asli population. Moreover, in light of growing ecotourism, which also encompasses stays at remote Orang Asli villages and canoeing on small streams (*10*), appropriate public health measures, such as rodent and snail control near tourist sites, should be implemented.

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Asian Musk Shrew as a Reservoir of Rat Hepatitis E Virus, China

To the Editor: Rat hepatitis E virus (HEV), a member of genus *Hepevirus* in the family *Hepeviridae*, was first detected in Norway rats in Germany in 2010 (*1, 2*). Since then, this rat HEVhas been detected in multiple wild rat species in the United States, Vietnam, Germany, and Indonesia (*3–7*). Studies have shown that rat HEV failed to infect rhesus monkeys and pigs,

suggesting that rat HEV is restricted to its natural host (*6, 8*). However, it is not known whether animals other than rats are susceptible to rat HEV.

The Asian musk shrew (*Suncus murinus*), also called the Asian house shrew, is a small mole-like mammal belonging to the family *Soricidae* (order Soricomorpha), and wild rats are classified in the family *Muridae* (order Rodentia). Musk shrews originated from the Indian subcontinent and are now found from southern Asia and Afghanistan to the Malay Archipelago and southern Japan. These shrews are commensal rodents, commonly found living in human households. We previously showed that rat HEV infection frequently occurs in wild rats in Zhanjiang City, Guangdong Province, China (*9*). Asian musk shrews share this same environment; thus, they can be exposed to rat HEV derived from wild rats.

To determine whether Asian musk shrews are a reservoir for rat HEV, we examined 260 shrews (112 males, 148 females) that were trapped in Zhanjiang City during December 2011–September 2012. Of the 260 trapped shrews, 147 were from Mazhang District (23 from a pig farm and 124 from the villages of Chiling, Chofa, Beigou, Huangwai, Houyang, and Nanpan) and 113 were from Chikan District.

Blood samples were collected from the shrews, and serum was separated by centrifugation (2,500 × *g* for 20 min at 4°C), and stored at −80°C until use. We tested the serum samples for the presence of HEV IgG and IgM antibodies by using an ELISA based on rat HEV-like particles, as described (*3*). Of the 260 samples, 27 (10.4%) were HEV IgG positive and 12 (4.6%) were HEV IgM positive. Of these, 3 IgG-positive and 1 IgM-positive samples were among the 113 samples (2.7% and 1.0%, respectively) collected from shrews in Chikan District, and 24 IgG-positive and 11 IgM-positive samples were among the 147 samples (16.3% and 7.5%, respectively) collected from shrews in 6 villages (124 total samples) and the pig farm (23 total samples) in Mazhang District. The IgG-positive rate was higher for shrews from Mazhang District than for those from Chikan District ($p<0.05$); the rates of IgM-positivity did not differ significantly. The IgG-positive rate among the 6 villages varied substantially (8.3%–71.4%) (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/19/8/13-0069-Techapp1.pdf). The IgG-positive rates were 11.6% (13/112) in male and 9.5% (14/148) in female shrews, respectively; the difference in rates between the sexes was not statistically significant.

A total of 12 IgM-positive serum samples were selected for HEV RNA testing by nested broad-spectrum reverse transcription PCR (*2*); results for 5 were positive (online Technical Appendix Table 2). The length of the nested reverse transcription PCR products was 334 nt. After the primer sequences were removed, we sequenced the remaining 281 nt corresponding to nt 4107–4387 in the C-terminal open reading frame 1 of the rat HEV genome (GU345042) (GenBank accession nos. KC473527–KC473531). Phylogenetic analysis indicated that the 5 HEV isolates were all classified into the same group as rat HEV and clearly separated into 2 clusters, A and C. Cluster A isolates were further divided into 2 subclusters, sub-A1 (CHZ-sRat-E-1107) and sub-A2 (CHZ-sRat-E-1133) (Figure). Strains CHZ-sRat-E-739, CHZ-sRat-E-1086, and CHZ-sRat-E-1129 belong to cluster C. These findings are of limited precision because of the shot sequence that was analyzed, and, thus, they may not be predictive of results obtained with complete genomes.

Rat HEV isolated from the *S. murinus* shrews shared 77.4%–99.6% nt sequence identity with other rat HEV strains; the sequences were especially similar to those of HEV isolates from wild rats in this area (GenBank accession nos. KC465990–KC466001) (online Technical Appendix Table 3). In addition, nucleotide sequences from subcluster A1 and A2 and cluster C rat and shrew strains shared 97.5%–99.6%, 96.8%–97.2%, and 94.0%–97.5% identity, respectively (online Technical Appendix Table 3). These results indicate that rat HEV infection occurs in *S. murinus* shrews and that these rodents are a reservoir for rat HEV.

Evidence indicates that rat HEV may be capable of inducing an immune response in humans; thus, this virus may be relevant to the epidemiology of HEV in humans (*10*). A key step in understanding this epidemiology is to know the reservoirs of rat HEV, especially reservoirs like *S. murinus* shrews, which live in close proximity to humans.

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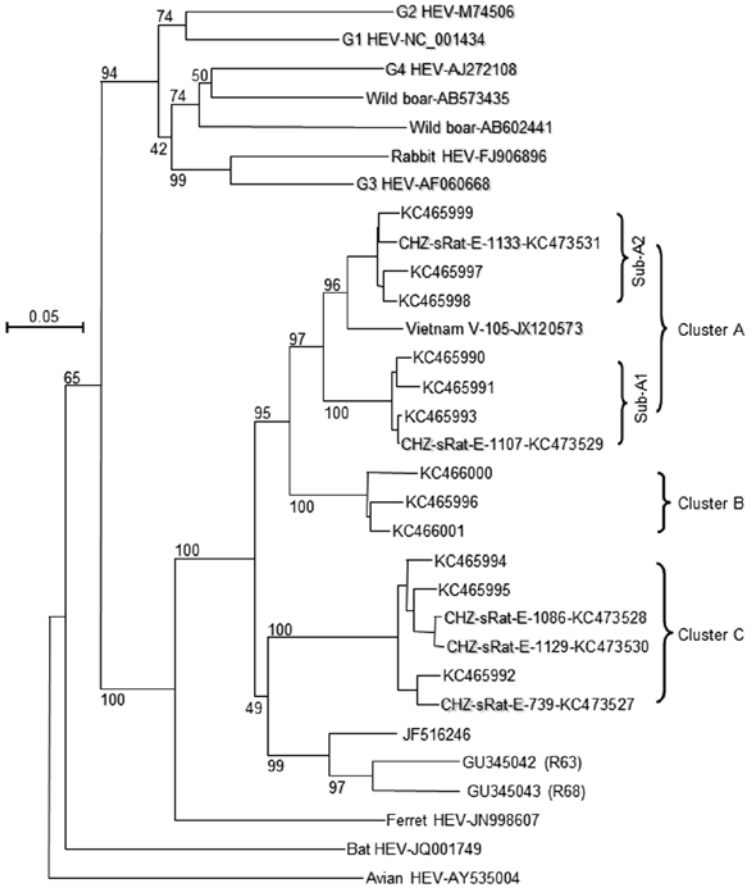

Figure. Phylogenetic analysis of rat hepatitis E virus (HEV) isolated from Asian musk shrews (*Suncus murinus*) in Zhanjiang City, China. Nucleic acid sequence alignment was performed by using ClustalX 1.81 (www.clustal.org). The genetic distance was calculated by using the Kimura 2-parameter method. The phylogenetic tree, with 1,000 bootstrap replicates, was generated by the neighbor-joining method based on the partial sequence (281 nt) of HEV open reading frame 1 of genotype 1–4, wild boar, rabbit, ferret, bat, avian, and rat HEV isolates. The scale bar indicates nucleotide substitutions per site.

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No Evidence for Hepatitis E Virus Genotype 3 Susceptibility in Rats

To the editor: Hepatitis E virus (HEV) is a positive-sense single-stranded RNA virus (genus *Hepevirus*, family *Hepeviridae*) (*1*). In humans, acute hepatitis infection caused by HEV is a serious public health concern in developing countries. Four HEV genotypes, G1–4, have been isolated from humans (*2*). G3 and G4 HEV have also been isolated from swine, wild boars, wild deer, and mongooses; these animals are thought to be the reservoirs of HEV (*3*). Direct evidence has indicated that HEV is transmitted from pigs or wild boars to humans; therefore, hepatitis E caused by G3 and G4 HEV infection is recognized as a zoonosis (*3*).

Although rats have long been suspected to be a potential reservoir for human HEV, no direct evidence has been found. The susceptibility of rats to human HEV genotypes is

controversial. For example, anti-HEV IgG has been detected in various rat species, including Norway (*Rattus norvegicus*), black (*Rattus rattus*), and cotton (*Sigmodon hispidus*) rats, by using ELISA with antigens derived from G1 HEV. These results suggest that HEV or HEV-like virus infections occur in wild rats. However, the virus genome has not been detected, and the source of the infection was confirmed in few cases; thus far, it is not clear whether the anti-HEV IgG was induced by HEV or other HEV-like viruses. The detection of a partial genome of G1 HEV from wild rats in Nepal was reported in 2002 (*4*); however, this report was retracted in 2006 because the isolated strain was determined to be a result of laboratory contamination. Recently, Lack et al. isolated strains of G3 HEV from a variety species of wild rats in the United States (*5*); this finding suggests that wild rats are hosts for G3 HEV. Maneerat et al. also reported that human HEV (presumably G1) was transmissible to Wistar laboratory rats (*6*). However, Purcell et al. recently reported that G1, G2, and G3 do not infect laboratory rats (*7*), and we found in a previous study that laboratory rats are not susceptible to G1, G3, or G4 HEV (*8*).

To further investigate the potential susceptibility of rats to infection with human HEV, we experimentally injected nude rats with G3 HEV and monitored virus growth. We used 2 samples of G3 HEV for the infection experiments, 1 derived from fecal specimens collected from a pig farm in Japan (GenBank accession no. DQ079632) and 1 derived from the supernatant of a hepatocarcinoma cell line, PLC/PRF/5, that was injected with the pig specimen. The infectivity of these samples was confirmed by experimental infections of cynomolgus monkeys (*9*; data not shown).

Six 15-week-old female nude rats (athymic rats, Long-Evans-run/run; Japan SLC, Inc., Hamamatsu, Japan) were used in this study. These rats, which are bred to be immunodeficient, are known to be susceptible to rat HEV, but it is unknown if they are susceptible to other types of HEV. All rats were negative for G3 HEV RNA and anti-HEV antibodies, as determined by nested reverse transcription PCR (*10*) and ELISA (*8*), respectively. Rats were housed individually in biosafety level 2 facilities. Experiments were reviewed by the ethics committee of the National Institute of Infectious Diseases (NIID) Japan and carried out according to the "Guidelines for animal experiments performed at NIID" under code 113060.

The 6 rats were randomly assigned to 2 groups, injected intravenously with 500 μL of an HEV sample suspension through the tail vein, and monitored for 3 months. The 3 rats in group 1 were injected with the sample derived from pig feces, which contained 5×10^4 copies of G3 HEV; the 3 rats in group 2 were injected with the cell culture supernatant sample, which contained 4×10^6 copies of G3 HEV. Serum samples were collected weekly for examination of HEV RNA and anti-HEV IgG and IgM and were also used to determine alanine aminotransferase values. Fecal samples were collected every 3 days to detect HEV RNA. The animals were humanly killed by exsanguination 91 days postinjection, liver tissues were collected, and a 10% tissue suspension was prepared as described (*8*).

For groups 1 and 2, all serum samples collected 1–13 weeks postinjection were negative for HEV RNA and anti-HEV IgG and IgM. HEV RNA also was not detected in fecal samples or liver tissues (Table). Alanine aminotransferase elevation was not observed in any serum samples.

In conclusion, even by using samples with high titers of HEV RNA in injection experiments, we were unable to cause infection with G3 HEV in immunodeficient nude rats. We found no evidence that rats are susceptible to infection with G3 HEV.

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Fatal Case of Enterovirus 71 Infection and Rituximab Therapy, France, 2012

To the Editor: Enterovirus 71 (EV-71) causes primarily asymptomatic or benign infections in children <5 years of age. However, it may cause severe and sometimes fatal neurologic complications, such as brainstem encephalitis and polio-like paralysis (*1*). Over the last 15 years, large outbreaks of EV-71 infection have been described in the Asia–Pacific region, associated with the regular emergence of new genetic lineages (*2*). Since the 1978 outbreak in Hungary, rare sporadic cases have been reported in Europe (*1*). In France, during 2000–2009, a total of 81 hospitalized patients with EV–17 infection were reported by the sentinel surveillance system, including 2 child deaths, 1 due to proven rhombencephalitis (*3*,*4*).

We report here a fatal case of EV-71 rhombencephalitis in an immunocompromised adult who was receiving rituximab therapy. Rituximab is a chimeric anti-CD20 monoclonal antibody that is widely used for treating B-cell lymphoma and an increasing number of autoimmune diseases. Since rituximab became commercially available, several infectious side-effects for the drug have been reported, including hepatitis B reactivation, progressive multifocal leukoencephalopathy, and enteroviral meningoencephalitis (*5*). The first 2 cases of rituximab-associated enteroviral meningoencephalitis were reported in 2003 (*6*), and 5 additional cases have been reported to date (*7,8*).

In May 2012, a 66-year-old woman was hospitalized in the neurology unit of Bordeaux University Hospital with a 10-day history of fever, asthenia, and psychomotor retardation. She had no history of travel and had not been in close contact with sick persons. She had received a diagnosis of grade I follicular lymphoma 3 years earlier, and it had been treated with 6 cycles of R-CHOP (rituximab, cyclophosphamide, hydroxydaunorubicin, oncovin, prednisolone). Since July 2010, the lymphoma had been in remission, and she had been receiving maintenance therapy with rituximab since that time. The most recent rituximab infusion had been administered in March 2012. Her condition was treated initially with broad-spectrum antibiotics and acyclovir. Still, aphasia, facial paralysis, spastic movements, and consciousness disorders rapidly developed. On day 6, she was transferred to the intensive care unit for ventilatory support.

On patient's admission, blood samples showed lymphopenia (0.64×10^3 cells/mm^3) and low immunoglobulin levels, i.e., IgG 4.5 g/L (reference range 6.75–12.8 g/L) and IgM 0.33 g/L (reference range 0.56–1.9 g/L). Three cerebrospinal fluid (CSF) samples were collected on days 1, 4, and 6. CSF leukocyte count rose from 5 to 89 cells/mm^3, with lymphocytes from 24% to 95%, and protein levels rose from 0.68 to 1.03 g/L (reference range 0.15–0.45 g/L). CSF glucose level varied from 3.5 to 4.5 mmol/L (reference range 2.7–3.9 mmol/L). Enterovirus RNA was detected in the patient's first 3 CSF samples and in CSF, stool specimens, and blood until 4 weeks after admission (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/19/8/13-0202-Techapp1.pdf). PCR assays of the first 3 CSF samples were negative for JC polyomavirus, herpes simplex virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, adenovirus, and *Toxoplasma gondii*. Serologic tests for parvovirus B19, mumps virus, and measles virus were IgM negative. Samples were also negative for antibodies against Hu, Ri, Yo, and voltage-gated potassium channel antigens. All bacterial cultures were negative. No evidence for central nervous system infiltration by lymphoma cells was found, on the basis of CSF cytology.

Results of brain magnetic resonance imaging (MRI) scans performed on days 2 and 6 were normal, despite the patient's consciousness disorders (Figure, panel A). However, on day 13, MRI scans showed bilateral and symmetric T2 and FLAIR hypersignals in the medulla, the pons, and the mesencephalon, compatible with rhomboencephalitis (Figure, panel B). On day 24, the MRI scan showed a supratentorial extension involving white matter, the insular cortex, and basal ganglia (Figure, panel C). The patient's neurologic condition deteriorated progressively, and she died of enteroviral rhomboencephalitis 32 days after admission.

The EV associated with the rhomboencephalitis was identified as an EV-71 genogroup C2 isolate by 1D gene complete sequencing and phylogenetic analysis (online Technical Appendix Figure; online Technical Appendix Table 2). The 1D gene sequences determined from cerebrospinal fluid and fecal specimens from the patient showed 95%–97% nucleotide

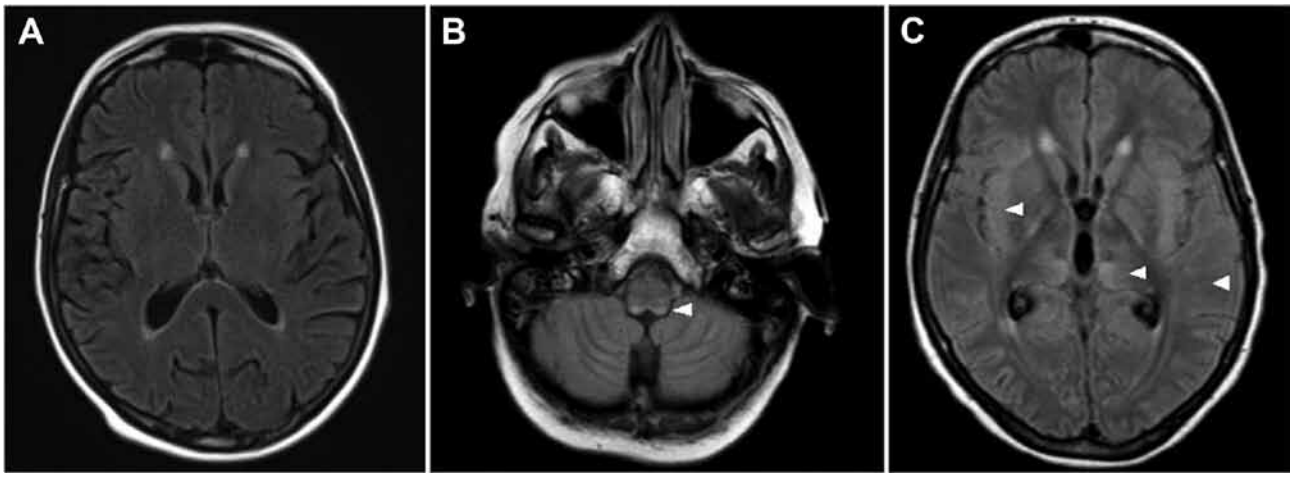

Figure. Magnetic resonance imaging axial flair sequence of brain of 66-year-old woman with fatal encephalitis, Bordeaux, France, 2012. A) No hypersignal at day 6. B) Bilateral posterior hypersignals in the medulla at day 13. C) Bilateral supratentorial hypersignals at day 24 in the cortex, the white matter, and the basal ganglia. Hypersignals are indicated by white arrowheads.

homology and clustered with 1D gene sequences from strains detected during 2006–2012 in France, the Netherlands, Germany, Spain, Canada, United Kingdom, and Singapore.

Only 7 cases of rituximab-associated EV encephalitis have been reported in the literature. Of the case-patients, 3 died from enteroviral meningoencephalitis, 1 showed partial neurologic improvement but died later from another infection (not specified), 2 suffered permanent sequelae, and 1 recovered completely (*6*–*8*).

The first case of fatal rituximab-associated EV-71 encephalitis was reported in Australia in 2011 (*8*). The Australian patient and the French patient reported here were adults, although most EV-71 encephalitis cases have been described in children (*1*). Neither adult patient exhibited neurogenic pulmonary edema. Both cases were associated with genogroup C2 EV-71 strains that were closely related to those that have been detected in recent years in Europe and worldwide (*3*).

Because invasive EV infections have been described in adults with hereditary or congenital defects in B-lymphocyte function, humoral immunity is likely to play a key role in EV infection control (*9*). Passive protection against lethal EV-71 infection in newborn mice by neutralizing antibodies is another convincing argument that the antibody-mediated response is critical (*10*). Thus, because rituximab is associated with long-lasting B-cell depletion and, in some patients, a decrease in immunoglobulin, it may lead to an increased risk for EV encephalitis. Although EV encephalitis seems to be rare in patients who receive rituximab treatment, cases may have been underdiagnosed. To detect this condition and prevent possible deaths, physicians should routinely screen for EV RNA in patients receiving anti-CD20 therapy who have neurologic symptoms and should consider the early administration of immunoglobulin.

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Norovirus Variant GII.4/Sydney/2012, Bangladesh

To the Editor: Noroviruses (NoVs) are the most common cause of foodborne and waterborne outbreaks of gastroenteritis in persons from all age groups in industrialized and developing countries (*1*). Although NoV outbreaks occur throughout the year, activity increases in the winter months, especially in the countries with a temperate climate. As expected, during the last few months of 2012, outbreaks of NoV gastroenteritis markedly increased in Europe and the United States (*2*–*4*). These increases corresponded with the emergence of a variant of genotype GII.4, Sydney/2012, which was first reported from Australia in March 2012 and, subsequently, in the United States, Belgium, Denmark, Scotland, and Japan (*2*,*5*–*7*).

We identified the NoV GII.4 variant Sydney/2012 through hospital surveillance on diarrhea etiology in Bangladesh in December 2011 and then throughout 2012. These strains came from 3 hospitals in Dhaka, Matlab, and Mirzapur, where ≈150,000 patients with diarrhea are treated annually. We randomly selected 795 fecal specimens from patients of all ages who sought treatment for diarrhea in these hospitals during 2010–2012 and detected NoV RNA in 90 (33.6%), 72 (27.9%), and 92 (34.2%) samples in 2010, 2011, and 2012, respectively, by performing real-time PCR (*8*). For characterization, we amplified and sequenced 108 samples on the basis of the capsid genes (*9*).

Ages of diarrhea patients with NoV infection ranged from 1 month to 91 years (median 15 months; mean 11.9 years). Most (66%) NoV-positive patients were <5 years of age. Infection rates were lowest in patients <3 months (2.1%) and 5–18 years (2.5%) of age. A high number of NoV infections were recorded in adults (28.8% in patients ≥18 years of age). NoVs were detected throughout the year, and no clear seasonal peaks were observed.

Overall, GII was the most predominant genogroup (66.1%), followed by GI (18.1%) and GIV (3.9%). Mixed infections were detected in 11.8% of samples. We observed a high diversity in the GII genogroup and identified at least 11 different genotypes within the group, in which GII.4 constituted 30.1% of all GII strains. Until December 2011, the GII.4 variant NewOrleans/2009 was the most predominant strain (Figure). However, the new GII.4 variant, Sydney/2012, replaced the old variant and appeared as the dominant strain in 2012. We constructed a phylogenetic tree on the basis of 1,026 bases around the junction region of *pol* and *cap* genes, and it revealed that the newly identified variant has evolved from previous NoV GII.4 variants Apeldoorn/2007 and NewOrleans/2009 (data not shown).

NoVs, old and new, remain a substantial threat to human health, with a new variant emerging every 2–3 years. The Sydney/2012 strain appears to have replaced the previously predominant strain, but its clinical effects and epidemiology are largely unknown and warrant further investigation.

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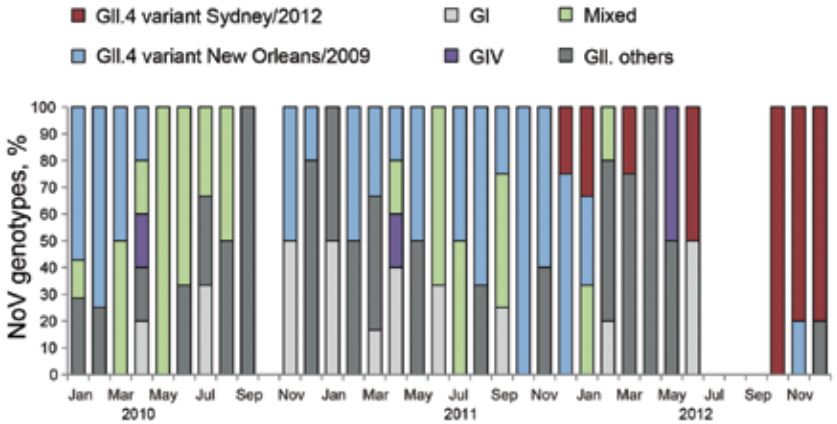

Figure. Distribution of 108 norovirus (NoV) genotypes in Bangladesh, 2010–2012. Bar chart shows the percentage of NoV genotypes. Mixed genotypes comprise NoV GI and GII. GI comprises GI.1, GI.3, GI.4, GI.5, and GI.9. GII.others comprises GII.2, GII.3, GII.4, GII.6, GII.10, GII.13, GII.16, GII.17, and GII.21.

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Norovirus GII.4/Sydney/2012 in Italy, Winter 2012–2013

To the Editor: Noroviruses (NoVs) are the major cause of acute gastroenteritis in children and adults; they are responsible for sporadic cases and outbreaks of gastroenteritis in various epidemiologic settings. NoVs can be classified genetically into at least 5 genogroups, GI to GV (*1*). Although >30 genotypes within genogroups GI, GII, and GIV can infect humans (*2*), a single genotype, GII.4, has been associated with most NoV-related outbreaks and sporadic cases of gastroenteritis worldwide (*3*).

GII.4 NoV strains continuously undergo genetic/antigenic diversification and periodically generate novel strains through accumulation of punctate mutations or recombination. New GII.4 variants emerge every 2–3 years (*4*). Increased incidence of NoV-related illness and/or outbreaks in various countries in late 2012 has been related to the emergence of a novel GII.4 variant, Sydney 2012. This variant was first identified in March 2012 in Australia (*5*).

The Italian Study Group for Enteric Viruses (ISGEV; http://isgev.net) monitors the epidemiology of enteric viruses in children through hospital-based surveillance (*6*–*8*). NoVs are monitored and characterized by multitarget analysis in the diagnostic regions A (open reading frame 1, polymerase) and C (open reading frame 2, capsid) of the NoV genome (*9*) and interrogation of the Norovirus Typing Tool database (www.rivm.nl/mpf/norovirus/typingtool). During November 2011–March 2012, the prevalence of sporadic NoV infections detected (in samples from newborns, infants, and children up to 5 years of age) by real-time reverse transcription PCR was 22.2% (121/545). A subset (≈50%) of the NoV-positive samples representative of the whole winter period was selected for sequence analysis, and 48 were successfully characterized in region A and region C.

Among these 48 NoV strains, 20 (41.7%) were characterized as the variant GII.4 New Orleans 2009, a smaller number, 6 (12.5%), displayed a New Orleans 2009 polymerase (pol) but 2 distinct GII.4 capsid sequences, which were not typeable in the Norovirus Typing Tool database, and only 2 (4.2%) GII.4 strains of the variant Den Haag 2006b were detected. Moreover, 4 sporadic cases in November 2011 and January 2012 and a small outbreak in February 2012 were related to a GII.Pe_GII.4 recombinant strain. After the set of sequences of GII.4 variants from the Norovirus Typing Tool database was updated (access to the updated database: April 11, 2013), 5 (10.4%) GII.Pe_GII.4 recombinant strains were characterized as variant Sydney 2012.

From April through October 2012, a total of 56 (7.6%) NoV-positive samples were detected from 737 analyzed samples, of which 34 (60.7%) NoV-positive samples could be sequenced. Of

these, 41.2% were characterized as GII.3 (mostly with a GII.Pb pol), 26.5% as GII.Pg_GII.1, and 17.6% as GII.4 variants. From spring to fall 2012, the variant New Orleans 2009 became the predominant GII.4 strain, and the variant Sydney 2012 strain apparently disappeared.

During November–December 2012 and January 2013, ISGEV detected NoV infection in 90 (28.9%) of 311 children hospitalized for gastroenteritis. This finding is comparable to a prevalence of 25.2% in the same period (November–January) of the 2011–12 winter season. A representative subset of 45 samples was randomly selected for sequencing, and 26 (74.3%) of 35 fully typed strains were characterized as GII.4 Sydney 2012, which suggested that the new NoV variant had become the predominant strain in Italy.

Our surveillance seem to mirror observations of a report from Denmark that documented the onset and circulation at low prevalence of the variant GII.4 Sydney 2012 at the beginning of 2012 with a marked increase in the prevalence only by the end of 2012 (*10*). Our surveillance detected the emergence of this variant in Italy at the end of 2011 and provided us with one of the earliest strains of the variant GII.4 Sydney 2012. This novel variant has a common ancestor with the NoV GII.4 variants Apeldoorn 2008 and New Orleans 2009 and has several amino acid changes on the main epitope in the capsid P2 domain (*10*).

Sequence analysis of these early strains of the GII.4 variant Sydney 2012 could help clarify the mechanisms driving its global emergence and spread. Continued surveillance for NoV infections through ISGEV and additional data on clinical and epidemiologic features will enable further assessment of the public health implications of the new variant GII.4 Sydney 2012 in Italy.

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Group C Betacoronavirus in Bat Guano Fertilizer, Thailand

To the Editor: Bats play a critical role in the transmission and origin of zoonotic diseases, primarily viral zoonoses associated with high case-fatality rates, including those caused by Nipah virus (NiV) and severe acute respiratory syndrome (SARS)–like coronavirus (CoV) infections (*1*). Recently, the World Health Organization (WHO) reported 44 confirmed cases of human infection with Middle East

respiratory syndrome CoV, resulting in 22 deaths. Full-genome and phylogenetic analyses of these Middle East respiratory syndrome CoVs have been published elsewhere (*2*). The identified viruses from 2 patients (previously referred to as England/Qatar/2012 and EMC/2012) are genetically related and belong to group C betacoronavirus, which is most related to CoVs from *Nycteris* bats in Ghana and *Pipistrellus* bats in Europe (*2*,*3*). In addition, bat CoVs HKU4 and HKU5 originated from *Tylonycteris pachypus* and *Pipistrellus abramus* bats, respectively, in the People's Republic of China (*4*). Bats are also known to harbor and transmit nonviral zoonotic pathogens, including the fungal pathogen *Histoplasma capsulatum,* which causes histoplasmosis in humans (*5*).

Bat guano is sold for use as a fertilizer in several countries, including Thailand, Indonesia, Mexico, Cuba, and Jamaica. The practice of collecting and harvesting bat guano may pose a considerable health risk because guano miners have a high level of contact and potential exposure to bat-borne pathogens. To assess pathogens in bat guano, we examined bat guano from a cave in the Khao Chong Phran Non-hunting Area (KCP-NHA) in Ratchaburi Province, Thailand, where bat guano was sold as agricultural fertilizer, for the presence of NiV, CoV, and *H. capsulatum* fungi. Bats from 14 species in 7 families have been found roosting within this area. *Tadarida plicata* bats are the most abundant species (2,500,000 bats), and 3 other species of bats found at the site each had thousands of members: *Taphozous melanopogon*, *Taphozous theobaldi*, and *Hipposideros larvatus*.

A random sample of dry bat guano, ≈100 g, was collected in a sterile plastic bag weekly from the main cave at KCP-NHA from September 2006 through August 2007. The specimens were sent for analysis by express mail (at room temperature within 2–3 days) to the WHO Collaborating Centre for Research and Training in the Viral Zoonoses Laboratory at Chulalongkorn University. Samples were frozen immediately at –80°C until nucleic acids were extracted and PCR assays were run. A total of 52 collected bat guano specimens were examined in this study.

Two aliquots of feces from each weekly specimen (104 samples total) were screened for CoV, NiV, and *H. capsulatum* by PCR. RNA was extracted from 10 mg of fecal pellet by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). CoV RNA was detected by using nested reverse transcription PCR with the degenerated primers to amplify the RNA-dependent RNA polymerase (*RdRp*) gene (*6*). NiV RNA was detected by duplex nested reverse transcription PCR (*7*). To detect *H. capsulatum* and other fungi, we extracted genomic DNA directly from bat guano by using the silica-guanidine thiocyanate protocol, NucliSense Isolation Reagent (bioMérieux, Boxtel, the Netherlands), according to the manufacturer's protocol. We tested for fungal ribosomal DNA (rDNA) in extracted total nucleic acid specimens by using the PCR protocol designed to amplify all rDNA from 4 major fungus phyla at the internal transcribed spacer 1 and 2 regions (*8*).

Four (3.8%) of 104 samples were positive for CoV. They were collected on September 2, 2006 (KCP9), October 26, 2006 (KCP12), November 14, 2006 (KCP15), and March 4, 2007 (KCP31). Three of the 4 positive CoV sequences (KCP9, KCP12, and KCP15) were identical at 152 nt of the *RdRp* region (ATCGTGCTATGCCTAATATGTGTAGGATTTTTGCATCTCTCATATTAGCTCGTAAACACAATACTTGTTGTAGTGTTTCAGACCGCTtTtATAGACTTGCaAACGAGTGTGCGCAAGTCTTGAGTGAGTATGTGCTATGTGGTGGTGGCTAT) and phylogenetically clustered with the group C betacoronavirus (Figure), with 76%, 80%, and 77% nt identity to bat CoV HKU4, bat CoV HKU5, and human CoV EMC and England1_CoV, respectively. The other CoV sequence (KCP31: ATCGTGCACTTCCCAATATGATACGCATGATTTCCGCCATGATTTTGGGATCAAAGCATGTTACTTGCTGTGACACATCTGATAAGTATTACCGTCTTTGTAATGAGCTtGCACAAGTTTTGACAGAGGTTGTTTATTCTAATGGTGGTTTC) showed 82% nt identity with bat CoV HKU8, an alphacoronavirus. Although we recognize that longer sequences or full genomes may alter the topology of the phylogeny slightly and give stronger branch support, we expect that the overall topology and placement of these CoVs would remain consistent. Samples from particular bat species could not be identified because bats of different species roost in this cave, and samples were pooled during collection for bat guano fertilizer. The detection of CoVs in bat guano from the KCP-NHA cave in Ratchaburi was consistent with the previous finding of alphacoronavirus from *Hipposideros armiger* bats from the same province in 2007, but those researchers tested fresh bat feces (*9*).

All bat guano samples screened by PCR were negative for NiV and *Histoplasma* spp. but were positive for group C betacoronavirus. The natural reservoir and complete geographic distribution of this CoV are currently unknown. Although we did not isolate live virus from these samples, the detection of nucleic acid and previous isolation of viruses from bat feces and urine (*10*) warrants some concern that guano miners might be exposed to bat pathogens in fresh excreta as well as in soil substances. We suggest that guano miners use preventive measures of personal hygiene and improved barrier protection to reduce the possibility of exposure to zoonotic pathogens.

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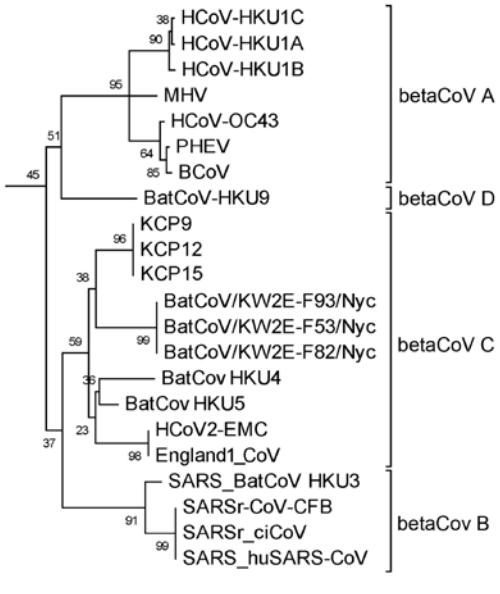

Figure. Phylogenetic tree of 3 coronaviruses (CoVs) isolated from bat guano collected in this study (KCP9, KCP12, and KCP15); 19 additional human and animal CoVs from the National Center for Biotechnology Information database are included. Construction of the tree was based on 152 nt of the *RNA-dependent RNA polymerase* gene region by maximum-likelihood method and GTR+I model with the 1,000 bootstrap resampling method implemented in MEGA5 (http://megasoftware.net/). Numbers on branches indicate percentages of bootstrap support from 1,000 replicates. The scale bar indicates the estimated 0.1 nt substitutions per site. HCoV-HKU1C, human CoV HKU1C (DQ415913); HCoV-HKU1A, human CoV HKU1A (DQ415903); HCoV-HKU1B, human CoV HKU1B (AY884001); MHV, murine hepatitis virus (NC001846); HCoV-OC43, human CoV OC43 (AY585229); PHEV, porcine hemagglutinating encephalomyelitis virus (DQ011855); BCoV, bovine CoV (AF391541); BatCoV-HKU9, *Rousettus* bat CoV HKU9 (NC009021); BatCoV/KW2E-F93/Nyc, *Nycteris* bat CoV (JX899383); BatCoV/KW2E-F53/Nyc, *Nycteris* bat CoV (JX899384); BatCoV/KW2E-F82/Nyc, *Nycteris* bat CoV (JX899382); BatCoV HKU4, *Tylonycteris* bat CoV HKU4 (NC009019); BatCoV HKU5, *Pipistrellus* bat CoV HKU5 (NC_009020); HCoV-EMC, human betacoronavirus 2c EMC/2012 (JX869059); England1_CoV, human betacoronavirus England 1 (NC_019843); SARS_BatCoV HKU3, severe acute respiratory syndrome (SARS)–related *Rhinolophus* bat CoV HKU3 (DQ022305); SARSr-CoV-CFB, SARS-related Chinese ferret badger CoV (AY545919); SARSr-ciCoV, SARS-related palm civet CoV (AY304488); SARS_huSARS-CoV, SARS human CoV (NC_004718). An expanded version is online at wwwnc.cdc.gov/EID/article/19/8/13-0119-F1.htm

PCR Detection of Microbial Pathogens

Mark Wilks, Editor

Humana Press, New York, New York, USA, 2012
ISBN-10: 1603273522
ISBN-13: 978:1603273527
Pages: 328; Price: US $84.00

This book covers general challenges of introducing primarily noncommercial PCRs and specific procedures into the laboratory, including sample treatment, extraction protocols, quality and quality assurance, and internal and external laboratory processes. The chapters on specific pathogens illustrate principles that could be applied in many diagnostic laboratories.

The editor's preface to this book is helpful in framing approaches to PCR pathogen detection methods. The focus is primarily on detection of bacterial pathogens, with the exception of *Pneumocystis* spp., and the case is made for using less expensive noncommercial strategies that enable more flexibility and customization. The book addresses the many parameters of nucleic acid preparation, buffer choice, primer construction, inhibition, cycling parameters, detection, and statistical analysis.

The ≈300 pages of text are divided in 21 chapters, of which the first 3 cover concepts of importance to all clinical laboratories using PCRs. The third chapter, which covers quality and quality assurance, is particularly comprehensive in its treatment of internal and external laboratory process and PCR controls. This chapter covers a variety of concepts, from Westguard rules for investigations of systematic and other errors, to proficiency testing, and includes many useful tables. Of importance to clinical laboratories and epidemiologic investigations alike, the authors make an essential point that up to 75% of errors in the testing process can be attributed to improper sample collection and transport of specimens, areas that often get less attention than assay quality control. The fourth chapter covers preanalytical and extraction protocols specifically for molecular detection of pathogens in whole blood, which is a particularly challenging specimen.

The remaining chapters cover a mixture of mostly real-time and some conventional PCRs targeting specific pathogens (sometimes by multiplex approaches), and 1 chapter describes a loop-mediated isothermal amplification method for detection of *Campylobacter* spp. The pathogens and techniques covered represent a good survey of approaches and vendor equipment choices. The quality of the chapters in this book varies widely, and some repetitive information is included. Overall, this book would be of interest to those involved in PCR principles and laboratory quality control. It contains examples of successful noncommercial diagnostic PCRs. If your pathogen(s) of interest are covered, it is an added bonus.

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Real-time PCR in Food Science: Current Technology and Applications

David Rodríguez-Lázaro, editor

Caister Academic Press/ Horizon Scientific Press, Poole, United Kingdom, 2013
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ISBN-13: 978-1908230157
Pages: 302; Price: US $319.00

This 302-page book describes methodologies and applications of real-time PCR in food science. In addition to detection of enteric pathogens, including foodborne and waterborne parasites, a section on food quality provides information on the use of this method to detect genetically modified organisms, allergens, and animal or plant species in food products. This book will be valuable to food scientists with an interest in real-time PCR, also known as quantitative PCR or qPCR, (not to be confused with reverse transcription PCR, or RT-PCR). This book provides a comprehensive overview of conventional and qPCR methods used to detect pathogens in contaminated foods, as well as their use in analysis of food integrity, including the detection of genetically modified organisms, allergens, and authentication of biological species in labeled foods. The book is divided into 3 sections.

The first section, comprising 4 chapters, provides a detailed examination of basic methodologies of qPCR. The inclusion of an introduction to these methodologies, sample preparations, assay design, and the role of controls is beneficial for new scientists and experienced readers. As a bonus, color plates are included.

The second and third sections span 14 chapters. Each of the chapters covering detection of enteric pathogens is organized similarly, which allows the reader to quickly

compare and locate information. Bacterial pathogens are discussed in separate chapters; viruses and foodborne and waterborne parasitic pathogens are covered in 2 comprehensive chapters. Each chapter includes background information about organism types, tables of published assays, current methods, the use of controls, approaches to determine limits of detection, and current challenges. A compelling chapter on standardization of qPCR methods compares the International Organization for Standardization and the European Organization for Standardization platforms, giving the reader a glimpse into international processes for creating standards.

Rounding out this text are chapters focused on the use of qPCR to detect allergens, gluten, and genetically modified organisms and chapters addressing authentication of animal or plant species present in labeled foods. This book provides an excellent, detailed guide for anyone interested in development and use of qPCR platforms for food safety, quality, and microbiology.

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Public Health in East and Southeast Asia: Challenges and Opportunities in the Twenty-First Century

Roger Detels, Sheena G. Sullivan, Chorh Chuan Tan, editors

University of California Press, Berkeley, California, USA, 2012
ISBN-10: 193816900X
ISBN-13: 978-1938169007
Pages: 360; Price: US $44.95

Writing a book about public health in East and Southeast Asia is a daunting challenge. Comprising Mongolia to the north and Indonesia to the south, with all the countries in between, East and Southeast Asia are home to >2 billion persons and include the full economic and development spectrum of nations in the 21st century. Because of this regional complexity, providing a cohesive, comprehensive overview of public health issues, which involves making generalizations while trying to provide the right level of detail and contrast, is an ambitious goal. However, it was not completely met by this text.

The book addresses such topics as the area's changing societal norms and lifestyles, emerging infectious and chronic diseases, nutrition, tobacco use, injuries, occupational health, health services, and globalization. Its strengths include the chapters on chronic diseases, tobacco, and injuries, which provide a good general overview of these issues in the region, with a detailed look at mental health issues. The chapters on infectious diseases are sparse in detail, however, and lack in-depth discussions of the context that places Asia at such high risk for becoming the source of pandemics.

A rather confusing organization places a description of the control of emerging and other communicable diseases in a separate section of the book (the health services section). However, besides a few redundancies in the chapters, thought-provoking discussions on economics are provided. Data from specific countries are presented somewhat randomly throughout the chapters, sometimes resulting in fragmented discussions.

This volume may be a useful addition to those studying public health issues in East and Southeast Asia, especially its sections on chronic diseases, injuries, and tobacco. Nonetheless, this book should be supplemented by more detailed texts for in-depth studies of individual countries or disease states.

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Catherine M. Howell (1892–1975) *Oyster Shuckers* (1934) (detail) Oil on canvas (101.4 cm × 91.6 cm) Smithsonian American Art Museum, Transfer from the US Department of the Interior, National Park Service, Washington, DC, USA

Ripped, Shucked, and Scattered

Polyxeni Potter

"The man sure had a palate covered o'er / With brass or steel, that on the rocky shore / First broke the oozy oyster's pearly coat / And risk'd the living morsel down his throat," wrote John Gay (1685–1732), one of many poets since antiquity who became fascinated with the subject. More recently, Irish bard Seamus Heaney after enjoying fresh oysters with some friends, was so moved that he wrote a poem, "Laying down a perfect memory / In the cool of thatch and crockery," and affirming the general idea that those of us who have never swallowed an oyster may not have lived life to the fullest. "Our shells clacked on the plates. / My tongue was a filling estuary, / My palate hung with starlight: / As I tasted the salty Pleiades / Orion dipped his foot into the water."

As if the experience were not enough, Heaney offered a little history, "Over the Alps, packed deep in hay and snow," he wrote, "the Romans hauled their oysters south to Rome." The proper way of moving oysters from place to place has not changed much. Nor has the process of growing, harvesting, shucking, or eating oysters changed. "Alive and violated, / They lay on their bed of ice: / Bivalves: the split bulb / And philandering sigh of ocean / Millions of them ripped and shucked and scattered."

Their silky texture and taste of the sea alone would have made oysters a popular food, not to mention their rich nutritional value and simple abundance. In the New World, Native Americans appreciated them, as did the invading Spaniards, even if only for the pearls. In the 1800s, consumption of the eastern oyster outpaced beef as a source of protein in some regions. In Louisiana, various ethnic groups settled in local parishes and contributed to the oyster industry. In the mid-1840s, fishermen started to gather seed oysters, plant them in favorable spots, and allow them to grow to market size in estuaries near the Mississippi River and in coastal areas farther west, creating one of the most successful oyster cultivation industries in the country. The modern harvesting processes came about in the early 1900s. As for readying oysters for market, despite attempts to mechanize the process, commercial oyster shucking remains the method of choice. Though experienced shuckers can glean large quantities of meat very quickly, efficiency comes at the expense of sound labor practices. Oyster shucking is marred, in the very least, by the monotony of processing and the cacophony of pounding blades.

This soul-testing occupation, labor-intensive and dangerous, usually in frigid environment and in the face of seemingly inexhaustible harvest, is what Catherine Howell captured in *Oyster Shuckers*, on this month's cover. In this scene, painted in New Orleans (as inscribed on the upper left canvas), workers go at the task leaned over an overloaded bench. Their faces and clothes are sympathetically cast in broad impressionist strokes and lit from the window. Despite the need to handle each specimen separately and the pressure to deliver the oyster whole and the shell undamaged, this is an assembly line. Abject boredom marks the vacant faces. This is piece work–the more oysters shucked, the more money made.

Not much is known about Catherine Howell, other than she studied at the Art Students League of New York and the School of the Art Institute of Chicago. Nor is it known why she selected oyster shucking as the subject of this painting. But, a native of East Feliciana Parish, Louisiana, she was clearly aware of the oyster industry amidst the poverty of her times. She was also interested in local history, having coauthored in 1936 The Perfect Blend of the Old and the New: a Story of the Famous Vieux Carré [French Quarter] of New Orleans.

Along with many others, Howell took advantage of the Public Works of Art Project (December 1933 to June 1934), a program, part of the New Deal, set up to support artists during the Great Depression. First of its kind, this program affirmed the value of art as a legitimate occupation needed in reconstructing a society unhinged by economic catastrophe. "Work must be found for artists as well as for longshoremen." Or as President Roosevelt's relief administrator put it, "They've got to eat just like other people." Applicants had to prove they were professional artists, and they had to pass a needs test. Most who took the job were young. After doing their work for the nation, they returned to local or regional occupations and remain mostly unknown today. The art they produced was for the most part conservative by modern

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standards, but at the time, "It was a revelation to many people in America that the country even had artists in it."

The newly hired workers were encouraged to paint the contemporary American scene: the cities and countryside, harbors and sidewalks, factories and coal mines, farms and orchards, church halls and baseball fields of everyday people at work and play, the cotton pickers, the restaurant and mill workers. Along the same lines, Catherine Howell's *Oyster Shuckers* was chosen by the Roosevelts to hang in the White House. In addition to supporting unemployed artists, the arts project aimed to improve the appearance of public buildings and embellish common areas, bringing to the local population pride in their surroundings. Among buildings that benefited in Howell's area were 18 Louisiana Post Offices, which received murals. The program was not without its critics, who decried having taxpayer money used for decoration.

Government programs, whether for the advancement of art or the promotion of public health, are always under scrutiny, sometimes for their perceived frivolity but most often for their cost-effectiveness or economic fallout. In addition to immediate financial benefit and value as a morale booster, New Deal art has left behind a precious legacy, an artistic record of the times. "One hundred years from now," President Roosevelt predicted, "my administration will be remembered for its art, not its relief." The same philosophy could well apply to public health. U.S. Government-funded disease surveillance systems, which have grown swiftly in scope and sophistication, are providing data for immediate improvements in health. At the same time, by exploring the effects of disease, as well as virus evolution and structure, vaccination, and other disease prevention measures, they also increase understanding of problems that have puzzled us since the beginning of time.

Author and philosopher Pliny the Elder discussed Roman fondness for oysters at great length. The best, he maintained, were found at the mouths of rivers. "It is hardly possible to say enough about them, for they have held first rank as a table delicacy for a long time." His compatriots generally ate oysters raw, sometimes served covered with snow, often in large quantities. Emperor Clodius Albinus, known for his gluttony, was said to consume 400 at one sitting. "Oysters must be permitted when wanted, but seldom, because they are cold and phlegmatic," wrote Greek physician Anthimus in his cookbook On the Observance of Foods. "But if oysters smell, and anyone eat of them, he has need of no other poison." Anthimus' observation in the 6th century was remarkably astute, despite the generally unreliable association between spoilage and safety.

Consumption of raw seafood has a long and storied past, and so does gastrointestinal illness associated with some shellfish, especially raw or undercooked oysters. One reason is their filter-feeding nature, which allows them to passively concentrate bacteria and viruses; another is their minimal processing and cooking before consumption. Advances in laboratory techniques and epidemiologic methods have honed in on the specific causes of enteric diseases, long perceived to be primarily bacterial or unknown. Contaminated oysters are now frequently implicated in norovirus outbreaks across the globe.

Despite sewage control and improvements in hygiene, enteric diseases caused by contaminated food and water or spread from person to person remain far too common. In the United States, norovirus is the leading cause of gastroenteritis. Food and friendship aside, in the case of oysters, poetry must still reside in a balanced combination of pleasure and responsibility. And as during the Public Works of Art Project, a long-term solution may also lie in art, this time the art of isolating pathogenic agents and gathering surveillance data.

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Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 70% passing score) and earn continuing medical education (CME) credit, please go to www.medscape.org/journal/eid. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the New Users: Free Registration link on the left hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/category/2922.html. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for AMA PRA Category 1 Credits™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the certificate and present it to your national medical association for review.

Article Title
Extended-Spectrum β-Lactamase–producing *Enterobacteriaceae* among Travelers from the Netherlands

CME Questions

1. You are seeing a 50-year-old woman for a routine physical exam before she embarks on a 3-month trip around the world. She is concerned regarding the possibility of illness during her travels. Based on the current study by Paltansing and colleagues, what should you consider regarding acquisition of multidrug-resistant *Enterobacteriaceae* (MDR-E) during travel?

A. Incident colonization occurred in approximately 80% of travelers
B. Incident colonization occurred in approximately 30% of travelers
C. MDR-E was not found among any individual prior to travel
D. Over 90% of MDR-E found after travel was carbapenemase-producing *Enterobacteriaceae* (CPE)

2. What can you tell this patient was the region most associated with colonization with MDR-E in the current study?

A. Eastern Europe
B. Sub-Saharan Africa
C. Asia
D. Central America

3. Which of the following was the most significant risk factor for colonization with MDR-E after returning from travel?

A. Colonization with MDR-E prior to travel
B. Older age
C. Recent antibiotic use
D. A history of diarrhea during travel

4. What else should you consider regarding the acquisition of extended-spectrum β-lactamase–producing Enterobacteriaceae (ESBL-E) during travel in the current study?

A. CTX-M enzymes were the predominant ESBLs
B. Coresistance to other antibiotics was rare among ESBL-E
C. ESBL-E was not found among any carrier after 6 months of follow-up
D. ESBL-E was not found among household contacts of carriers

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 70% passing score) and earn continuing medical education (CME) credit, please go to **www.medscape.org/journal/eid**. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the New Users: Free Registration link on the left hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/category/2922.html. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for *AMA PRA Category 1 Credits*™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the certificate and present it to your national medical association for review.

Article Title
Effects and Clinical Significance of GII.4 Sydney Norovirus, United States, 2012–2013

CME Questions

1. You are seeing a 30-year-old man for a 2-day history of acute gastroenteritis symptoms. Which would you consider regarding the epidemiology of norovirus infections as you evaluate this patient?

A. Noroviruses are the most common cause of epidemic gastroenteritis worldwide
B. Norovirus outbreaks are equally spread throughout the calendar year
C. Most norovirus outbreaks are caused by genotype GI.2
D. New norovirus strains inevitably lead to new outbreaks

2. What was the most common setting for infection with norovirus in the current study?

A. Day care center
B. Healthcare setting
C. School
D. Workplace

3. According to the current study, which of the following statements regarding norovirus outbreaks during the 2012–2013 season is most accurate?

A. The number of outbreaks was 5 times higher in 2012–2013 compared with previous years
B. Outbreaks occurred earlier in the year in 2012–2013 compared with previous years
C. Outbreaks were 50% longer in 2012–2013 compared with previous years
D. Genotype GII.4 Sydney accounted for far more outbreaks by February 2013

4. Which of the following statements regarding the clinical presentation and outcomes of norovirus infection in the current study is most accurate?

A. GII.4 Sydney was associated with a higher rate of vomiting and abdominal cramping compared with non-GII.4 Sydney infections
B. GII.4 Sydney was associated with a higher rate of fever compared with non-GII.4 Sydney infections
C. GII.4 Sydney was associated with a higher rate of outpatient visits compared with non-GII.4 Sydney infections
D. GII.4 Sydney was associated with a higher risk of mortality compared with non-GII.4 Sydney infections

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

EMERGING INFECTIOUS DISEASES

Upcoming Issue

Acute Encephalitis Syndrome Surveillance, Kushinagar District, Uttar Pradesh, India, 2011–2012

Detection of Diphtheritic Polyneuropathy by Acute Flaccid Paralysis Surveillance, India

Nodding Syndrome

Postexposure Prophylaxis with a Third Dose of Measles-Mumps-Rubella Vaccine during a Mumps Outbreak, New York

Antigenic and Molecular Characterization of Avian Influenza A(H9N2) Viruses, Bangladesh

Protection by Face Masks against Influenza A(H1N1)pdm09 Virus on Trans-Pacific Passenger Aircraft, 2009

Divergent Astrovirus Associated with Neurologic Disease in Cattle

Continued Evolution of West Nile Virus, Houston, Texas, 2002–2012

Underreporting of Viral Encephalitis and Viral Meningitis, Ireland, 2005–2008

High Rates of *Mycobacterium tuberculosis* among Socially Marginalized Immigrants in Low-Incidence Area, Italy, 1991–2010

Plasmodium falciparum Mutant Haplotype Infection during Pregnancy Associated with Reduced Birthweight, Tanzania

High Estimated Incidence of Encephalitis in England

Enzootic and Epizootic Rabies Associated with Vampire Bats, Peru

Staged Molecular Analysis of Unexplained Central Nervous System Infections

Quinto Tiberio Angelerio and New Measures for Controlling Plague in 16th-Century Alghero, Sardinia

Microsporidial Keratoconjunctivitis Outbreak after Rugby Tournament, Singapore

Novel Bunyavirus in Domestic and Captive Farmed Animals, Minnesota, USA

Successful treatment with benznidazole of chagasic encephalitis in a pregnant woman with AIDS

Mycobacterium chelonae Abscesses Associated with Biomesotherapy

Spread of Neisseria meningitidis Serogroup W Clone, China

Human Parainfluenza Virus Type 3 in Wild Primates

Complete list of articles in the Septemeber issue at
http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

August 24–28, 2013
2013 Infectious Disease Board Review Course
McLean, Virginia, USA
http://www.IDBoardReview.com

September 5–10, 2013
Options for the Control of Influenza VIII
Cape Town, South Africa
http://www.isirv.org

September 10–13, 2013
ICAAC 2013
Interscience Conference on Antimicrobial Agents and Chemotherapy
Denver, Colorado, USA
http://www.icaac.org

October 2–6, 2013
2nd annual IDWeek
San Francisco, CA, USA
A combined meeting of the Infectious Diseases Society of America (IDSA), the Society for Healthcare Epidemiology of America (SHEA), the HIV Medicine Association (HIVMA) and the Pediatric Infectious Diseases Society (PIDS.
http://idweek.org/

November 2–6, 2013
American Public Health Association APHA's 141st Annual Meeting and Exposition
Boston, MA, USA
http://www.apha.org

November 4–7, 2013
3rd ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications
Copenhagen, Denmark
http://www.asm.org/conferences

November 5–7, 2013
ESCAIDE 2013
European Scientific Conference on Applied Infectious Disease Epidemiology
Stockholm, Sweden
http://www.escaide.eu

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

Announcements may be posted on the journal Web page only, depending on the event date.

EMERGING INFECTIOUS DISEASES®

JOURNAL BACKGROUND AND GOALS

What are "emerging" infectious diseases?

Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include

- New infections resulting from changes or evolution of existing organisms.
- Known infections spreading to new geographic areas or populations.
- Previously unrecognized infections appearing in areas undergoing ecologic transformation.
- Old infections reemerging as a result of antimicrobial resistance in known agents or breakdowns in public health measures.

Why an "Emerging" Infectious Diseases journal?

The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and West Nile virus infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

Emerging Infectious Diseases represents the scientific communications component of CDC's efforts against the threat of emerging infections. However, even as it addresses CDC's interest in the elusive, continuous, evolving, and global nature of these infections, the journal relies on a broad international authorship base and is rigorously peer-reviewed by independent reviewers from all over the world.

What are the goals of Emerging Infectious Diseases?

1) Recognition of new and reemerging infections and understanding of factors involved in disease emergence, prevention, and elimination. Toward this end, the journal

   - Investigates factors known to influence emergence: microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.
   - Reports laboratory and epidemiologic findings within a broader public health perspective.
   - Provides swift updates of infectious disease trends and research: new methods of detecting, characterizing, or subtyping pathogens; developments in antimicrobial drugs, vaccines, and prevention or elimination programs; case reports.

2) Fast and broad dissemination of reliable information on emerging infectious diseases. Toward this end, the journal

   - Publishes reports of interest to researchers in infectious diseases and related sciences, as well as to public health generalists learning the scientific basis for prevention programs.
   - Encourages insightful analysis and commentary, stimulating global interest in and discussion of emerging infectious disease issues.
   - Harnesses electronic technology to expedite and enhance global dissemination of emerging infectious disease information.

Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles, see below and visit http://wwwnc.cdc.gov/eid/pages/author-resource-center.htm.

Emerging Infectious Diseases is published in English. To expedite publication, we post some articles online ahead of print. Partial translations of the journal are available in Japanese (print only), Chinese, French, and Spanish (http://wwwnc.cdc.gov/eid/pages/translations.htm).

Instructions to Authors

Manuscript Submission. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid). Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch), verifying the word and reference counts, and confirming that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist.

Manuscript Preparation. For word processing, use MS Word. List the following information in this order: title page, article summary line, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, and figure legends. Appendix materials and figures should be in separate files.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit editable figures as separate files (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .tif or .jpeg files. Do not embed figures in the manuscript file. Use Arial 10 pt. or 12 pt. font for lettering so that figures, symbols, lettering, and numbering can remain legible when reduced to print size. Place figure keys within the figure. Figure legends should be placed at the end of the manuscript file.

Videos. Submit as AVI, MOV, MPG, MPEG, or WMV. Videos should not exceed 5 minutes and should include an audio description and complete captioning. If audio is not available, provide a description of the action in the video as a separate Word file. Published or copyrighted material (e.g., music) is discouraged and must be accompanied by written release. If video is part of a manuscript, files must be uploaded with manuscript submission. When uploading, choose "Video" file. Include a brief video legend in the manuscript file.

Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome.

Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no abstract, figures, or tables. Include biographical sketch.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. Provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

Announcements. We welcome brief announcements of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.