EMERGING INFECTIOUS DISEASES

Tracking trends and analyzing new and reemerging infectious disease issues around the world

A peer-reviewed journal published by the National Center for Infectious Diseases

Vol. 5, No. 5, Sept–Oct 1999

Foodborne Illness

Pandemic Influenza

C. parvum in Oysters

DEPARTMENT OF HEALTH AND HUMAN SERVICES

CDC

CENTERS FOR DISEASE CONTROL AND PREVENTION

EMERGING INFECTIOUS DISEASES

Tracking trends and analyzing new and reemerging infectious disease issues around the world

A peer-reviewed journal published by the National Center for Infectious Diseases

Volume 5, Number 5
September–October 1999

Cover: Jan Steen. Girl Eating Oysters (c.1658/1660). Reprinted with permission of Mauritshuis, The Hague, the Netherlands.

Letters

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

Perspectives

Synopses

Research

EMERGING INFECTIOUS DISEASES

Tracking trends and analyzing new and reemerging infectious disease issues around the world

A peer-reviewed journal published by the National Center for Infectious Diseases

Volume 5, Number 5
September–October 1999

Dispatches

Book Review

News and Notes

EMERGING INFECTIOUS DISEASES

Tracking trends and analyzing new and reemerging infectious disease issues around the world

A peer-reviewed journal published by the National Center for Infectious Diseases

The journal is distributed electronically and in hard copy and is available **at no charge**.

☐ YES, I would like to receive Emerging Infectious Diseases.

Please print your name and business address in the box and return by fax to 404-639-3075 or mail to
EID Editor
CDC/NCID/MS C12
1600 Clifton Road, NE
Atlanta, GA 30333

Moving? Please give us your new address (in the box) and print the number of your old mailing label here_____

In Index Medicus/Medline, Current Contents, Excerpta Medica, and other databases

Editorial Board

Abdu F. Azad, Baltimore, Maryland, USA
Johan Bakken, Duluth, Minnesota, USA
Barry J. Beaty, Ft. Collins, Colorado, USA
Gus Birkhead, Albany, New York, USA
Martin J. Blaser, Nashville, Tennessee, USA
S.P. Borriello, London, United Kingdom
Donald S. Burke, Baltimore, Maryland, USA
Charles Calisher, Ft. Collins, Colorado, USA
Arturo Casadevall, Bronx, New York, USA
Thomas Cleary, Houston, Texas, USA
Barnett L. Cline, New Orleans, Louisiana, USA
J. Stephen Dumler, Baltimore, Maryland, USA
Durland Fish, New Haven, Connecticut, USA
Richard L. Guerrant, Charlottesville, Virginia, USA
Brian Gushulak, Geneva, Switzerland
Scott Halstead, Bethesda, Maryland, USA
Seyed Hasnain, New Delhi, India
David L. Heymann, Geneva, Switzerland
Walter Hierholzer, New Haven, Connecticut, USA
Dagmar Hulìnskà, Prague, Czech Republic
Peter B. Jahrling, Frederick, Maryland, USA
Suzanne Jenkins, Richmond, Virginia, USA
Mohamed A. Karmali, Toronto, Ontario, Canada
Richard Krause, Bethesda, Maryland, USA
Bruce R. Levin, Atlanta, Georgia, USA
Myron Levine, Baltimore, Maryland, USA
Stuart Levy, Boston, Massachusetts, USA
John E. McGowan, Jr., Atlanta, Georgia, USA
Patrick S. Moore, New York, New York, USA
Philip P. Mortimer, London, United Kingdom
Fred A. Murphy, El Macero, California, USA
Barbara E. Murray, Houston, Texas, USA
James M. Musser, Houston, Texas, USA
Neal Nathanson, Philadelphia, Pennsylvania, USA
Rosanna W. Peeling, Winnipeg, Manitoba, Canada
David H. Persing, Rochester, Minnesota, USA
Richard Platt, Boston, Massachusetts, USA
Didier Raoult, Marseille, France
David Relman, Palo Alto, California, USA
Rebecca Rico-Hesse, San Antonio, Texas, USA
Connie Schmaljohn, Frederick, Maryland, USA
Robert Shope, Galveston, Texas, USA
Peter Small, Stanford, California, USA
Bonnie Smoak, US Army Medical Research Unit, Kenya
Rosemary Soave, New York, New York, USA
P. Frederick Sparling, Chapel Hill, North Carolina, USA
G. Thomas Strickland, Baltimore, Maryland, USA
Jan Svoboda, Prague, Czech Republic
Robert Swanepoel, Sandringham, South Africa
Phillip Tarr, Seattle, Washington, USA
Lucy Tompkins, Stanford, California, USA
Elaine Tuomanen, New York, New York, USA
David Walker, Galveston, Texas, USA
Burton W. Wilcke, Jr., Burlington, Vermont, USA
Mary E. Wilson, Cambridge, Massachusetts, USA
Washington C. Winn, Jr., Burlington, Vermont, USA

Liaison Representatives

David Brandling-Bennett, WHO, USA
Gail Cassell, Lilly Research Lab, USA
Joseph Losos, Dept. Health, Canada
Gerald L. Mandell, U. Va. Sch. Med., USA
William J. Martone, NFID, USA
Mahomed Patel, NCEPH, Australia
Roberto Tapia-Conyer, Sec. de Salud, México
Kaye Wachsmuth, USDA, USA

Editors

Joseph E. McDade, Editor-in-Chief
Atlanta, Georgia, USA

Stephen S. Morse, Perspectives Editor
New York, New York, USA

Phillip J. Baker, Synopses Editor
Bethesda, Maryland, USA

Stephen Ostroff, Dispatches Editor
Atlanta, Georgia, USA

Patricia M. Quinlisk, Letters Editor
Des Moines, Iowa, USA

Polyxeni Potter, Managing Editor
Atlanta, Georgia, USA

International Editors

Patrice Courvalin
Paris, France

Keith Klugman
Johannesburg, Republic of South Africa

Takeshi Kurata
Tokyo, Japan

S.K. Lam
Kuala Lumpur, Malaysia

John S. MacKenzie
Brisbane, Australia

Hooman Momen
Rio de Janeiro, Brazil

Sergey V. Netesov
Novosibirsk Region, Russian Federation

V. Ramalingaswami
New Delhi, India

Diana Walford
London, United Kingdom

Editorial and Computer Support

Maria T. Brito
Teresa M. Hood
Ava W. Navin
Dwight Williams

Electronic Access

Retrieve the journal electronically on the World Wide Web (WWW), through file transfer protocol (FTP), or by electronic mail (e-mail).

Access the journal at http://www.cdc.gov/eid or from the CDC home page (http://www.cdc.gov), or download it through anonymous FTP at ftp.cdc.gov (files can be found in the directory pub/EID).

To subscribe to an e-mail list, send an e-mail to listserv@cdc.gov with the following in the body of your message: subscribe listname (e.g., subscribe EID-TOC). EID-TOC will send announcements of new table of contents automatically to your e-mail box.

Emerging Infectious Diseases

Emerging Infectious Diseases is published four to six times a year by the National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, Mailstop C-12, Atllanta, GA 30333, USA. Telephone 404-639-4856, fax 404-639-3075, e-mail eideditor@cdc.gov.

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of CDC or the institutions with which the authors are affiliated.

All material published in Emerging Infectious Diseases is in the public domain and may be used and reprinted without special permission; proper citation, however, is appreciated.

Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

∞ Emerging Infectious Diseases is printed on acid free paper that meets the requirements of ANSI/NISO 239.48-1992 (Permanence of Paper).

EMERGING INFECTIOUS DISEASES

Tracking trends and analyzing new and reemerging infectious disease issues around the world

A peer-reviewed journal published by the National Center for Infectious Diseases

The journal is distributed electronically and in hard copy and is available **at no charge**.

☐ YES, I would like to receive Emerging Infectious Diseases.

Please print your name and business address in the box and return by fax to 404-639-3075 or mail to
EID Editor
CDC/NCID/MS C12
1600 Clifton Road, NE
Atlanta, GA 30333

Moving? Please give us your new address (in the box) and print the number of your old mailing label here_____

Using a Spatial Filter and a Geographic Information System to Improve Rabies Surveillance Data

Andrew Curtis
Louisiana State University, Baton Rouge, Louisiana, USA

The design and coordination of antirabies measures (e.g., oral vaccine and disease awareness campaigns) often depend on surveillance data. In Kentucky, health officials are concerned that the raccoon rabies epizootic that has spread throughout the east coast since the late 1970s could enter the state. The quality of surveillance data from Kentucky's 120 counties, however, may not be consistent. This article presents a geographic model that can be used with a geographic information system (GIS) to assess whether a county has a lower number of animals submitted for rabies testing than surrounding counties. This technique can be used as a first step in identifying areas needing improvement in their surveillance scheme. This model is a variant of a spatial filter that uses points within an area of analysis (usually a circle) to estimate the value of a central point. The spatial filter is an easy-to-use method of identifying point patterns, such as clusters or holes, at various geographic scales (county, intraurban), by using the traditional circle as an area of analysis or a GIS to incorporate a political shape (county boundary).

Two raccoon rabies epizootics have been spreading from separate sites in the eastern United States. The original site was in Florida in the 1950s (1), the second in West Virginia/ Virginia in 1977 as a result of the importation of rabid raccoons from Florida by hunters (2). In 1994, the two rabies waves met in North Carolina (3). Kentucky has so far not been affected by the epizootic because of geographic barriers—the Appalachian Mountains in the east and the Ohio River to the north of the state.

Raccoon rabies could enter Tennessee and from there move relatively unhindered into the Bluegrass area of central Kentucky (4). Raccoons are the wild animal most frequently submitted for rabies testing in Kentucky (150, 145, and 169 animals in 1995-97, respectively), even though the annual number of positive cases remains low (1 to 2 cases per year). These positive cases appear to occur as a result of spillover infection with skunk rabies, a variant of the virus that differs from the current raccoon rabies epizootic on the eastern seaboard. The high number of raccoons submitted for testing, however, indicates potential for an increase in raccoon rabies similar to that in eastern seaboard states (5-7). In New York State, for example, the number of rabid raccoons increased from 0 in 1985 to >2,000 in 1993.

Surveillance data are useful in coordinating the placement of oral vaccination containment lines to limit the spread of the disease or quickly identify clusters of rabies beyond the containment zone. These data usually are provided by county officials or by the public after animal-human interaction (bite, scratch) or after an encounter with an animal exhibiting rabies symptoms. These data underrepresent the actual extent of the disease, because not all animals with rabies interact with people and not all interactions are reported (5,8,9). The quality of surveillance data varies among counties for several reasons (10, unpub. data). According to multivariate analysis, the number of animals submitted for testing was positively correlated with the number of people living in the county and negatively correlated with distance to the state testing facility in Frankfort (unpub. data).

Address for correspondence: Andrew Curtis, E110 Howe/ Russell Geoscience Complex, Louisiana State University, Baton Rouge, LA 70803-4105, USA; fax: 225-388-4420; e-mail: acurti1@lsu.edu.

We describe a method that can improve the quality of surveillance data by identifying counties that submit lower numbers of animals for testing than their surrounding counties. This method is a variation of a spatial filter that is calculated within a geographic information system (GIS).

Using a Spatial Filter in a GIS

When counties that submit few animals for rabies testing are surrounded by counties with high numbers of submissions, underreporting is suspected. Different methods of data analysis can produce different visual (mapped) results. Conclusions, and resulting policy actions, therefore may vary according to the method chosen.

Figure 1 shows three maps that can be generated from the rabies surveillance data:

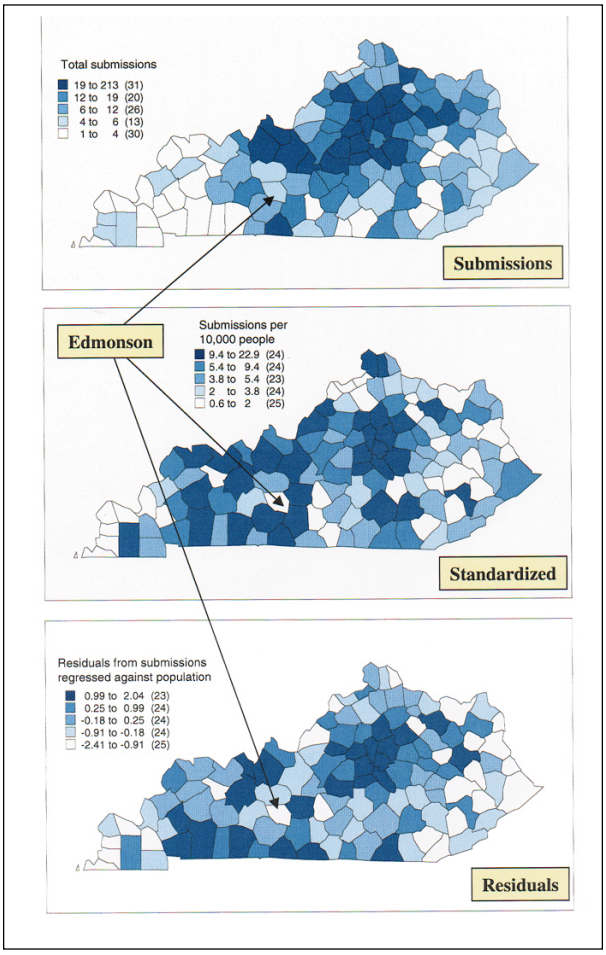


Figure 1. Comparison of data analysis for identifying counties with low submission rates.

actual numbers of submissions; standardized submissions (submissions per 10,000 human population); and the mapped residuals of a regression of submissions compared with the human population. Results can also vary according to the classification scheme chosen, that is, the quantity and range of data assigned to each color category on the map. The size and shape of the area investigated (county units, zip codes, census tracts) may also produce different results, depending on the chosen political unit—an effect known in geographic research as the Modifiable Area Unit Problem (11).

Spatial filter models are methods of exploratory spatial analysis that smooth point data, allowing values for central data points to be calculated. The traditional spatial filter technique, as applied to medical research, involves placing a grid over the investigated area, with the intersections of the grid lines providing the centers of a series of overlapping circles. Rates are then calculated for these circles to give a continuous surface. This approach has the potential to better replicate distribution of a disease, because diseases do not usually follow political boundaries. An important aspect of the spatial filter is the size of the circle, i.e., the area over which the analysis is performed, which can affect the analysis result (12).

Applying the Spatial Filter to the Number of Animals Submitted for Rabies Testing in Kentucky

In this Kentucky example, the spatial filter is the county to be investigated and a sphere of influence around it. GIS modifies the shape of the filter to include a buffer that follows the exact shape of the county. The size of the filter is the extension of the buffer beyond the county boundary. The total number of points (numbers of animals submitted for rabies testing) that fall within the filter is then randomly distributed across the area, and this randomization is repeated 100 times. If the number of randomly generated points in the investigated county is lower than the actual number of surveillance points in fewer than 5 of the 100 random runs, there is a 95% chance that a significantly low frequency (number of animals submitted for testing) was reported from that county.

Applying the Spatial Filter to Surveillance Data in Kentucky

The different maps of animals submitted for rabies testing in Kentucky for 1997 (Figure 1) confirm that several counties appear to submit low numbers of animals. For example, one county we investigated, Edmonson, although appearing to be a 'hole' in both the map of raw animal submission numbers and the map of residuals generated by the regression of submissions compared with the human population, does not appear to have underreported when the data are standardized by the human population. A benefit of the spatial filter is that it can be applied to any variable with a spatial location. This analysis depends on an initial visual interpretation because the data are aggregated at the county level.

In 1997, two animals were submitted for testing from Edmonson County. The surrounding five counties submitted 98 animals for testing, for a total of 100 submissions from the six-county area (Figure 2a). The first step is to randomly distribute all submissions across the county of origin (Figure 2b). Using the actual origin of the animal would improve the analysis, as the more traditional spatial filter method could be used; however, this information is not available from the submissions record. The second step is to layer a grid of coordinates across the six-county area (Figure 2c). This grid consists of closely packed coordinates onto which the submissions for each county can be randomly assigned. The spatial filter is then centered on Edmonson County and a buffer of 5 km is drawn. The buffer size of 5 km provides a standard buffer that could be used for any county, and which would, on average, match the area of an investigated county. The randomly distributed animal submissions for each of the surrounding five counties are then displayed to see how many animals were submitted for testing from the buffered area (Figure 2d). The simulation can be repeated several times, and a histogram can be used to identify the most frequent number of animal submissions from the buffer being chosen for the filter analysis. For this example, 12 animals were allocated to the area of Edmonson County and its surrounding 5-km buffer (two from Edmonson County and 10 from the buffer area). Within a GIS these 12 points are randomly redistributed across the area of Edmonson County plus buffer. This simulation procedure is

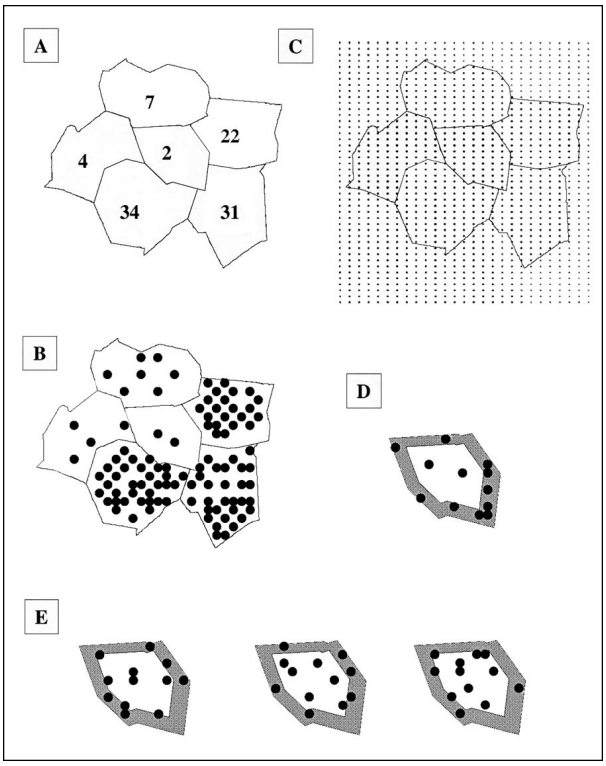


Figure 2. Application of spatial filter with a geographic information system.

repeated 100 times (Figure 2e displays 5, 6, and 8 submissions falling on the central county). The number of points falling just within Edmonson County for each of the random runs was recorded. In 100 runs, the fewest number of points (animal submissions) allocated to Edmonson County was three. Thus, the number of submissions is significantly low compared with those from the surrounding counties. Our analysis did not replicate the number of submissions from Edmonson County in 1997 in any of the 100 simulations generated from a data distribution that matched the surveillance data of 1997.

The lower count from Edmonson County may result from a smaller human population, more limited animal habitat, or fewer human-animal interactions. The landscape of the buffer may also differ from that of the surrounding counties, making it poor habitat for raccoons. This technique identifies 'holes' in data, prompting further investigation for causes.

Improvements to the Filter

The most important improvement in using the spatial filter technique is that current

submission records should start to contain precise spatial locations. Surveillance data from other states contain a spatial location, such as the distance from a road intersection (6), obtained in postrabies confirmation interview. A Global Positioning System, or even a systematic method to locate submissions according to a paper map reference system, would allow the spatial filter to be calculated as a series of overlapping circles that do not depend on political boundaries.

The technique presented in this article provides an investigator, such as a state health official, with a quick and accurate method of identifying statistically significant data holes in a surface of animals submitted for rabies testing. A decision can then be made as to whether the 'hole' is expected or needs further investigation. This technique can also be applied to any point data surface where the identification of a 'hole' is important, e.g., if one part of a suburb has fewer Lyme disease cases than its surrounding areas.

Acknowledgments

The author thanks M.B. Auslander for his continued support and R. Mitchelson for his critical review of this manuscript.

This work was funded by a research grant from Morehead State University.

Dr. Curtis is an instructor at the Department of Geography and Anthropology at Louisiana State University. His research interests include developing spatial analysis within a GIS environment, with a particular emphasis on medical data.

References

1. Bigler WJ, McLean RG, Trevino HA. Epizootiologic aspects of raccoon rabies in Florida. Am J Epidemiol 1973;98:326-35.
2. Jenkins SR, Winkler WG. Descriptive epidemiology from an epizootic of raccoon rabies in the middle Atlantic states, 1982-1983. Am J Epidemiol 1987;126:429-37.
3. Krebs JW, Strine TW, Smith JS, Rupprecht CE, Childs JE. Rabies surveillance in the United States during 1994. J Am Vet Med Assoc 1995;207:1562-75.
4. Taylor L. Raccoons expected to spread risk in Ky. Lexington Herald-Leader May 25, 1998; Business Monday p. 11.
5. Wilson ML, Bretsky PM, Cooper GH Jr, Egbertson SH, Van Kruiningen HJ, Cartter ML. Emergence of raccoon rabies in Connecticut, 1991-1994: spatial and temporal characteristics of animal infection and human contact. Am J Trop Med Hyg 1997;57:457-63.
6. Hubbard DR. A descriptive epidemiological study of raccoon rabies in a rural environment. J Wildl Dis 1985;21:105-10.
7. Beck A, Felser S, Glickman L. An epizootic of rabies in Maryland, 1982-84. Am J Public Health 1987;77:42-44.
8. Krebs JW, Wilson ML, Childs JE. Rabies-epidemiology, prevention, and future research. Journal of Mammalogy 1985;76:681-94.
9. Smith JS. New aspects of rabies with emphasis on epidemiology, diagnosis, and prevention of the disease in the United States. Clin Microbiol Rev 1996;Apr:166-176.
10. Heidt G, Ferguson D, Lammers J. A profile of reported skunk rabies in Arkansas: 1977-1979. J Wildl Dis 1982;18:269-77.
11. Curtis A, MacPherson AD. The zone definition problem in survey research: an empirical example from New York State. The Professional Geographer 1996;48:310-20.
12. Scott DW. Multivariate density estimation: theory, practice and visualization. New York: J. Wiley; 1992.

Food-Related Illness and Death in the United States

Paul S. Mead, Laurence Slutsker, Vance Dietz, Linda F. McCaig, Joseph S. Bresee, Craig Shapiro, Patricia M. Griffin, and Robert V. Tauxe
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

To better quantify the impact of foodborne diseases on health in the United States, we compiled and analyzed information from multiple surveillance systems and other sources. We estimate that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year. Known pathogens account for an estimated 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths. Three pathogens, *Salmonella, Listeria*, and *Toxoplasma*, are responsible for 1,500 deaths each year, more than 75% of those caused by known pathogens, while unknown agents account for the remaining 62 million illnesses, 265,000 hospitalizations, and 3,200 deaths. Overall, foodborne diseases appear to cause more illnesses but fewer deaths than previously estimated.

More than 200 known diseases are transmitted through food (1). The causes of foodborne illness include viruses, bacteria, parasites, toxins, metals, and prions, and the symptoms of foodborne illness range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes. In the United States, foodborne diseases have been estimated to cause 6 million to 81 million illnesses and up to 9,000 deaths each year (2-5). However, ongoing changes in the food supply, the identification of new foodborne diseases, and the availability of new surveillance data have made these figures obsolete. New, more accurate estimates are needed to guide prevention efforts and assess the effectiveness of food safety regulations.

Surveillance of foodborne illness is complicated by several factors. The first is underreporting. Although foodborne illnesses can be severe or even fatal, milder cases are often not detected through routine surveillance. Second, many pathogens transmitted through food are also spread through water or from person to person, thus obscuring the role of foodborne transmission. Finally, some proportion of foodborne illness is caused by pathogens or agents that have not yet been identified and thus cannot be diagnosed. The importance of this final factor cannot be overstated. Many of the pathogens of greatest concern today (e.g., *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes, Cyclospora cayetanensis*) were not recognized as causes of foodborne illness just 20 years ago.

In this article, we report new estimates of illnesses, hospitalizations, and deaths due to foodborne diseases in the United States. To ensure their validity, these estimates have been derived by using data from multiple sources, including the newly established Foodborne Diseases Active Surveillance Network (FoodNet). The figures presented include estimates for specific known pathogens, as well as overall estimates for all causes of foodborne illness, known, unknown, infectious, and noninfectious.

Data Sources

Data sources for this analysis include the Foodborne Diseases Active Surveillance Network (FoodNet) (6), the National Notifiable Disease Surveillance System (7), the Public Health Laboratory Information System (8), the Gulf Coast States Vibrio Surveillance System (9), the Foodborne Disease Outbreak Surveillance System (10), the National Ambulatory Medical Care Survey (11), the National Hospital Ambulatory Medical Care Survey (12-14), the

Address for correspondence: Paul S. Mead, Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention, Mail Stop A38, 1600 Clifton Road, Atlanta, GA 30333, USA; fax: 404-639-2205; e-mail: pfm0@cdc.gov.

National Hospital Discharge Survey (15), the National Vital Statistics System (16), and selected published studies.

Established in 1996, FoodNet is a collaborative effort by the Centers for Disease Control and Prevention, the U.S. Department of Agriculture, the U.S. Food and Drug Administration, and selected state health departments. FoodNet conducts active surveillance for seven bacterial and two parasitic foodborne diseases within a defined population of 20.5 million Americans (6). Additional surveys conducted within the FoodNet catchment area provide information on the frequency of diarrhea in the general population, the proportion of ill persons seeking care, and the frequency of stool culturing by physicians and laboratories for selected foodborne pathogens.

The National Notifiable Disease Surveillance System (7) and the Public Health Laboratory Information System (8) collect passive national surveillance data for a wide range of diseases reported by physicians and laboratories. The Gulf Coast States Vibrio Surveillance System collects reports of *Vibrio* infections from selected states (9), and the Foodborne Disease Outbreak Surveillance System receives data from all states on recognized foodborne illness outbreaks (defined as two or more cases of a similar illness resulting from ingestion of a common food) (10).

As components of the National Health Care Survey, the National Ambulatory Medical Care Survey and the National Hospital Ambulatory Medical Care Survey measure health care use in various clinical settings, including physician offices and hospital emergency and outpatient departments (11-14). These surveys collect information on patient characteristics, patient symptoms or reasons for visit, provider diagnosis, and whether the patient was hospitalized. Up to three symptoms are recorded using a standard classification (17), and up to three provider diagnoses are recorded according to the International Classification of Diseases, 9th Revision, Clinical Modifications (ICD-9-CM,18) (Table 1).

The National Hospital Discharge Survey, another component of the National Health Care Survey, is a representative annual sample of discharge records from approximately 475 nonfederal short-stay hospitals (15). The information collected includes up to seven principal discharge diagnoses classified by ICD-9-CM codes (18). Because these data include information on condition at discharge, they can be used as a source of information on in-hospital deaths. Additional information on food-related deaths was obtained from the National Vital Statistics System, which collects death certificate data on causes of death classified by 3- or 4-digit ICD-9 codes (16).

Table 1. ICD-9-CM codes and associated conditions

Code	Condition
001	Cholera
002	Typhoid fever
003	*Salmonella*
004	Shigellosis
005.0	Staphyloccocal food poisoning
005.1	Botulism
005.2-005.3	Other *Clostridia*
005.4	*Vibrio parahaemolyticus*
005.8-005.9	Other and unspecified bacterial food poisoning
006	Amebiasis
007.1	Giardiasis
007.0, 007.2-007.9	Other protozoal intestinal infections
008.00, 008.09	Misc. *Escherichia coli*
008.01	Enteropathogenic *E. coli*
008.02	Enterotoxigenic *E. coli*
008.03	Enteroinvasive *E. coli*
008.04	Enterohemorrhagic *E. coli*
008.43	*Campylobacter*
008.44	*Yersinia*
008.41-2, 008.46-9, 008.5	Misc. bacterial
008.61	Rotavirus
008.62	Adenovirus
008.63	Norwalk virus
008.64	Other small round structured viruses
008.65	Calicivirus
008.66	Astrovirus
008.67	Enterovirus
008.69, 008.8	Other virus
009.	Ill-defined intestinal infections
558.9	Other noninfectious gastroenteritis

In addition to information from these formal surveillance systems, we used data from two published population-based studies. The Tecumseh study was conducted from 1965 through 1971 in 850 households in Tecumseh, Michigan, with an emphasis on households with young children (19). Households were telephoned weekly to identify incident cases of self-defined diarrhea, vomiting, nausea, or stomach upset. The Cleveland study was conducted among a selected group of 86 families followed from 1948 through 1957 (20). A family member

recorded occurrences of gastrointestinal illnesses and associated symptoms on a monthly tally sheet. Both studies also collected information on extraintestinal illnesses (e.g., respiratory illness). Other studies with similar designs were not included in our analysis, either because they were relatively small or because they did not provide information on the desired endpoints.

The Study

Food-Related Illness and Death from Known Pathogens

Total Cases

To estimate the total number of foodborne illnesses caused by known pathogens, we determined the number of reported cases for each pathogen, adjusted the figures to account for underreporting, and estimated the proportion of illnesses specifically attributable to foodborne transmission. Although data from various periods were used, adjustments for changes in population size had minimal effect on the final estimates and were therefore omitted.

Cases may be reported in association with documented foodborne outbreaks, through passive surveillance systems (e.g., the National Notifiable Disease Surveillance System, the Public Health Laboratory Information System), or through active surveillance systems (e.g., FoodNet). Sporadic illness caused by some pathogens (e.g., *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*) is not reportable through passive or active systems; hence, the only cases reported are those related to outbreaks. For these pathogens, we have assumed that if diagnosed sporadic cases were reported, the total number would be 10 times the number of outbreak-related cases. This multiplier is based on experience with pathogens for which data are available on both sporadic and outbreak-associated cases (e.g., reported cases of *Salmonella* or *Shigella*, Table 2). For all pathogens, the number of outbreak-related cases was calculated as the average annual number of such cases reported to CDC from 1983 to 1992, the most recent years for which published outbreak data are available. For pathogens also under passive surveillance, we used the average number of cases reported to CDC from 1992 through 1997, and for pathogens under active surveillance through FoodNet, we used the average rate observed for the surveillance population from 1996 to 1997 and applied this to the total 1997 U.S. population (with some modification for *E. coli* O157:H7; Appendix).

Irrespective of the surveillance system, many cases of foodborne illness are not reported because the ill person does not seek medical care, the health-care provider does not obtain a specimen for diagnosis, the laboratory does not perform the necessary diagnostic test, or the illness or laboratory findings are not communicated to public health officials. Therefore, to calculate the total number of illnesses caused by each pathogen, it is necessary to account for underreporting, i.e., the difference between the number of reported cases and the number of cases that actually occur in the community. For *Salmonella*, a pathogen that typically causes nonbloody diarrhea, the degree of underreporting has been estimated at ~38 fold (Voetsch, manuscript in preparation) (21). For *E. coli* O157:H7, a pathogen that typically causes bloody diarrhea, the degree of underreporting has been estimated at ~20 fold (22). Because similar information is not available for most other pathogens, we used a factor of 38 for pathogens that cause primarily nonbloody diarrhea (e.g., *Salmonella, Campylobacter*) and 20 for pathogens that cause bloody diarrhea (e.g., *E. coli* O157:H7, *Shigella*). For pathogens that typically cause severe illness (i.e., *Clostridium botulinum, Listeria monocytogenes*), we arbitrarily used a far lower multiplier of 2, on the assumption that most cases come to medical attention. Details of the calculations for each specific pathogen and rationale are provided in the Appendix. Where information from both active and passive reporting was available, we used the figure from active surveillance when estimating the total number of cases.

Having estimated the number of cases caused by each pathogen, the final step was to estimate for each the percentage of illness attributable to foodborne transmission. The total number of cases was then multiplied by this percentage to derive the total number of illnesses attributable to foodborne transmission. The rationale for each estimate is presented in the Appendix; although precise percentages are generally difficult to justify, in most instances there is ample support for the approximate value used.

Results are presented in Tables 2 and 3. Known pathogens account for an estimated 38.6

Table 2. Reported and estimated[a] illnesses, frequency of foodborne transmission, and hospitalization and case-fatality rates for known foodborne pathogens, United States

Disease or Agent	Estimated total cases	Reported Cases by Surveillance Type			% Foodborne transmission	Hospitalization rate	Case-fatality rate
		Active	Passive	Outbreak			
Bacterial							
Bacillus cereus	*27,360*		*720*	72	*100*	0.006	0.0000
Botulism, foodborne	*58*		29		*100*	0.800	0.0769
Brucella spp.	*1,554*		111		*50*	0.550	0.0500
Campylobacter spp	*2,453,926*	*64,577*	37,496	146	*80*	0.102	0.0010
Clostridium perfringens	*248,520*		*6,540*	654	*100*	0.003	0.0005
Escherichia coli O157:H7	*73,480*	*3,674*	2,725	500	*85*	0.295	0.0083
E. coli, non-O157 STEC	*36,740*	*1,837*			*85*	0.295	0.0083
E. coli, enterotoxigenic	*79,420*		*2,090*	209	*70*	0.005	0.0001
E. coli, other diarrheogenic	*79,420*		*2,090*		30	0.005	0.0001
Listeria monocytogenes	*2,518*	*1,259*	373		*99*	0.922	0.2000
Salmonella Typhi[b]	*824*		412		*80*	0.750	0.0040
Salmonella, nontyphoidal	*1,412,498*	*37,171*	37,842	3,640	*95*	0.221	0.0078
Shigella spp.	*448,240*	*22,412*	17,324	1,476	*20*	0.139	0.0016
Staphylococcus food poisoning	*185,060*		*4,870*	487	*100*	0.180	0.0002
Streptococcus, foodborne	*50,920*		*1,340*	134	*100*	0.133	0.0000
Vibrio cholerae, toxigenic	*54*		27		*90*	0.340	0.0060
V. vulnificus	*94*		47		*50*	0.910	0.3900
Vibrio, other	*7,880*	*393*	112		*65*	0.126	0.0250
Yersinia enterocolitica	*96,368*	*2,536*			*90*	0.242	0.0005
Subtotal	*5,204,934*						
Parasitic							
Cryptosporidium parvum	300,000	*6,630*	2,788		*10*	0.150	0.005
Cyclospora cayetanensis	*16,264*	*428*	98		*90*	0.020	0.0005
Giardia lamblia	*2,000,000*	*107,000*	22,907		*10*	n/a	n/a
Toxoplasma gondii	*225,000*		15,000		*50*	n/a	n/a
Trichinella spiralis	*52*		26		100	0.081	0.003
Subtotal	*2,541,316*						
Viral							
Norwalk-like viruses	*23,000,000*				*40*	n/a	n/a
Rotavirus	*3,900,000*				*1*	n/a	n/a
Astrovirus	*3,900,000*				*1*	n/a	n/a
Hepatitis A	*83,391*		27,797		*5*	0.130	0.0030
Subtotal	*30,883,391*						
Grand Total	***38,629,641***						

[a]Numbers in italics are estimates; others are measured.
[b]>70% of cases acquired abroad.

million illnesses each year, including 5.2 million (13%) due to bacteria, 2.5 million (7%) due to parasites, and 30.9 million (80%) due to viruses (Table 2). Overall, foodborne transmission accounts for 13.8 million of the 38.6 million illnesses (Table 3). Excluding illness caused by *Listeria*, *Toxoplasma*, and hepatitis A virus (three pathogens that typically cause nongastrointestinal illness), 38.3 million cases of acute gastroenteritis are caused by known pathogens, and 13.6 million (36%) of these are attributable to foodborne transmission. Among all illnesses attributable to foodborne transmission, 30% are caused by bacteria, 3% by parasites, and 67% by viruses.

Hospitalizations

To estimate the number of hospitalizations due to foodborne transmission, we calculated for each pathogen the expected number of hospitalizations among reported cases by

Table 3. Estimated illnesses, hospitalizations, and deaths caused by known foodborne pathogens, United States

	Illnesses			Hospitalizations			Deaths		
Disease or agent	Total	Food-borne	% of total foodborne	Total	Food-borne	% of total foodborne	Total	Food-borne	% of total foodborne
Bacterial									
Bacillus cereus	27,360	27,360	0.2	8	8	0.0	0	0	0.0
Botulism, foodborne	58	58	0.0	46	46	0.1	4	4	0.2
Brucella spp.	1,554	777	0.0	122	61	0.1	11	6	0.3
Campylobacter spp.	2,453,926	1,963,141	14.2	13,174	10,539	17.3	124	99	5.5
Clostridium perfringens	248,520	248,520	1.8	41	41	0.1	7	7	0.4
Escherichia coli O157:H7	73,480	62,458	0.5	2,168	1,843	3.0	61	52	2.9
E. coli, non-O157 STEC	36,740	31,229	0.2	1,084	921	1.5	30	26	1.4
E. coli, enterotoxigenic	79,420	55,594	0.4	21	15	0.0	0	0	0.0
E. coli, other diarrheogenic	79,420	23,826	0.2	21	6	0.0	0	0	0.0
Listeria monocytogenes	2,518	2,493	0.0	2,322	2,298	3.8	504	499	27.6
Salmonella typhi	824	659	0.0	618	494	0.8	3	3	0.1
Salmonella, nontyphoidal	1,412,498	1,341,873	9.7	16,430	15,608	25.6	582	553	30.6
Shigella spp.	448,240	89,648	0.6	6,231	1,246	2.0	70	14	0.8
Staphylococcus food poisoning	185,060	185,060	1.3	1,753	1,753	2.9	2	2	0.1
Streptococcus, foodborne	50,920	50,920	0.4	358	358	0.6	0	0	0.0
Vibrio cholerae, toxigenic	54	49	0.0	18	17	0.0	0	0	0.0
V. vulnificus	94	47	0.0	86	43	0.1	37	18	1.0
Vibrio, other	7,880	5,122	0.0	99	65	0.1	20	13	0.7
Yersinia enterocolitica	96,368	86,731	0.6	1,228	1,105	1.8	3	2	0.1
Subtotal	5,204,934	4,175,565	30.2	45,826	36,466	59.9	1,458	1,297	71.7
Parasitic									
Cryptosporidium parvum	300,000	30,000	0.2	1,989	199	0.3	66	7	0.4
Cyclospora cayetanensis	16,264	14,638	0.1	17	15	0.0	0	0	0.0
Giardia lamblia	2,000,000	200,000	1.4	5,000	500	0.8	10	1	0.1
Toxoplasma gondii	225,000	112,500	0.8	5,000	2,500	4.1	750	375	20.7
Trichinella spiralis	52	52	0.0	4	4	0.0	0	0	0.0
Subtotal	2,541,316	357,190	2.6	12,010	3,219	5.3	827	383	21.2
Viral									
Norwalk-like viruses	23,000,000	9,200,000	66.6	50,000	20,000	32.9	310	124	6.9
Rotavirus	3,900,000	39,000	0.3	50,000	500	0.8	30	0	0.0
Astrovirus	3,900,000	39,000	0.3	12,500	125	0.2	10	0	0.0
Hepatitis A	83,391	4,170	0.0	10,841	90	0.9	83	4	0.2
Subtotal	30,833,391	9,282,170	67.2	123,341	21,167	34.8	433	129	7.1
Grand Total	38,629,641	13,814,924	100.0	181,177	60,854	100.0	2,718	1,809	100.0

multiplying the number of reported cases by pathogen-specific hospitalization rates from FoodNet data (23, 24), reported outbreaks (10, 25), or other published studies (Appendix). Not all illnesses resulting in hospitalization are diagnosed or reported. Health-care providers may not order the necessary diagnostic tests, patients may have already taken antibiotics that interfere with diagnostic testing, or the condition leading to hospitalization may be a sequela that develops well after resolution of the actual infection (e.g., *Campylobacter*-associated Guillain-Barré syndrome). Therefore, to account for underreporting, we doubled the number of hospitalizations among reported cases to derive for each pathogen an estimate of the total number of hospitalizations. Finally, we multiplied this figure by the proportion of infections attributable to foodborne transmission. Because of gaps in the available data, this approach could not be used for some parasitic and viral diseases (Appendix).

Overall, the pathogens listed in Table 2 cause an estimated 181,177 hospitalizations each year, of which 60,854 are attributable to foodborne transmission (Table 3). Excluding hospitalizations for infection with *Listeria*, *Toxoplasma*, and hepatitis A virus, 163,015 hospitalizations for acute gastroenteritis are caused by known pathogens, of which 55,512 (34%) are attributable to foodborne transmission. Overall, bacterial pathogens account for 60% of hospitalizations attributable to foodborne transmission, parasites for 5%, and viruses for 34%.

Deaths

Like illnesses and hospitalizations, deaths are also underreported. Precise information on food-related deaths is especially difficult to obtain because pathogen-specific surveillance systems rarely collect information on illness outcome, and outcome-specific surveillance systems (e.g., death certificates) grossly underreport many pathogen-specific conditions. To estimate the number of deaths due to bacterial pathogens, we used the same approach described for hospitalizations: first calculating the number of deaths among reported cases, then doubling this figure to account for unreported deaths, and finally multiplying by the percentage of infections attributable to foodborne transmission. As with hospitalization, this approach could not be used for some parasitic and viral diseases.

Overall, the specified pathogens cause an estimated 2,718 deaths each year, of which 1,809 are attributable to foodborne transmission (Table 3). Excluding death due *to Listeria*, *Toxoplasma*, and hepatitis A virus, the number of deaths due to pathogens that cause acute gastroenteritis is 1,381, of which 931 (67%) are attributable to foodborne transmission. Bacteria account for 72% of deaths associated with foodborne transmission, parasites for 21%, and viruses for 7%. Five pathogens account for over 90% of estimated food-related deaths: *Salmonella* (31%), *Listeria* (28%), *Toxoplasma* (21%), Norwalk-like viruses (7%), *Campylobacter* (5%), and *E. coli* O157:H7 (3%).

Food-Related Illness and Death from Unknown Pathogens

Some proportion of gastrointestinal illness is caused by foodborne agents not yet identified. This conclusion is supported by well-documented foodborne outbreaks of distinctive illness for which the causative agent remains unknown (e.g., Brainerd diarrhea) (26), by the large percentage of foodborne outbreaks reported to CDC for which no pathogen is identified (25), and by the large number of new foodborne pathogens identified in recent years.

To estimate food-related illness and death from unknown pathogens, we used symptom-based data to estimate the total number of acute gastrointestinal illnesses and then subtracted from this total the number of cases accounted for by known pathogens; this difference represents the illness due to acute gastroenteritis of unknown etiology. To determine how much of this illness was due to foodborne transmission, we used the percentages of foodborne transmission as determined above for acute gastroenteritis caused by known pathogens.

Total Cases

To determine the rate of acute gastroenteritis in the general population, we used data on the frequency of diarrhea from the 1996 to 1997 FoodNet population survey. This survey did not collect data on the rate of vomiting among persons without diarrhea, however, so we relied on the Tecumseh and Cleveland studies for information on the frequency of this symptom. Because young children were overrepresented in the Tecumseh and Cleveland studies relative to the current U.S. population, rates of illness for these studies were age-adjusted. For the Tecumseh data, we used the reported age- and symptom-specific rates. For the Cleveland study, we used the method described by Garthright (27) to derive an overall age-adjusted rate of gastrointestinal illness; we then multiplied this rate by the relative frequency of symptoms to derive age-adjusted rates for specific symptoms.

In the 1996-97 FoodNet population survey, the overall rate of diarrhea was 1.4 episodes per person per year, and the rate of diarrheal illness, defined as diarrhea (3 loose stools per 24-hour period) lasting >1 day or interfering with normal activities, was 0.75 episodes per person per year (H. Herikstad, manuscript in preparation). We used the lower 0.75 rate for our analysis. To this we added the average age-adjusted rate of vomiting without diarrhea from the Tecumseh and Cleveland studies (0.30, Table 4) to derive an overall estimate of 1.05 episodes per person per year of acute gastrointestinal illness characterized by diarrhea, vomiting, or both.

Previous studies have shown that some cases of acute gastrointestinal illness are accompanied by respiratory symptoms; although the causes of these illnesses are generally unknown, such cases have traditionally been attributed to respiratory pathogens (20,27). Data on the frequency of concomitant respiratory symptoms were not collected in the 1996-97 FoodNet survey but were 20% to 27% among patients with acute gastroenteritis in the Tecumseh and Cleveland studies. Therefore, we adjusted downward our estimate of acute gastroenteritis by 25%, yielding a final estimate of 0.79 (1.05 X 0.75)

Table 4. Frequency of gastrointestinal illness in the general population, in episodes per person per year, as determined by three studies

	FoodNet Population Survey	Tecumseh Study		Cleveland Study	
Symptom	Age adjusted	Crude	Age adjusted	Crude	Age adjusted
Diarrhea or vomiting	--	0.98	0.81	1.28	0.87
Diarrhea, any	0.75	0.63	0.52	0.83	0.56
Without vomiting	0.61	0.40	0.33	0.48	0.33
With vomiting	0.14	0.23	0.19	0.35	0.23
Vomiting without diarrhea	--	0.35	0.29	0.45	0.31

episodes of acute gastroenteritis per person per year. Extrapolated to a population of 267.7 million persons, the U.S. resident population in 1997 (28), this rate is equivalent to 211 million episodes each year in the United States.

As determined previously, 38.3 million of these 211 million episodes of acute gastroenteritis are attributable to known pathogens. A small proportion of the remaining 173 million episodes can be accounted for by known, noninfectious agents (e.g., mycotoxins, marine biotoxins); however, most are attributable to unknown agents. Because we cannot directly ascertain how many of these illnesses of unknown etiology are due to foodborne transmission, we used the relative frequency of foodborne transmission for known pathogens as a guide. For illnesses of known etiology, foodborne transmission accounts for 36% of total cases. Applying this percentage yields an estimate of 62 million cases of acute gastroenteritis of unknown etiology (36% of 173 million) due to foodborne transmission each year.

Hospitalizations

The National Ambulatory Medical Care Survey/the National Hospital Ambulatory Medical Care Survey data were searched for visits due to symptoms of diarrhea, vomiting, or gastrointestinal infection (reason for visit classification {RVC} codes 1595, 1530, 1540) (17) and for visits resulting in a diagnosis of infectious enteritis (ICD-9-CM codes 001-009.3; Table 1). Visits associated with respiratory symptoms (RVC codes 1400-1499) or a diagnosis of influenza (ICD-9-CM code 487) were excluded. Data for the years 1992 to 1996 were combined before analysis. Overall, these criteria yielded an average of 15,810,905 visits annually from 1992 through 1996, of which an average of 1,246,763, or 7.9%, resulted in hospitalization. This figure is equivalent to a rate of 4.7 hospitalizations per 1,000 person-years.

The National Hospital Discharge Survey data were searched by using diagnostic codes for infectious gastroenteritis of known cause (ICD-9-CM codes 001-008; Table 1), with the exception of the code for *Clostridium difficile* colitis (ICD9 008.45), a common form of nosocomially acquired diarrhea. In addition, we included the nonspecific ICD-9-CM diagnosis codes 009 (infectious gastroenteritis) and 558.9 (other and unspecified noninfectious gastroenteritis and colitis). Despite the description, many of the illnesses attributed to ICD-9-CM code 558.9 are likely to be either infectious or due to agents possibly transmitted by food. For example, in the absence of laboratory testing, sporadic cases of viral gastroenteritis may be coded as 558.9. Under the previous ICD-8 classification, these same cases would have been assumed to be infectious and coded as 009 (29, 30). Data for the years 1992 to 1996 were weighted according to National Center for Health Statistics criteria and averaged to derive national estimates of annual hospitalizations. Records with a diagnosis of respiratory illness were not excluded because of the high incidence of respiratory infections among hospitalized patients.

Considering all listed diagnoses, the National Hospital Discharge Survey data for the years 1992 to 1996 yielded an annual average of 616,337 hospital discharges with a diagnosis of gastrointestinal illness. Included in this figure are 193,084 cases of gastroenteritis with an identified pathogen and an additional 423,293 cases of gastroenteritis of unknown etiology (Table 5). Converted to a rate, the total number is equivalent to 2.3 hospitalizations per 1,000 person-years. Because these data depend on the recording of a diagnosis and not just a symptom, it is likely that they underestimate the rate of hospitalization for acute gastroenteritis. This view is supported by FoodNet population survey data indicating a rate of approximately 7.2 hospitalizations per 1,000 person-years for

Table 5. Average annual hospitalizations and deaths for gastrointestinal illness by diagnostic category, National Hospital Discharge Survey, 1992–1996

	1st diagnosis		All diagnoses	
Cause of enteritis[a]	Hospitalizations	Deaths	Hospitalizations	Deaths
Bacterial (001-005, 008-008.5)	27,987	148[b]	54,953	1,139
Viral (008.6-008.8)	82,149	0[b]	132,332	194[b]
Parasitic (006-007)	2,806	82[b]	5,799	127[b]
Unknown etiology (009, 558.9)	186,537	868[b]	423,293	5,148
Total	299,479	1,898	616,377	6,608

[a]ICD-9-CM code.
[b]Estimate unreliable due to small sample size.

diarrheal illness (H. Herikstad, manuscript in preparation). These data were not included here because they omit hospitalizations for vomiting alone and are not easily adjusted for concomitant respiratory symptoms. Averaging the rates from the National Ambulatory Medical Care Survey/ National Hospital Ambulatory Medical Care Survey and National Hospital Discharge Survey yields a final estimate of 3.5 hospitalizations per 1,000 person-years, equivalent to 936,726 hospitalizations annually for acute gastroenteritis. As noted previously, 163,153 of these hospitalizations can be attributed to known causes of acute gastroenteritis, yielding an estimated 773,573 hospitalizations for acute gastroenteritis caused by unknown agents. Applying the relative frequency of foodborne transmission as determined for known pathogens yields an estimated 263,015 hospitalizations (34% of 773,573) for acute gastroenteritis due to foodborne transmission of unknown agents.

Deaths

Multiple-cause-of-death data (16) and information on in-hospital-death data (National Hospital Discharge Survey) were used. ICD-9-CM codes 001-008 were employed to identify deaths due to diagnosed infectious gastroenteritis and ICD-9-CM codes 009 and 558 to identify deaths due to gastroenteritis of unknown etiology.

Death certificate data for the years 1992 to 1996 yielded an annual average of 6,195 total deaths, of which 1,432 (23%) were due to specific causes of gastroenteritis and 4,763 (77%) to undiagnosed causes of gastroenteritis. For the same years and ICD-9-CM codes, the average annual in-hospital deaths for all-listed diagnoses totaled 6,608, of which 1,460 were due to specific and 5,148 (77%) undiagnosed causes of gastroenteritis (Table 5). Averaging the totals for all causes from death certificate and National Hospital Discharge Survey data and adjusting to the 1997 U.S. census estimates, we estimated that gastroenteritis contributed to the death of 6,402 persons in the United States in 1997.

A total of 1,386 of these deaths can be explained by known causes of acute gastroenteritis (see above). Thus an estimated 5,016 deaths from acute gastroenteritis are caused by unknown agents. Applying the relative frequency of foodborne transmission as determined for known pathogens yields an estimated 3,360 deaths (67% of 5,016) due to acute gastroenteritis caused by foodborne transmission of unknown agents.

Overall Food-Related Illness and Death

We summed illness attributable to foodborne gastroenteritis caused by known and unknown pathogens, yielding an estimate of 76 million illnesses, 318,574 hospitalizations, and 4,316 deaths. Adding to these figures the nongastrointestinal illness caused by *Listeria*, *Toxoplasma*, and hepatitis A virus, we arrived at a final national estimate of 76 million illnesses, 323,914 hospitalizations, and 5,194 deaths each year (Figure 1).

Conclusions

The nature of food and foodborne illness has changed dramatically in the United States over the last century. While technological advances such as pasteurization and proper canning have all but eliminated some disease, new causes of foodborne illness have been identified. Researchers have used various methods to estimate the illnesses and deaths due to foodborne diseases in the United States. In 1985, Archer and Kvenberg coupled information on underreporting of salmonellosis with data on other foodborne pathogens to derive estimates of 8.9 million illnesses due to known pathogens and 24 million to 81 million illnesses due to all foodborne agents (2). In 1987, Bennett et al. computed incidence

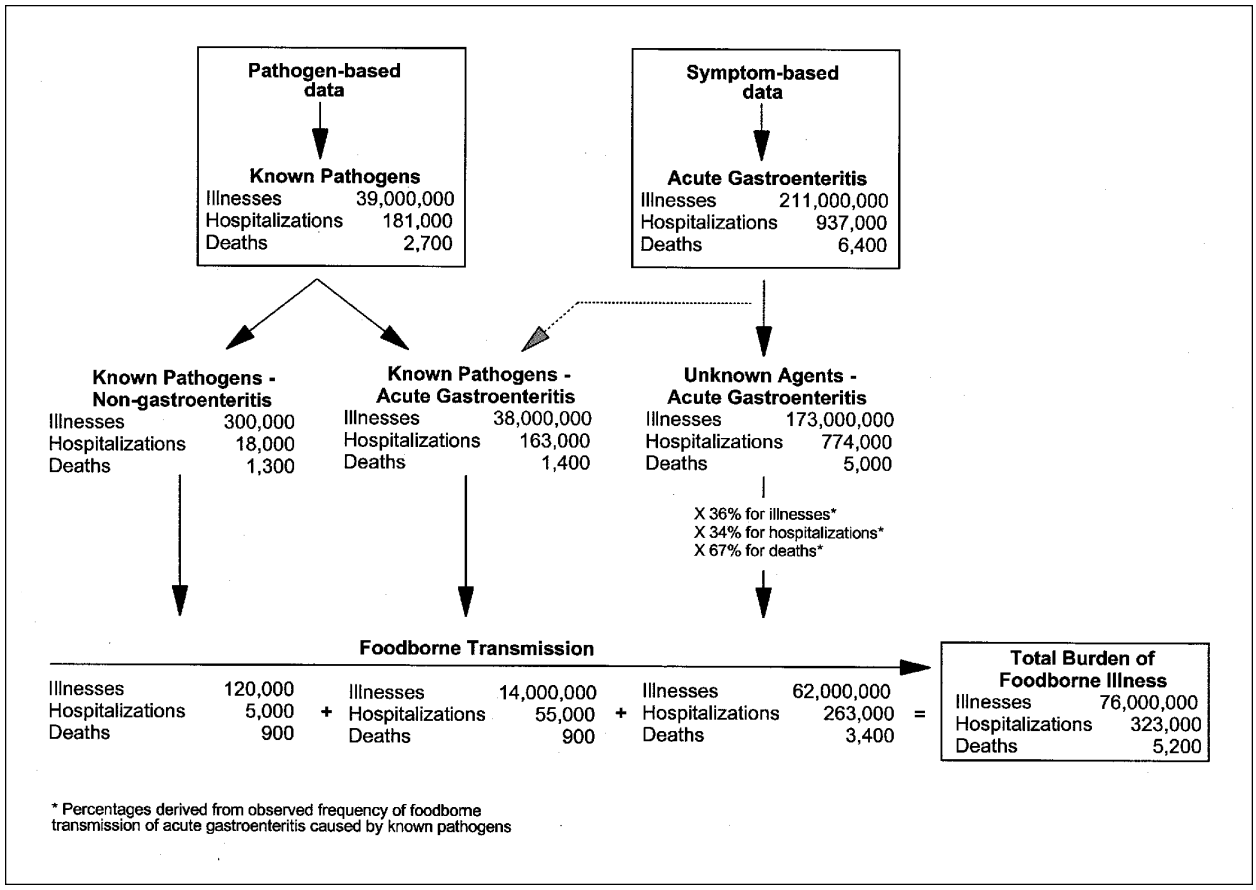


Figure. Estimated frequency of foodborne illness in the United States.

figures for all known infectious diseases and determined the proportion of each due to various modes of transmission. Summing these figures, they concluded that foodborne transmission of known pathogens caused 6.5 million illnesses and up to 9,000 deaths each year (3). In 1989, Todd used a combination of methods, including extrapolation from Canadian surveillance data, to derive an estimate of 12.5 million foodborne illnesses and 522 related deaths each year (4). Finally, in 1994, a task force convened by the Council for Agricultural Science and Technology (CAST) reviewed available studies and estimated the overall number of food-related illnesses at 33 million cases per year (5). These various estimates often refer to different entities. The estimates of 6.5 million and 8.9 million refer to illness caused by known pathogens, whereas the estimate of 33 million refers to all causes of foodborne illnesses, known and unknown, infectious and noninfectious.

Our estimates are based on data from a wide variety of sources and differ from previous estimates in several respects. For known pathogens, our estimate of 13.8 million illnesses per year is substantially higher than the previous estimates of 6.5 million and 8.9 million (2, 3), an increase attributable largely to our inclusion of foodborne illness caused by Norwalk-like viruses. For foodborne illness of all etiologies, our estimate of 76 million illnesses is within the range proposed by Archer and Kvenberg (2) but considerably higher than the point estimate of 33 million presented in the CAST report (5). Both our estimate and the CAST estimate assume that foodborne transmission accounts for ~35% of acute gastroenteritis cases caused by unknown agents. The disparity between the two stems from differences in the estimated annual frequency of acute gastroenteritis overall: 211 million cases for our estimate, 99 million for the CAST estimate.

Whereas our estimates of illness are generally higher than those of previous studies, our estimates of death are generally lower. We estimate that foodborne illness causes 5,020

deaths annually (1,810 deaths due to known pathogens and 3,210 deaths due to unknown agents), a total that is slightly more than half the 9,000 deaths estimated by Bennett et al. (3). The Bennett estimate includes 2,100 deaths due to campylobacteriosis, 1,200 deaths due to staphylococcal food poisoning, and 1,000 deaths due to trichinosis: our total for all three of these diseases is 101 deaths. Our estimated case-fatality rates for several other diseases are also lower than those used in the Bennett report, either because better data are available or perhaps because treatment has improved.

Our analysis suggests that unknown agents account for approximately 81% of foodborne illnesses and hospitalizations and 64% of deaths. Among cases of foodborne illness due to known agents, Norwalk-like viruses account for over 67% of all cases, 33% of hospitalizations, and 7% of deaths. The assumptions underlying the Norwalk-like viruses figures are among the most difficult to verify, and these percentages should be interpreted with caution (Appendix). Other important causes of severe illness are *Salmonella* and *Campylobacter*, accounting for 26% and 17% of hospitalizations, respectively. The leading causes of death are *Salmonella*, *Listeria,* and *Toxoplasma*, which together account for 1,427, or more than 75% of foodborne deaths caused by known pathogens. Many of the deaths due to toxoplasmosis occur in HIV-infected patients; recent advances in HIV treatment may greatly reduce deaths due to toxoplasmosis.

Of necessity, our analysis entails a number of assumptions. The first major assumption concerns the degree of underreporting. Well-documented estimates of underreporting are not available for most pathogens; therefore, we relied on multipliers derived for salmonellosis and other diseases. For salmonellosis, the multiplier of 38 has been independently derived by investigators in the United States using different data sources. The U.S. figure is five to tenfold higher than multipliers for *Salmonella* and *Campylobacter* recently derived in Great Britain (31). However, this difference is nearly or wholly offset by far higher per capita rates of reported infections in Great Britain. Nevertheless, when extrapolated to other pathogens, these multipliers may result in under- or overestimates, and clearly studies such as those conducted for *Salmonella* are needed to develop better multipliers for these other diseases. However, in our analysis, changing the multipliers for individual diseases has a minimal effect on the overall estimate of foodborne illness.

Our second set of assumptions concerns the frequency of foodborne transmission for individual pathogens. We have used published studies when available, but these are rare. As with underreporting multipliers, errors affect estimates for individual pathogens but have minimal effect on the estimate of overall illness and death from foodborne diseases. The one notable exception is the estimate for Norwalk-like viruses. Because these viruses account for an especially large number of illnesses, changes in the percentage attributed to foodborne transmission have a major effect on our overall estimates. For example, if the actual number of infections due to foodborne transmission were 30% rather than 40%, the overall estimate would decrease from 76 million to 63 million illnesses per year. Interestingly, our overall estimate is influenced far less by the Norwalk-like virus case estimate itself. It would require a 100-fold reduction in the estimated number of Norwalk-like virus cases to reduce the overall estimate from 76 million to 63 million.

A third assumption concerns the frequency of acute gastroenteritis in the general population. The rate we used is based in part on recent data from the FoodNet population survey, a retrospective survey involving more than 9,000 households. The overall rate of diarrhea as recorded by the survey was 1.4 episodes per person per year; however, we used the survey's far lower rate of 0.75 episodes of diarrheal illness per person per year. Furthermore, we limited our definition of acute gastroenteritis to symptoms of diarrhea or vomiting and reduced the rate to account for concomitant respiratory symptoms. As a result, our final assumed rate of 0.79 episodes of acute gastroenteritis per person per year is very similar to respiratory-adjusted estimates derived from the prospectively conducted Tecumseh (0.74) and Cleveland (0.71) studies (27). All three studies are based on household surveys, and thus the rates of illness are not influenced by changes in health-care delivery. Compared with rates of diarrheal illness from studies conducted in Great Britain, our estimated rate is higher than in one recent study (31) but lower than another (32).

In addition to these assumptions, our analysis has several limitations. Differences in

available surveillance information prevented us from using the same method to estimate illness and death from bacterial, parasitic, and viral pathogens. Furthermore, because of a paucity of surveillance information, we did not include specific estimates for some known, occasionally foodborne pathogens (e.g., *Plesiomonas, Aeromonas, or Edwardsiella)*, nor did we develop specific estimates for known noninfectious agents, such as mushroom or marine biotoxins, metals, and other inorganic toxins. However, many of these agents cause gastroenteritis and are therefore captured in our overall estimate of foodborne illness. With the exception of a few important pathogens (Appendix), we have not estimated the number of cases of chronic sequelae, although these may be part of the overall burden of foodborne diseases. Finally, future research will refine our assumptions and allow for more precise estimates.

Methodologic differences between our analysis and previously published studies make it difficult to draw firm conclusions regarding overall trends in the incidence of foodborne illness. In general, the differences between our estimates and previously published figures appear to be due primarily to the availability of better information and new analyses rather than real changes in disease frequency over time. For example, *E. coli* O157:H7 was estimated to cause 10,000 to 20,000 illnesses annually, based on studies of patients visiting a physician for diarrhea. Recent FoodNet data have allowed a more detailed estimation of mild illnesses not resulting in physician consultation. Our estimate of nearly 74,000 illnesses per year incorporates these milder illnesses and should not be misconstrued as demonstrating a recent increase in *E. coli* O157:H7 infections. Whatever the limitations on retrospective comparisons, the estimates presented here provide a more reliable benchmark with which to judge the effectiveness of ongoing and future prevention efforts.

Further refinements of foodborne disease estimates will require continued and improved active surveillance. Beginning in 1998, the FoodNet population survey was modified to capture cases of vomiting not associated with diarrhea; further enhancement to capture concomitant respiratory symptoms should refine the FoodNet survey data. Expansion of laboratory diagnostic capacity could lead to better detection of certain pathogens, estimates of the degree of underreporting for additional diseases, and estimates of the proportion of specific diseases transmitted through food. Heightened surveillance for acute, noninfectious foodborne diseases, such as mushroom poisoning and other illnesses caused by biotoxins, could further improve estimates of illness and death from foodborne illness. Emergency department-based surveillance systems (33) or poison control center-based surveillance might provide such information. Finally, identifying new causes of enteric illness and defining the public health importance of known agents (e.g., enteroaggregative *E. coli*) would improve foodborne disease prevention efforts.

Appendix

Methods, assumptions, and references for pathogen-specific estimates

Bacterial Pathogens

Pathogen: *Bacillus cereus*
Reported cases: Cases not routinely reported. Because it is a mild illness, reported cases assumed to be 10 times the average annual number of outbreak-related cases reported to CDC, 1983-1992 (10,25).
Total cases: Assumed to be 38 times the number of reported cases by extrapolation from studies of salmonellosis.
Hospitalization rate: Determined from outbreaks reported to CDC, 1982-1992 (10,25) and (CDC, unpub. data).
Case-fatality rates: Determined from outbreaks reported to CDC, 1982-1992 (10,25), including those associated with nursing homes (34).
Percent foodborne: Although infection occasionally occurs through other routes, case estimates presented are based on foodborne outbreaks and are therefore assumed to reflect only foodborne transmission.

Pathogen: *Clostridium botulinum*
Reported cases: Average annual number of cases of foodborne botulism reported to CDC, 1992-1997 (7).
Total cases: Because it is a severe illness, assumed to be two times the number of reported cases.
Hospitalization rate: Determined from outbreaks reported to CDC, 1982-1992 (10,25) and (CDC, unpub. data).
Case-fatality rate: Based on outbreaks reported to CDC, 1982-1992 (10,25).
Percent foodborne: 100% by definition.

Pathogen: *Brucella* spp.
Reported cases: Average annual number of cases reported to CDC, 1992-1997 (7).
Total cases: Assumed to be 14 times reported cases, based on published estimates that 4% to 10% of cases are reported (35).
Hospitalization rate: Determined from outbreaks reported to CDC, 1982-1992 (10,25) and (CDC, unpub. data).
Case-fatality rate: Historically 2% to 5% (36).
Percent foodborne: Overall, consumption of milk or cheese products from Mexico implicated in 45% of cases reported from California from 1973 to 1992 (37). Because the proportion of cases due to foodborne transmission was higher

in the latter half of this period, we assumed that currently 50% of cases are foodborne.
Comments: Reports from California or Texas account for most of cases in recent years.

Pathogen: *Campylobacter* spp.
Reported cases: Outbreak-related cases based on reports to CDC, 1983-1992 (10,25). Passive surveillance estimate based on average number of cases reported to CDC, 1992-1994 (CDC, unpub. data). Active surveillance estimate based on extrapolation of average 1996-1997 FoodNet rate (24.1 cases per 100,000 population) to 1997 U.S. population (23).
Total cases: Assumed to be 38 times the number of reported cases, based on studies of salmonellosis. Resulting estimate is roughly comparable with midpoint rate estimate from Tauxe (38) for *C. jejuni* (1,020 cases per 100,000 population), applied to 1997 population. Assumes minimal contribution from non-jejuni *Campylobacter*.
Hospitalization rate: Based on hospitalization rate for culture-confirmed cases reported to FoodNet, 1996-1997 (23,24).
Case-fatality rate: Based on case-fatality rate for culture-confirmed cases reported to FoodNet, 1996-1997 (23,24).
Percent foodborne: Although waterborne outbreaks occur, foodborne transmission accounts for most of the sporadic cases (38).
Comments: Guillain-Barré syndrome (GBS) is an acute flaccid paralysis that can occur several weeks after infection with various agents, including *Campylobacter*. The incidence of GBS has been estimated at 1.7 cases per 100,000 population, and serologic studies suggest that ~30% of patients with GBS have evidence of recent infection with *Campylobacter* (39). Based on these figures, we estimate that ~1,360 cases of *Campylobacter*-associated GBS occurred in the United States in 1997.

Pathogen: *Clostridium perfringens*
Reported cases: Cases not routinely reported. Because it is a mild illness, number of reported cases assumed to be 10 times the average annual number of outbreak-related cases reported to CDC, 1983-1992 (10,25).
Total cases: Assumed to be 38 times the number of reported cases, by extrapolation from studies of salmonellosis.
Hospitalization rate: Determined from outbreaks reported to CDC, 1982-1992 (10,25) and (CDC, unpub. data).
Case-fatality rate: Based on reported outbreaks, 1983-1992 (10,25).
Percent foodborne: 100% (40). Case estimates presented are based on foodborne outbreaks and therefore reflect foodborne transmission of *C. perfringens*, type A.

Pathogen: *Escherichia coli* O157:H7
Reported cases: Passive surveillance estimate based on average number of cases reported to CDC through the National Electronic Telecommunications System for Surveillance (NETSS), 1995-1998; data from the Public Health Laboratory Information System (PHLIS) were used for those states not reporting to NETSS during this time period (7). Passive surveillance data for 1998 are provisional. Active surveillance estimate based on an extrapolation of a weighted average of the FoodNet rate for the years 1996-1997 to the 1997 U.S. population (23,24). A weighted average was used because the overall FoodNet rate is disproportionately influenced by a high rate in a single northern state with a relatively small population. Because the incidence of infection is thought to be generally higher in northern states (41), we weighted the crude rate derived from FoodNet by the total population of each participating state. The weighted rate (1.34 cases per 100,000 population) was used when extrapolating the FoodNet rate to the total U.S. population.
Total cases: Studies conducted in FoodNet sites suggest that 13-27 cases of *E. coli* O157:H7 infection occur in the community for each confirmed case that is reported (22). To estimate total cases, we multiplied the number of reported cases, as determined through active surveillance, by 20, the midpoint of this estimate.
Hospitalization rate: Based on the hospitalization rate for culture-confirmed cases reported to FoodNet, 1996-1997 (23,24).
Case-fatality rate: Case-fatality rate based on mortality associated with sporadic cases reported to FoodNet, 1996-1997 (23,24).
Percent foodborne: Based on outbreaks of known source reported to CDC, 1982-1997 (CDC, unpub. data). Person-to-person transmission assumed to be secondary to foodborne transmission (2).
Comments: Our estimate of total cases is considerably higher than previous estimates based on patients seeking care for diarrhea. Our estimate includes patients with far milder illness and should not be interpreted as indicating an increase in incidence. Hemolytic uremic syndrome (HUS) occurs in ~4% of all reported cases. Based on our estimate of total cases and active surveillance cases, between 2,954 and 147 patients are expected to contract HUS each year.

Pathogen: *E. coli*, Shiga toxin-producing serogroups other than O157 (STEC)
Reported cases: Cases not routinely reported; many clinical laboratories cannot identify.
Total cases: Assumed to be half as common as infection with *E. coli* O157:H7. Early studies suggest that the incidence of non-O157 STEC infections is 20%-30% that of *E. coli* O157:H7 in North America (42, 43); however, more recent studies using different techniques suggest that this figure should be 50% (44,45).
Hospitalization rate: Assumed to be comparable with *E. coli* O157:H7, but may be lower (46).
Case-fatality rate: Assumed to be comparable with *E. coli* O157:H7, but may be lower (46).
Percent foodborne: Assumed to be comparable with *E. coli* O157:H7.
Comment: Although non-O157 STEC can cause hemolytic uremic syndrome, the relative frequency of this complication is unknown. Reports from Canada suggest that non-O157 STEC are the cause of at least 7% (47) and possibly as many as 20% (48) of HUS cases.

Pathogen: *E. coli,* enterotoxigenic
Reported cases: Not routinely reported. Outbreak-related cases based on average for 18 outbreaks reported to CDC from 1975 through 1997 (CDC, unpub. data). Reported cases assumed to be 10 times the number of outbreak-related cases.
Total cases: Assumed to be 38 times the number of reported cases by extrapolation from studies of salmonellosis.
Hospitalization rate: Low; assumed to be 0.5% of cases.
Case-fatality rate: Serious illness is generally restricted to infants in developing countries. Based on experience with reported outbreaks, assumed to be 1 in 10,000 cases in the United States.
Percent foodborne: Nearly all outbreaks reported to CDC from 1975 through 1997 have been foodborne (CDC, unpub. data); many sporadic cases are associated with travel to other

countries where both water and foodborne exposures are likely.

Pathogen: *E. coli,* other diarrheogenic
Reported cases: Not routinely reported. Assumed to be at least as common as enterotoxigenic *E. coli* (ETEC) based on limited information from studies in North America and Europe (49).
Total cases: Assumed equal to ETEC.
Hospitalization rate: Assumed equal to ETEC.
Case-fatality rate: Assumed equal to ETEC.
Percent foodborne: Very little data available. As few foodborne outbreaks have been reported, it is assumed that only 30% of cases are foodborne.
Comment: This category includes enteropathogenic, enteroaggregative, and enteroinvasive *E. coli*, as well as poorly defined pathogenic groups (50). Although little is known about the incidence of these infections in the United States, these pathogens have been linked to both outbreaks and sporadic illnesses. Limited studies suggest that the importance of some of these organisms in the United States is seriously underestimated (see Nataro and Kaper [49]). Although clearly a heterogeneous collection of organisms, we assume that these pathogens as a group have similar modes of transmission and mortality rates as ETEC.

Pathogen: *Listeria monocytogenes*
Reported cases: Rates from FoodNet, 1996-1997, (23,24) and comparable sentinel site surveillance (51), extrapolated to the 1997 U.S. population.
Total cases: Because it is a severe illness, assumed to be 2 times the number of reported cases.
Hospitalization rate: Based on hospitalization rate for culture-confirmed cases reported to FoodNet, 1996-1997 (23,24).
Case-fatality rate: Based on published reports (51), 1996-1997 FoodNet data (23,24), and recent outbreaks (CDC, unpub. data).
Percent foodborne: Although foodborne transmission accounts for all reported domestic outbreaks (52), the potential for nosocomial transmission has been demonstrated (53).
Comments: Figures include both perinatal and nonperinatal disease. FoodNet data on hospitalization indicate that nearly 90% of reported cases result in hospitalization (24).

Pathogen: *Salmonella* Typhi
Reported cases: Average number of cases reported to CDC, 1992-1997 (7).
Total cases: Because it is a severe illness, assumed to be two times the number of reported cases.
Hospitalization rate: Rate of hospitalization based on published outbreak reports (54,55).
Case-fatality rate: Based on outcomes of 2,254 cases reviewed by Mermin (56).
Percent foodborne: Although waterborne outbreaks have been reported in the United States, foodborne transmission is believed to account for most cases (3).
Comments: Over 70% percent of reported cases are associated with foreign travel (56).

A. Pathogen: *Salmonella*, nontyphoidal
B. Reported cases: Outbreak-related cases based on reports to CDC, 1983-1992 (10,25). Passive surveillance estimate based on average number of cases reported to CDC, 1992-1997 (57). Active surveillance estimate based on extrapolation of the average 1996-1997 FoodNet rate to the 1997 U.S. population (23).
Total cases: Assumed to be 38 times the number of reported cases based on FoodNet data (Voetsch, manuscript in preparation) and the "sequential surveillance artifact" multiplier derived by Chalker and Blaser (21).
Hospitalization rate: Based on hospitalization rate for culture-confirmed cases reported to FoodNet, 1996-1997 (23,24).
Case-fatality rate: Average case-fatality rate among cases reported to FoodNet, 1996-1997 (23,24). This rate is lower than the previously published rate of 1.3% (58).
Percent foodborne: Although occasionally associated with exposure to pets, reptiles, and contaminated water, salmonellosis is primarily a foodborne disease (59).

Pathogen: *Shigella* spp.
Reported cases: Outbreak-related cases based on reports to CDC, 1983-1992 (10,25). Passive surveillance estimate based on average number of cases reported annually to CDC, 1992-1997 (57). Active surveillance estimate based on extrapolation of average 1996-1997 FoodNet rate to the 1997 U.S. population (23).
Total cases: Because *Shigella* frequently causes bloody diarrhea, total cases assumed to be 20 times the number of reported cases, based on similarity to *E. coli* O157:H7.
Hospitalization rate: Based on hospitalization rate for culture-confirmed cases reported to FoodNet, 1996-1997 (23,24).
Case-fatality rate: Average case-fatality rate among cases reported to FoodNet, 1996-1997 (23,24).
Percent foodborne: Assumed to be 20%. Although most cases are due to person-to-person transmission (60), foodborne outbreaks are responsible for a substantial number of cases (61).

Pathogen: *Staphylococcus aureus* (enterotoxin)
Reported cases: Not routinely reported. Assumed to be 10 times the number of foodborne outbreak-related cases reported to CDC, 1983-1992 (10,25).
Total cases: Assumed to be 38 times the number of reported cases, by extrapolation from studies of salmonellosis.
Hospitalization rate: Determined from outbreaks reported to CDC, 1982-1992 (10,25), (CDC, unpub. data), and published reports (62).
Case-fatality rate: Determined from reported outbreaks to CDC, 1977-1992 (10,25,63).
Percent foodborne: 100% by definition. Case estimates presented are based on foodborne outbreaks and therefore reflect foodborne transmission.
Comment: The number of outbreak-associated cases of staphylococcal food poisoning reported to CDC has decreased substantially since 1973 (Bean and Griffin, 1990). This decrease is unlikely to be an artifact of decreased recognition: there has been no compensatory increase in the number of foodborne outbreaks of unknown etiology with an incubation period consistent with staphylococcal food poisoning (CDC, unpub. data).

Pathogen: *Streptococcus,* Group A
Reported cases: Not routinely reported. Assumed to be 10 times the number of foodborne outbreak-related cases reported to CDC, 1982-1992 (10,25).
Total cases: Assumed to be 38 times the number of reported cases, by extrapolation from studies of salmonellosis.
Hospitalization rate: Determined from outbreaks reported

to CDC, 1982-1992 (10,25) and CDC, unpub. data.
Case-fatality rate: Determined from outbreaks reported to CDC, 1982-1992 (10).
Percent foodborne: 100% foodborne by definition. Case estimates presented are based on foodborne outbreaks and therefore reflect foodborne transmission.

Pathogen: *Vibrio cholerae*, toxigenic O1 or O139
Reported cases: Based on cases reported to CDC, 1988-1997 (7).
Total cases: Assumed that the number of clinically significant illnesses is two times the number of reported cases.
Hospitalization rate: Based on cases reported to CDC, 1992-1994 (64).
Case-fatality rate: Based on cases reported to CDC, 1992-1994 (64).
Percent foodborne: Assumed to be primarily foodborne. Most reported cases linked to foodborne outbreaks, and at least 65% of sporadic cases may be foodborne (64).
Comments: 96% of cases acquired abroad (64).

Pathogen: *Vibrio vulnificus*
Reported cases: Cases reported to CDC from 22 states, 1988-1996 (65).
Total cases: Because it is a severe illness, assumed to be two times the number of reported cases.
Hospitalization rate: Based on overall rate among cases reported to CDC, 1988-1996 (65).
Case-fatality rate: Based on overall rate among cases reported to CDC, 1988-1996; death rate higher among cases due to foodborne transmission (65).
Percent foodborne: Based on Shapiro et al. (65).
Comment: Most cases are reported by Gulf States (Florida, Alabama, Louisiana, Texas).

Pathogen: *Vibrio,* other spp.
Reported cases: Passive surveillance estimate based on cases reported to CDC, 1988-1996 (CDC, unpub. data). Active surveillance estimate based on 1996 FoodNet rate extrapolated to the 1997 U.S. population (23). FoodNet data from 1997 not included because of a large outbreak of *Vibrio parahaemolyticus* infections that could falsely elevate the overall rate.
Total cases: Because it is a moderately severe illness, total cases assumed to equal 20 times the reported cases, a degree of underreporting comparable with *E. coli* O157:H7 infections.
Hospitalization rate: Based on rate among non-*vulnificus*, non-*cholerae* O1 cases reported by Hlady (66).
Case-fatality rate: Based on rate among non-*vulnificus*, non-*cholerae* O1 cases reported by Hlady (66).
Percent foodborne: Based on history of shellfish consumption for cases reported by Hlady (66).
Comment: Because of larger sample size, data from Hlady (66) used in preference to FoodNet data for hospitalization and death rates.

Pathogen: *Yersinia enterocolitica*
Reported cases: Active surveillance estimate based on extrapolation of average 1996-1997 FoodNet rate to the 1997 U.S. population (23,24).
Total cases: Assumed to be 38 times the number of reported cases, based on studies of salmonellosis.
Hospitalization rate: Based on the hospitalization rate for culture-confirmed cases reported to FoodNet, 1996-1997 (23,24).
Case-fatality rate: Low, assumed to be 0.5% (23).
Percent foodborne: Assumed to be 90%. Nearly all reported outbreaks in United States have been linked to contaminated foods, and pork is specifically believed to be the source of most infections (67).

Parasitic Pathogens

Pathogen: *Cryptosporidium parvum*
Reported cases: Passive surveillance estimate based on the average annual number of cases reported to CDC, 1995-1997 (7). Active surveillance estimate based on extrapolation of the average 1997-98 FoodNet rate to the 1997 U.S. population (6,24).
Total cases: Published studies suggest that ~2% of all stools tested for *Cryptosporidium* are positive (68, 69). We assume this rate of infection applies to all patients visiting a health-care provider for acute gastroenteritis. Using an estimate of ~15 million physician visits for diarrhea each year (see text), we estimate there are approximately 300,000 cases of cryptosporidiosis per year. This figure is 45-fold higher than the estimated number of reported cases based on FoodNet active surveillance, a multiplier only slightly larger than the one used for salmonellosis.
Hospitalization rate: Based on the hospitalization rate for culture-confirmed cases reported to FoodNet, 1997-1998 (6,24).
Case-fatality rate: Average case-fatality rate among cases reported to FoodNet, 1997-1998 (6,24).
Percent foodborne: Based on very limited information (70-72), we assume that 10% of cases are attributable to foodborne transmission, with the rest due to consumption of contaminated water or person-to-person transmission.
Comment: Cryptosporidiosis in AIDS is associated with a severe protracted course of diarrhea (73).

Pathogen: *Cyclospora cayetanensis*
Reported cases: Passive surveillance estimate based on average annual number of cases reported to CDC, 1995-1997 (7). Active surveillance estimate based on extrapolation of average 1997-1998 FoodNet rate to the 1997 U.S. population (6,24).
Total cases: Assumed to be 38 times the number of reported cases based on studies of salmonellosis.
Hospitalization rate: Based on the hospitalization rate for culture-confirmed cases reported to FoodNet, 1997 (24).
Case-fatality rate: Very low (74,75). Assumed to be 0.05%, comparable with *Clostridium perfringens*.
Percent foodborne: Assumed 90% foodborne, based on recent reported outbreaks (74,75).

Pathogen: *Giardia lamblia*
Reported cases: Not routinely reported.
Total cases: Sensitive surveillance in two sites (Vermont and Wisconsin) suggests a rate of 40 cases per 100,000 persons per year (76,77). In addition, an estimated 5% of all cases are reported. Thus, approximately 100,000 cases will be detected each year, representing 2,000,000 actual cases.
Hospitalization rate: An estimated 5,000 cases per year are severe enough to require hospitalization.
Case-fatality rate: Exceedingly low. Assumed to be no more than 10 deaths annually.
Percent foodborne: Assumed to be 10%. Recreational water is probably the major source of transmission (76-78); however, several foodborne outbreaks have been reported (79,80).

Pathogen: *Toxoplasma gondii*
Reported cases: Not routinely reported.

Total cases: Based on national serologic data collected during the 1994 NHANES, approximately 40% of persons ≥60 years old are seropositive for toxoplasmosis (CDC, unpub. data). Assuming equal rates of infection over time, at least 0.6% of the population experiences an acute infection each year, representing approximately 1,500,000 infections per year. Approximately 15% of infections are symptomatic.
Hospitalization rate: Varies widely according to host immune status. Data from NHDS indicate that from 1992 to 1996, toxoplasmosis was the first listed diagnosis for approximately 5,000 hospital discharges each year. We have used this figure as a conservative estimate of the number of actual hospitalizations.
Case-fatality rate: Varies widely according to host immune status. Of the approximately 5,000 hospital discharges annually for which toxoplasmosis is the first listed diagnosis, approximately 750 involve a deceased patient. We have used this figure as a conservative estimate of the number of actual deaths.
Percent foodborne: Although the proportion associated with eating contaminated food varies by geographic region, we assume an overall average of 50%. Recent unpublished data from Europe suggest that 60% of acute infections are from contaminated food (Ruth Gilbert, pers. comm.).
Comment: Typically, infection with *Toxoplasma gondii* produces an asymptomatic illness or a mild viral-like febrile illness with lymphadenopathy. Acute diarrhea is not commonly associated with acute infection. Estimates from the Massachusetts Department of Health suggest that one case of congenital toxoplasmosis occurs for every 10,000 births (81). Extrapolating to 4,000,000 live births in the United States, an estimated 400 children are born with congenital toxoplasmosis. Based on calculations by investigators from Stanford University, each year approximately 6,000 women who experience an acute infection during pregnancy and who do not receive treatment give birth to a child with congenital toxoplasmosis, which results in chronic sequelae (82). During an outbreak of toxoplasmosis in British Columbia, of an estimated 2,900-7,700 infections, 19 cases of retinitis were reported. If there are at least 150,000 symptomatic cases annually, from 300 to 1,050 cases (0.2% to 0.7%, respectively) of ocular toxoplasmosis could occur. If there are 300,000 cases, from 600 to 2,100 ocular cases could occur. Thus, there could be from 300 to 2,100 ocular cases of toxoplasmosis annually. An estimated 4,000 persons with AIDS develop *Toxoplasma* encephalitis annually. In summary, from (400+300+4,000) = 4,700 to (6,000+2,100+4,000) = 12,100 persons develop chronic sequelae due to toxoplasmosis each year.

Pathogen: *Trichinella spiralis*
Reported cases: Based on NETSS surveillance data, approximately 40 cases are reported annually.
Total cases: Because it can be a severe illness, assumed to be two times the number of reported cases.
Hospitalization rate: Based on outbreak-related cases reported to CDC, 1982-1992 (10).
Case-fatality rate: Assumed to be 0.3% based on data from a large series in Europe.
Percent foodborne: 100% (83)
Comment: Clinically, acute trichinosis may be asymptomatic or may have acute gastrointestinal symptoms, followed by a parenteral phase of fever and myalgias. In 10% to 20% of cases neurologic or cardiac symptoms develop, many severe and potentially leading to chronic illness.

Viral Pathogens

Pathogen: Rotavirus
Reported cases: Not routinely reported.
Total cases: Because every child has at least one symptomatic infection (84-86), the number of cases is assumed to equal the 1997 U.S. birth cohort (3.9 million).
Hospitalizations: 50,000 (87,88).
Case-fatality rate: Very low: 20 to 40 deaths per year (89).
Percent foodborne: probably very low (<1%) (90).

Pathogen: Astrovirus
Reported cases: Not routinely reported.
Total cases: Because every child has at least one symptomatic infection, the number of cases is assumed to equal the 1997 US birth cohort (3.9 million).
Hospitalizations: Assumed to equal 25% of number of hospitalizations for rotavirus (= 12,500) (91).
Case-fatality rate: Very low (<10 deaths per year).
Percent foodborne: Probably very low (<1%) (91).

Pathogen: Norwalk-like viruses (NLV).
Reported cases: Not routinely reported.
Total cases: Very few data are available for assessing the disease burden associated with Norwalk-like viruses, and very few studies have been conducted using the most sensitive diagnostics for NLVs. One community-based study from the Netherlands found 17% of cases of acute gastroenteritis were associated with Norwalk-like viruses, compared with 6% of controls, using reverse transcriptase polymerase chain reaction (RT-PCR) for detection of NLVs (92). An Australian study detected NLVs in 15% of hospitalized patients using immune electron microscopy (93). Studies have generally been conducted exclusively among young children or used less sensitive detection methods (electron microscopy); in these studies, NLVs have been detected in ~1% to 5% of participants (94-98). However, a recent study incorporating RT-PCR for viral detection among children 2 months to 2 years of age found that 21% of cases of acute gastroenteritis were associated with NLVs (99). Given these data, we assume that 11% of all episodes of acute primary gastroenteritis are due to NLVs (using the data from the best of the studies) (92).
Hospitalizations: NLV assumed to account for 11% of 452,000 annual hospitalizations for viral gastroenteritis (100).
Case-fatality rate: Low. NLV assumed to account for 11% of an estimated 2,800 fatal cases of viral gastroenteritis each year (100).
Percent foodborne: We assume that the proportion of all NLV-associated illness that is foodborne is 40%. This estimate is based on a recent report which found that 47% of NLV-associated acute gastroenteritis outbreaks in the United States in which the modes of transmission were known were foodborne (101). Since we would assume that foodborne-associated outbreaks might be more likley to be reported than Norwalk-like virus-associated outbreaks with other mechanisms of spread, the proportion was lowered to 40%. This estimate is in general agreement with other reviews (102-104). No data are available to directly determine the proportion of cases of NLV-associated disease attributable to foodborne transmission.

Pathogen: Hepatitis A
Reported cases: Based on cases reported to CDC, 1992-1997 (7).

Total cases: Assumed to be three times the number of reported cases (105).
Hospitalizations: Thirteen percent; based on data from CDC Sentinel Counties Studies (106);
Case-fatality rate: 0.3%; based on data from the viral Hepatitis Surveillance Program and the CDC Sentinel Counties Studies (105,107). Deaths calculated by applying the case-fatality rate to reported cases.
Percent foodborne: Foodborne transmission accounts for approximately 5% of outbreaks of known source (105). Note that the source is not determined in approximately 50% of hepatitis A outbreaks, and foodborne transmission could account for a far higher percentage of cases.

Acknowledgments

We thank Fred Angulo, Beth Bell, Thomas Breuer, Cindy Friedman, Roger Glass, Eric Mintz, Steven Ostroff, Morris Potter, David Swerdlow, Tom Van Gilder, and two anonymous reviewers for their comments.

Dr. Mead is a medical epidemiologist with the Foodborne and Diarrheal Diseases Branch, CDC, in Atlanta, Georgia. His professional interests include infectious diseases surveillance, outbreak investigations, and interventions to prevent foodborne illness.

References

1. Bryan FL. Diseases transmitted by foods. Atlanta: Centers for Disease Control; 1982.
2. Archer DL, Kvenberg JE. Incidence and cost of foodborne diarrheal disease in the United States. J Food Protect 1985;48:887-94.
3. Bennett J, Holmberg S, Rogers M, Solomon S. Infectious and parasitic diseases. In: Amler R, Dull H, editors. Closing the gap: the burden of unnecessary illness. New York: Oxford Univ Press; 1987: 102-14.
4. Todd ECD. Preliminary estimates of costs of foodborne disease in the United States. J Food Protect 1989;52:595-601.
5. Foodborne pathogens: risks and consequences. Ames, IA: Council of Agricultural Science and Technology; 1994.
6. 1998 FoodNet Surveillance Results. Preliminary Report. Atlanta: Centers for Disease Control and Prevention; 1999.
7. Summary of notifiable diseases, United States, 1997. MMWR Morb Mortal Wkly Rep 1997;46(54).
8. Bean NH, Martin SM, Bradford H. PHLIS: an electronic system for reporting public health data from remote sites. Am J Public Health 1992;82:1273-76.
9. Levine W, Griffin P, Gulf Coast Vibrio Working Group. Vibrio infections on the Gulf Coast: results of first year regional surveillance. J Infect Dis 1993;167:479-83.
10. Foodborne disease outbreaks, 5-year summary, 1983-1987. MMWR 1992;39 (SS-1):15-57.
11. Woodwell DA. National Ambulatory Medical Care Survey: 1996 Summary. Advance data from vital and health statistics; no. 295. Hyattsville, Maryland: National Center for Health Statistics; 1997.
12. McCaig LF, McLemore T. Plan and operation of the National Hospital Ambulatory Medical Care Survey. Hyattsville: National Center for Health Statistics; 1994.
13. McCaig LF. National Hospital Ambulatory Medical Care Survey: 1996 Outpatient Department Summary. Advance data from vital and health statistics: no. 294. Hyattsville, Maryland: National Center for Health Statistics; 1997.
14. McCaig LF, Stussman BJ. National Hospital Ambulatory Medical Care Survey: 1996 Emergency Department Summary. Advance data from vital and health statistics: no. 293. Hyattsville, Maryland: National Center for Health Statistics; 1997.
15. Graves EJ, Gillium BS. Detailed diagnoses and procedures, National Hospital Discharge Survey, 1995. National Center for Health Statistics. Vital Health Stat 1997;13.
16. NCHS. Public use data tape documentation. Multiple cause of death for ICD-9. Hyattsville, Maryland: Public Health Service; 1998.
17. Schneider D, Appleton L, McLemore T. A reason for visit classification for ambulatory care. Hyattsville, Maryland: National Center for Health Statistics; 1979.
18. Public Health Service and Health Care Financing Administration. International classification of diseases, 9th Revision, Clinical Modification. Washington D.C.: Public Health Service; 1991.
19. Monto AS, Koopman JS. The Tecumseh Study. XI. Occurrence of acute enteric illness in the community. Am J Epidemiol 1980;112:323-333.
20. Dingle JH, Badger GF, Jordan W. Gastrointestinal illness. In: Illness in the home. A study of 25,000 illness in a group of Cleveland families. Cleveland: The Press of Western Reserve University; 1964: 129-61.
21. Chalker R, Blaser M. A review of human salmonellosis: III. Magnitude of *Salmonella* infection in the United States. Rev Infect Dis 1988;10:111-24.
22. Hedberg C, Angulo F, Townes J, Vugia D, Farley M, FoodNet. Differences in *Escherichia coli* O157:H7 annual incidence among FoodNet active surveillance sites. Baltimore, MD; 1997 June 22-26, 1997.
23. 1996 Final FoodNet surveillance report. Atlanta: Centers for Disease Control and Prevention; 1998.
24. 1997 Final FoodNet surveillance report. Atlanta: Centers for Disease Control and Prevention; 1998.
25. Surveillance for foodborne-disease outbreaks—United States, 1988-1992. MMWR 1996;45(No. SS-5):2-55.
26. Parsonnet J, Wanke CA, Hack H. Idiopathic chronic diarrhea. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL, editors. Infections of the gastrointestinal tract. New York: Raven Press, Ltd; 1995: 311-23.
27. Garthright WE, Archer DL, Kvenberg JE. Estimates of incidence and cost of infectious diseases in the United States. Pub Health Reports 1988;103:107-15.
28. Population estimates. Available at <http://www.census.gov/population/www/estimates/popest.html> ed: Bureau of the Census, Economics and Statistics Administration, US Department of Commerce.
29. Helmick CG, Griffin PM, Addiss DG, Tauxe RV, Juranek DD. Infectious diarrheas. In: Everhart JE, editor. Digestive diseases in the United States: epidemiology and impact. U.S. Department of Health and Human Service, National Institutes of Health, National Institute of Diabetes and Digestive Diseases. Washington, D.C.: U.S. Government Printing Office; 1994: 85-123.

30. Dugger BC, Lewis WF. Comparability of diagnostic data coded by the 8th and 9th revisions of the international classification of diseases. Washington, D.C.: U.S. Government Printing Office 1987: DHHS publication no. (PHS) 87-1378. (Vital and health statistics: series 2, no. 104).
31. Wheeler JG, Sethi D, Cowden JM, Wall PG, Rodrigues LC, Tompkins DS, et al. Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive. BMJ 1999;318:1046-50.
32. Feldman RA, Banatvala N. The frequency of culturing stools from adults with diarrhea in Great Britain. Epidemiol Infect 1994;113:41-4.
33. Talan DA, Moran GJ, Mower WR, Newdow M, Ong S, Slutsker L, et al. Emergency ID NET: an emergency department-based emerging infections sentinel network. Ann Emerg Med 1998;32:703-11.
34. Levine W, Smart J, Archer D, Bean N, Tauxe R. Foodborne disease outbreaks in nursing homes, 1975 through 1987. JAMA 1991;266:2105-09.
35. Taylor JP, Perdue JN. The changing epidemiology of human brucellosis in Texas. Am J Epidemiol 1989;130:160-5.
36. Dalrymple-Champneys W. Brucella infection and undulant fever in man. London: Oxford University Press; 1960.
37. Chomel B, DeBess E, Mangiamele D, Reilly K, Farver T, Sun R, et al. Changing trends in the epidemiology of human brucellosis in California from 1973 to 1992: a shift toward foodborne transmission. J Infect Dis 1994;170:1216-23.
38. Tauxe R. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin, Blaser M, Tompkins L, editors. *Campylobacter jejuni*: current status and future trends; 1992. p. 9-19.
39. Mishu B, Blaser MJ. Role of infection due to *Campylobacter jejuni* in the initiation of Guillain-Barré syndrome. Clin Infec Dis 1993;17:104-8.
40. Bartlett JG. Gas gangrene (other *Clostridium*-associated diseases). In: Mandell GL, Douglas RG, Bennett JE, editors. Principles and practice of infectious diseases. Third ed. New York: Churchill Livingstone; 1990: 1850-60.
41. Slutsker L, Ries AA, Greene KD, Wells JG, Hutwagner L, Griffin PM. *Escherichia coli* O157:H7 diarrhea in the United States: clinical and epidemiologic features. Ann Intern Med 1997;126:505-13.
42. Pai CH, Ahmed N, Lior H, Johnson WM, Sims HV, Woods DE. Epidemiology of sporadic diarrhea due to verocytotoxin-producing *Escherichia coli*: a two-year prospective study. J Infect Dis 1988;157:1054-7.
43. Bokete TN, O'Callahan CM, Clausen CR, Tang NM, Tran N, Moseley SL, et al. Shiga-like toxin-producing *Escherichia coli* in Seattle children: a prospective study. Gastroenterology 1993;105:1724-31.
44. Acheson DWK, Breuker SD, Donohue-Rolfe A, Kozak K, Yi A, Keusch GT. Development of a clinically useful diagnostic enzyme immunoassay for enterohemorrhagic *Escherichia coli* infection. In: Karmali MA, Goglio AG, editors. Recent advances in verocytotoxin-producing *Escherichia coli* infections. Amsterdam: Elsevier Science B. V.; 1994: 109-12.
45. Park CH, Gates KM, Vandel NM, Hixon DL. Isolation of Shiga-like toxin producing *Escherichia coli* (O157 and non-O157) in a community hospital. Diagn Microbiol Infect Dis 1996;26:69-72.
46. Tarr PI, Neill MA. Perspective: the problem of non-O157:H7 shiga toxin (Verocytotoxin)-producing *Escherichia coli* [comment]. J Infect Dis 1996;174:1136-9.
47. Rowe PC, Orrbine E, Lior H, Wells GA, McLaine PN. A prospective study of exposure to verotoxin-producing *Escherichia coli* among Canadian children with haemolytic uraemic syndrome. The CPKDRC co-investigators. Epidemiol Infect 1993;110:1-7.
48. Johnson R, Clark R, Wilson J, Read S, Rhan K, Renwick S, et al. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verocytoxigenic *Escherichia coli*. J Food Protect 1996;59:1112-22.
49. Nataro JP, Kaper JB. Diarrheogenic *Escherichia coli*. Clin Microbiol Rev 1998;11:1-60.
50. Hedberg CW, Savarino SJ, Besser JM, Paulus CJ, Thelen VM, Myers LJ, et al. An outbreak of foodborne illness caused by *Escherichia coli* O39:NM, an agent not fitting into the existing scheme for classifying diarrheogenic *E. coli*. J Infect Dis 1997;176:1625-8.
51. Tappero J, Schuchat A, Deaver K, Mascola L, Wenger J. Reduction in the incidence of human listeriosis in the United States. Effectiveness of prevention efforts. JAMA 1995;273:1118-22.
52. Slutsker L, Schuchat A. Listeriosis in Humans. In: Ryser E, Marth E, editors. *Listeria*, listeriosis, and food safety. New York: Marcel Dekker; 1999: 75-96.
53. Schuchat A, Lizano C, Broome C, Swaminathan B, Kim C, Winn K. Outbreak of neonatal listeriosis associated with mineral oil. Pediatr Infect Dis J 1991;10:183-9.
54. Hoffman TA, Ruiz CJ, Counts GW, Sachs JM, Nitzkin JL. Waterborne typhoid fever in Dade County, Florida. Clinical and therapeutic evaluation of 105 bacteremic patients. Am J Med 1975;59:481-7.
55. Klotz SA, Jorgensen JH, Buckwold FJ, Craven PC. Typhoid fever. An epidemic with remarkably few clinical signs and symptoms. Arch Intern Med 1984;144:533-7.
56. Mermin J, Townes J, Gerber M, Dolan N, Mintz E, Tauxe R. Typhoid fever in the United States, 1985-1994. Arch Intern Med 1998;158:633-638.
57. Laboratory confirmed Salmonella surveillance. Annual Summary, 1997. Atlanta, Georgia: Centers for Disease Control and Prevention; 1999.
58. Cohen M, Tauxe R. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. Science 1986;234:964-9.
59. Tauxe R. *Salmonella*: a postmodern pathogen. J Food Protect 1991;54:563-8.
60. DuPont HL. Shigella species. In: Mandell GL, Douglas RG, Bennett JE, editors. Principles and practice of infectious diseases. 3rd ed. New York: Churchill Livingstone; 1990: 1716-22.
61. Black RE, Craun GF, Blake PA. Epidemiology of common-source outbreaks of shigellosis in the United States, 1961-1975. Am J Epidemiol 1978;108:47-52.
62. Levine WC, Bennett RW, Choi Y, Henning KJ, Rager JR, Hendricks KA, et al. Staphylococcal food poisoning caused by imported canned mushrooms. J Infect Dis 1996;173:1263-7.

63. Holmberg S, Blake P. Staphylococcal food poisoning in the United States. JAMA 1984;251:487-9.
64. Mahon B, Mintz E, Greene K, Wells J, Tauxe R. Reported cholera in the United States, 1992-1994. JAMA 1996;276:307-12.
65. Shapiro RL, Altekruse S, Hutwagner L, Bishop R, Hammond R, Wilson S, et al. The role of Gulf Coast oysters harvested in warmer months in *Vibrio vulnificus* infections in the United States, 1988-1996. Vibrio Working Group. J Infect Dis 1998;178:752-9.
66. Hlady W, Klontz K. The epidemiology of *Vibrio* infections in Florida, 1981-1993. J Infect Dis 1996;173:1176-83.
67. Ostroff S. Yersinia as an emerging infection: epidemiologic aspects of Yersiniosis. Contributions to Microbiology & Immunology 1995;13:5-10.
68. Skeels MR, Sokolow R, Hubbard CV, Andrus JK, Baisch J. Cryptosporidium infection in Oregon public health clinic patients, 1985-88: the value of statewide laboratory surveillance. Am J Public Health 1990;80:305-8.
69. Roberts CL, Morin C, Addiss DG, Wahlquist SP, Mshar PA, Hadler JL. Factors influencing Cryptosporidium testing in Connecticut. J Clin Microbiol 1996;34:2292-3.
70. Petersen C. Cryptosporidium and the food supply. Lancet 1995;345:1128-9.
71. Djuretic T, Wall PG, Nichols G. General outbreaks of infectious intestinal disease associated with milk and dairy products in England and Wales: 1992 to 1996. Commun Dis Rep CDR Wkly 1997;7:R41-5.
72. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider--Connecticut and New York, October 1996. MMWR 1997;46:4-8.
73. Petersen C. Cryptosporidiosis in patients infected with the human immunodeficiency virus [see comments]. Clin Infect Dis 1992;15:903-9.
74. Herwaldt BL, Ackers ML. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. The Cyclospora Working Group [see comments]. N Engl J Med 1997;336:1548-56.
75. Herwaldt BL, Beach MJ. The return of *Cyclospora* in 1997: another outbreak of cyclosporiasis in North America associated with imported raspberries. Cyclospora Working Group [see comments]. Ann Intern Med 1999;130:210-20.
76. Addiss DG, Davis JP, Roberts JM, Mast EE. Epidemiology of giardiasis in Wisconsin: increasing incidence of reported cases and unexplained seasonal trends. Am J Trop Med Hyg 1992;47:13-9.
77. Birkhead G, Vogt RL. Epidemiologic surveillance for endemic *Giardia lamblia* infection in Vermont. The roles of waterborne and person-to-person transmission. Am J Epidemiol 1989;129:762-8.
78. Dennis DT, Smith RP, Welch JJ, Chute CG, Anderson B, Herndon JL, et al. Endemic giardiasis in New Hampshire: a case-control study of environmental risks. J Infect Dis 1993;167:1391-5.
79. Petersen LR, Cartter ML, Hadler JL. A food-borne outbreak of *Giardia lamblia*. J Infect Dis 1988;157:846-848.
80. Osterholm MT, Forfang JC, Ristinen TL, Dean AG, Washburn JW, Godes JR, et al. An outbreak of foodborne giardiasis. N Engl J Med 1981;304:24-8.
81. Guerina NG, Hsu HW, Meissner HC, Maguire JH, Lynfield R, Stechenberg B, et al. Neonatal serologic screening and early treatment for congenital *Toxoplasma gondii* infection. The New England Regional Toxoplasma Working Group [see comments]. N Engl J Med 1994;330:1858-63.
82. Wong SY, Remington JS. Toxoplasmosis in pregnancy [see comments]. Clin Infect Dis 1994;18:853-61.
83. Capo V, Despommier DD. Clinical aspects of infection with *Trichinella* spp. Clinical Microbiology Reviews 1996;9:47-54.
84. Tucker A, Haddix A, Bresee J, Holman R, Parashar U, Glass R. Cost-effectiveness analysis of a rotavirus immunization program for the United States. JAMA 1998;279:1371-76.
85. Rodriguez WJ, Kim HW, Brandt CD, Schwartz RH, Gardner MK, Jeffries B, et al. Longitudinal study of rotavirus infection and gastroenteritis in families served by a pediatric medical practice: clinical and epidemiologic observations. Pediatr Infect Dis J 1987;6:170-6.
86. Gurwith M, Wenman W, Hinde D, Feltham S, Greenberg H. A prospective study of rotavirus infection in infants and young children. J Infect Dis 1981;144:218-24.
87. Parashar UD, Holman RC, Clarke MJ, Bresee JS, Glass RI. Hospitalizations associated with rotavirus diarrhea in the United States, 1993 through 1995: surveillance based on the new ICD-9-CM rotavirus-specific diagnostic code. J Infect Dis 1998;177:13-7.
88. Jin S, Kilgore PE, Holman RC, Clarke MJ, Gangarosa EJ, Glass RI. Trends in hospitalizations for diarrhea in United States children from 1979 through 1992: estimates of the morbidity associated with rotavirus. Pediatr Infect Dis J 1996;15:397-404.
89. Kilgore PE, Holman RC, Clarke MJ, Glass RI. Trends of diarrheal disease-associated mortality in US children, 1968 through 1991. JAMA 1995;274:1143-48.
90. Kapikian AZ, Chanock RM. Rotaviruses. In: Fields BN, DM DMK, Howley PM, et al, editors. Fields Virology. 3rd ed. Philadelphia: Lippincott-Raven; 1996: 1657-708.
91. Glass RI, Noel J, Mitchell D, Herrmann JE, Blacklow NR, Pickering LK, et al. The changing epidemiology of astrovirus-associated gastroenteritis: a review. Arch Virol - Suppl 1996;12:287-300.
92. Koopmans M, van Duynhoven Y, van de Heide R, et al. Molecular detection and epidemiology of Norwalk-like viruses and Sapporo-like viruses in the Netherlands. Presented at the International Workshop on Human Caliciviruses, Atlanta, Georgia, USA; 1999 Mar 29-31.
93. Grohman G. Viral diarrhoea in children in Australia. In: Tzipori S, et al, editors. Infectious diarrhoea of the young. New York: Elsevier Science Publishers; 1985.
94. Wolfaardt M, Taylor MB, Booysen HF, Englebrecht L, Grabow WOK, Jiang X. Incidence of human calicivirus and rotavirus infection in patients with gastroenteritis in South Africa. J Med Virol 1997;51:290-6.
95. Vial P, Kotloff KL, Tall BD, Morris JG, Levine MM. Detection by immune electron microscopy of 27-nm viral particles associated with community-acquired diarrhea in children. J Infect Dis 1989;161:571-3.
96. Donneli G, Ruggeri FM, Tinari A, Marziano ML, Menichella D, Caione D, et al. A three-year diagnostic and epidemiologic study on viral infantile diarrhoea in Rome. Epidemiol Infect 1988;100:311-20.

97. Riepenhoff-Talty M, Saif LJ, Barrett HJ, Suzuki H, Ogra PL. Potential spectrum of etiologic agents of viral enteritis in hospitalized patients. J Clin Microbiol 1983;17:352-6.
98. Suzuki H, Konno T, Kutsuzawa T, Imai A, Tazawa F, Ishida N, et al. The occurrence of calicivirus in infants with acute gastroenteritis. J Med Virol 1979;4:321-6.
99. Pang X, Joensuu J, Vesikari T. Human calicivirus-associated sporadic gastroenteritis in Finnish children less than 2 years of age followed prospectively during a rotavirus vaccine trial. Pediatr Infect Dis J 1999;18:420-6.
100. Mounts A, Holman R, Clarke M, Bresee J, Glass R. Trends in hospitalizations associated with gastroenteritis among adults in the United States, 1979-1995. Epi Infect . In press 1999.
101. Fankhauser RL NJ, Monroe SS, Ando T, Glass RI. Molecular epidemiology of "Norwalk-like viruses" in outbreaks of gastoenteritis in the United States. J Infect Dis 1998;178:1571-8.
102. Kaplan JE, Feldman R, Campbell DS, Lookabaugh C, Gary WG. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. Am J Pub Health 1982;72:1329-2.
103. Sekine S, Okada S, Hayashi Y. Prevalence of small round structured virus infections in acute gastroenteritis outbreaks in Tokyo. Microbiol Immunol 1989;33:207-17.
104. Viral Gastroenteritis Sub-Committee of the PHLS Virology Committee. Outbreaks of gastroenteritis associated with SRSVs. PHLS Microbiol Digest 1998;10:1-8.
105. Hepatitis surveillance report no. 56. Atlanta: U.S. Department of Health and Human Services, Public Health Service, CDC. 1996.
106. Bell BP, Shapiro CN, Alter MJ, Moyer LA, Judson FN, Mottram K, et al. The diverse patterns of hepatitis A epidemiology in the United States. Implications for vaccination strategies. J Infect Dis 1998;178:1579-84.
107. Hoofnagle JH, Carithers RL, Shapiro C, Ascher N. Fulminant hepatic failure. Summary of a workshop. Hepatology 1995:21:240-252.

Infections Associated with Eating Seed Sprouts: An International Concern

Peter J. Taormina,* Larry R. Beuchat,* and Laurence Slutsker†
*University of Georgia, Griffin, Georgia, USA; and †Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Recent outbreaks of *Salmonella* and *Escherichia coli* O157:H7 infections associated with raw seed sprouts have occurred in several countries. Subjective evaluations indicate that pathogens can exceed 10^7 per gram of sprouts produced from inoculated seeds during sprout production without adversely affecting appearance. Treating seeds and sprouts with chlorinated water or other disinfectants fails to eliminate the pathogens. A comprehensive approach based on good manufacturing practices and principles of hazard analysis and critical control points can reduce the risk of sprout-associated disease. Until effective measures to prevent sprout-associated illness are identified, persons who wish to reduce their risk of foodborne illness from raw sprouts are advised not to eat them; in particular, persons at high risk for severe complications of infections with *Salmonella* or *E. coli* O157:H7, such as the elderly, children, and those with compromised immune systems, should not eat raw sprouts.

With changing food production and eating habits, new pathogens and newly recognized vehicles of infection have emerged. Recent outbreaks of foodborne illness associated with eating fresh produce have heightened concerns that these foods may be an increasing source of illness (1). In the last decade, multiple outbreaks linked to raw seed sprouts have occurred in countries throughout the world (Table 1). Raw seed sprouts have become a popular food item in the United States; in a recent population-based survey, 7% of respondents had eaten alfalfa sprouts in the 5 days before the interview (2). We summarize the epidemiologic and microbiologic data from these outbreaks and review efforts to prevent sprout-associated illness.

Sprout-Associated Outbreaks

Seed sprouts have been implicated as vehicles of transmission in outbreaks of foodborne illness (Table 1). One of the first reported outbreaks, in 1973, was associated with sprouts grown by using a home sprouting kit (3). Soy, mustard, and cress sprouts submitted by one person with gastrointestinal illness were found to contain large numbers of aerobic spore-forming bacteria. Bacteriologic examination of seeds in previously unopened sprouting kits revealed that the soy seeds were contaminated with *Bacillus cereus* in pure culture, while the mustard and cress seeds had *B. cereus* as a minor part of their flora. After germination, all the sprouts contained large numbers of the pathogen. Fecal specimens from patients were not analyzed for *B. cereus* because the laboratory that processed the samples did not consider it an enteric pathogen. Bacteriologic investigation revealed that during seed germination *B. cereus* proliferated to $>10^7$ per g of sprouts. In 1987, Harmon et al. (4) recovered *B. cereus* from 57% of commercially sold alfalfa, mung bean, and wheat seeds intended for sprout production.

Salmonellosis

In 1988, raw mung bean sprouts were implicated in an epidemiologic study as the cause of an outbreak of *Salmonella* Saint-Paul infection in the United Kingdom (5). In addition, *S.* Virchow was isolated from samples of raw bean sprouts and was associated with seven cases of infection. Sprouts were produced from mung bean seeds imported mainly from Australia and Thailand. In a retail survey of mung bean sprouts in Thailand, several

Address for correspondence: Peter J. Taormina, Center for Food Safety and Quality Enhancement, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797, USA; fax: 770-229-3216; e-mail: taormina@cfsqe.griffin.peachnet.edu.

Table 1. Reported outbreaks of illness associated with seed sprouts, 1973–1998

Year	Pathogen	No. of culture-confirmed cases[a]	Location	Type of sprout	Likely source of contamination	Ref.
1973	*Bacillus cereus*	4	1 U.S. state	Soy, cress, mustard	Seed	3
1988	*Salmonella* Saint-Paul	143	United Kingdom	Mung	Seed	5
1989	*S.* Gold-Coast	31	United Kingdom	Cress	Seed and/or sprouter	7
1994	*S.* Bovismorbificans	595	Sweden, Finland	Alfalfa	Seed	8,9
1995	*S.* Stanley	242	17 U.S. states, Finland	Alfalfa	Seed	10
1995-96	*S.* Newport	133[b]	>7 U.S. states, Canada, Denmark	Alfalfa	Seed	11
1996	*S.* Montevideo and *S.* Meleagridis	~500	2 U.S. states	Alfalfa	Seed and/ or sprouter	13
1996	*Escherichia coli* O157:H7	~6,000	Japan	Radish	Seed	16
1997	*E. coli* O157:H7	126	Japan	Radish	Seed	17
1997	*S.* Meleagridis	78	Canada	Alfalfa	Seed	15
1997	*S.* Infantis and *S.* Anatum	109	2 U.S. states	Alfalfa, mung, other	Seed	14
1997	*E. coli* O157:H7	85	4 U.S. states	Alfalfa	Seed	18
1997-98	*S.* Senftenberg	52	2 U.S. states	Clover, alfalfa	Seed and/or sprouter	*
1998	*E. coli* O157:NM	8	2 U.S. states	Clover, alfalfa	Seed and/or sprouter	*
1998	*S.* Havana, *S.* Cubana, and *S.* Tennessee	34	5 U.S. states	Alfalfa	Seed and/or sprouter	*

[a]The number of culture-confirmed cases represents only a small proportion of the total illness in these outbreaks, as many ill persons either do not seek care or do not have a stool culture performed if they do seek care.
[b]Includes only culture-confirmed cases in Oregon and British Columbia.
*Mohle-Boetani J., pers. comm.

serotypes of *Salmonella* were isolated from 8.7% of samples tested (6).

An outbreak of *S.* Gold-Coast in England and Wales in 1989 was associated with eating mustard cress sprouts grown from seed imported from The Netherlands. The outbreak serotype was isolated during routine sampling of cress sprouts from the factory 2 weeks before the outbreak occurred (7). Cultures of cress seeds did not yield the pathogen.

In Finland, eight sprout-borne *Salmonella* outbreaks occurred from 1980 to 1997 (8). In 1994, two large outbreaks of salmonellosis were linked to alfalfa sprouts (282 cases in Sweden and 210 cases in Finland) (9). Both outbreaks were caused by *S.* Bovismorbificans; the implicated sprouts were grown from Australian alfalfa seeds.

In 1995, a large international outbreak of *S.* Stanley infections in Finland and 17 states in the United States was caused by alfalfa sprouts grown from contaminated seeds (10). *S.* Stanley isolates from patients in Finland and the United States had an indistinguishable DNA pattern by pulsed-field gel electrophoresis (PFGE) and an unusual antimicrobial resistance pattern that was identical among outbreak strains but differed from *S.* Stanley strains isolated from nonoutbreak-related cases. Sprouts that caused the outbreaks in both countries were grown from seeds obtained from the same shipper in The Netherlands, suggesting the seeds were contaminated at some point during growing, harvesting, or processing.

In late 1995 and early 1996, outbreaks of salmonellosis in Denmark and Oregon and British Columbia, Canada, were associated with eating alfalfa sprouts contaminated with *S.* Newport (11). Patients in this multinational outbreak had eaten alfalfa sprouts grown from four separately numbered lots of alfalfa seeds. The seeds implicated in the North American outbreaks were shipped by the same Dutch firm

implicated in the *S.* Stanley outbreak. A retrospective study determined that substantial increases in *S.* Newport infections occurred in Denmark and several states in the United States during the time that these seeds were likely to have been sprouted and eaten (11). PFGE patterns of *S.* Newport isolates from the Oregon and British Columbia outbreaks were indistinguishable from each other (11) and from isolates obtained during *S.* Newport outbreaks in late 1995 in Georgia and Vermont in the United States and in June 1995 in Denmark. Cultures of the implicated seeds yielded *S.* Newport (12).

In June 1996, the largest recorded sprout-associated outbreak in the United States occurred in California, resulting in >450 culture-confirmed cases of infection with *Salmonella* serotypes Montevideo and Meleagridis (13). The same strain of *S.* Meleagridis was isolated from patients and from alfalfa sprouts obtained from retail stores and the sprouting facility. Investigation at the sprouter revealed unsanitary sprouting practices and suboptimal employee hygiene. At the farm where the implicated alfalfa seed was grown, chicken manure was used to fertilize the field before planting. Horses grazed in adjacent fields, and their manure was collected and stored next to the alfalfa field.

An outbreak of *Salmonella* serotypes Infantis and Anatum, which occurred from February through June of 1997 in Kansas and Missouri, was associated with eating contaminated alfalfa sprouts produced by a local sprouter (14). On the basis of epidemiologic, traceback, and laboratory findings, the source of *Salmonella* contamination in this outbreak was determined to be alfalfa seeds.

In October 1997 in Alberta, Canada, an outbreak of *S.* Meleagridis infections was linked to eating alfalfa sprouts, and the outbreak serotype was isolated from retail product (15). During the same period, cases of *S.* Meleagridis infection with the same phage type occurred in persons who had eaten sprouts produced by sprouters in two other provinces but grown from the same alfalfa seed lot as the one implicated in Alberta.

In Northern California, in late 1997 and June 1998, two clusters of *S.* Senftenberg infections were associated with eating an alfalfa and clover sprout mixture; because the two types of sprouts were always mixed before sale, it was not possible to determine which type of seed was implicated (Mohle-Boetani J, pers. comm.). Cultures of clover and alfalfa seeds used to grow the implicated sprouts did not yield *S.* Senftenberg.

In May 1998, a cluster of *S.* Havana infections among patients in Arizona and California was linked to eating alfalfa sprouts (Mohle-Boetani J, pers. comm.). An outbreak of *S.* Cubana infections occurred from May to September 1998 among residents of Arizona, California, and New Mexico, also linked to eating alfalfa sprouts from the same grower implicated in the *S.* Havana outbreak. Alfalfa sprouts eaten by patients in both clusters were grown from the same seed lot, and cultures of seed from this implicated lot yielded *S.* Havana, *S.* Cubana, and *S.* Tennessee (Mohle-Boetani J, pers. comm.).

Enterohemorrhagic *Escherichia coli* Infection

Escherichia coli O157:H7 infection has also been related to eating sprouts. In the world's largest reported outbreak of *E. coli* O157:H7 infections, which occurred in Japan in 1996, white (daikon) radish sprouts were epidemiologically linked to approximately 6,000 of the nearly 10,000 cases reported (16). The pathogen was not detected in cultures of implicated seeds. In the following year, white radish sprouts were again implicated in an outbreak of *E. coli* O157:H7 infection affecting 126 people in Japan (17).

In July 1997, simultaneous outbreaks of *E. coli* O157:H7 infection in Michigan and Virginia were linked by independent epidemiologic investigations with eating alfalfa sprouts grown from the same lot of seeds (18). Molecular subtyping by PFGE revealed that strains from outbreaks in both states were indistinguishable. The simultaneous occurrence of two geographically distinct outbreaks linked to the same lot of alfalfa seeds and caused by the same strain of *E. coli* O157:H7 strongly suggested that contaminated seeds were the source.

In June 1998, a cluster of *E. coli* O157:NM infections in Northern California and Arizona was associated with eating an alfalfa and clover sprout mixture produced by the same sprouter implicated in the *S.* Senftenberg outbreak (Mohle-Boetani J, pers. comm.). *E. coli* O157:NM isolates from the patients had indistinguishable PFGE patterns.

Attempts to Control Microorganisms During Sprouting

Alfalfa and other types of seeds intended for sprouting are considered raw agricultural

commodities. Seeds are harvested and transported from fields to sprouting facilities by methods similar to those used by the cereal grain and fresh produce industries. Grains, fruits, and vegetables may become contaminated with pathogenic microorganisms, e.g., *B. cereus, Salmonella*, or *E. coli* O157:H7, while growing in fields or orchards or during harvesting, handling, processing, and distribution (19,20). Alfalfa seeds generally contain 10^2 to 10^5 aerobic mesophiles per gram (21,22). Piernas and Guiraud (23) reported that the microflora on rice seed exceeded 10^7 colony-forming units (cfu)/g. This naturally occurring population can rapidly increase during germination and sprouting, which is characterized by high moisture and a temperature generally in the range of 21°C to 25°C. Consequently, if seeds become contaminated with a pathogen, the sprouting process provides excellent conditions for its growth and distribution.

Populations of microorganisms on other seeds and sprouts have been studied. Potter and Ehrenfeld (24) detected non-O157 *E. coli* in 5 of 48 samples of mung bean seeds and mature bean sprouts, indicating possible fecal contamination. Alfalfa sprouts and bean sprouts in retail stores have been shown to contain microbial populations of 10^8 to 10^9 cfu/g (25); 6 of 23 retail samples of alfalfa sprouts contained $>10^5$ fecal coliforms per gram. Onion sprouts can contain $>10^9$ aerobic microorganisms per gram (20). Mung bean sprouts from restaurants may contain $>10^6$ cfu/g (26). Jaquette et al. (27) demonstrated that populations of *S.* Stanley in the range of 10^2 to 10^3 cfu/g can increase slightly during 6 hours of soaking, by approximately 10^3 cfu/g during a 24-hour germination period, and by an additional 10^1 cfu/g during a 72-hour sprouting stage, resulting in a 5- to 6-log overall amplification during the sprouting process. Pooled *Salmonella* serotypes inoculated onto mung beans and alfalfa seeds increased substantially during seed germination (21).

Growth characteristics of *E. coli* O157:H7 on radish sprouts have been studied. Itoh et al. (28) demonstrated the presence of *E. coli* O157:H7 not only on the surfaces but also in the inner tissues and stomata of cotyledons of radish sprouts grown from seeds inoculated with the bacterium. When radish seeds or radish sprout roots were soaked in a suspension of *E. coli* O157:H7, the edible parts (cotyledons and hypocotyl) became heavily contaminated (>7 log cfu/g) (29). Taormina and Beuchat (30) showed that *E. coli* O157:H7 inoculated onto alfalfa seeds reached 10^6 to 10^7 cfu/g within 48 hours after the sprouting process began. Populations on mature sprouts subsequently held at 9±2°C for 6 days remained essentially unchanged. Growth of *E. coli* O157:H7 to 10^7 cfu/g of alfalfa sprouts has also been reported by Ingram et al. (31).

Chemical Treatment as an Intervention

Numerous studies have been done to determine the effectiveness of a wide range of chemicals in killing pathogenic bacteria on seed sprouts and seeds intended for sprout production (Table 2). The efficacy of these chemicals as influenced by concentration, temperature, and time of exposure to contaminated seeds has been investigated. No single treatment has been demonstrated to reliably reduce populations of pathogens by more than approximately three logs.

Piernas and Guiraud (32) investigated different methods of disinfection of rice seeds. They observed 10^2 to 10^3 reductions in aerobic plate counts from rice seeds after treatment with 1,000 ppm NaOCl or 10,000 ppm (1%) H_2O_2 at room temperature. Ethanol was very effective in killing naturally occurring microorganisms, although it inhibited seed germination. Becker and Holzapfel (33) surveyed commercial prepackaged sprouts (alfalfa, lentils, wheat, peas, raphanus, sunflower, mung bean, and red radish) and found Enterobacteriaceae and pseudomonads to be the dominant groups of bacteria, with counts of 10^4 to 10^5 cfu/g. Washing sprouts in water did not remove bacteria; this treatment has been shown to reduce numbers of *E. coli* and *Salmonella* by no more than 1 log (24).

Treatment of bean sprouts with ozone has been shown to decrease microbial populations (34). Chlorine treatment, however, is ineffective in killing large numbers of naturally occurring microflora on seeds. Splittstoesser et al. (35) reported that treatment of sprouting mung beans with soak and rinse water containing 100 ppm chlorine reduced the natural microflora by <1 log; treatment of mature sprouts with 5,000 ppm chlorine resulted in a 2-log decrease (36).

The efficacy of chemicals in killing *Salmonella* on alfalfa seeds has been reported by several researchers. Jaquette et al. (27) evaluated chlorine and hot water treatments for their effectiveness in killing *S.* Stanley

Table 2. Control of microorganisms in seed sprouts, by type of treatment and treatment results

Organism, origin	Treatment	Results of treatment	Ref.
Aerobic bacteria, rice seeds	1,000 ppm NaOCl or 10,000 ppm H_2O_2	10^2 to 10^3 reductions in aerobic plate counts; germination inhibited	32
Enterobacteriaceae, pseudomonads, commercial sprouts	Washing in water	Ineffective in removing bacteria	33
Aerobic bacteria, mung bean sprouts	100 ppm chlorine or 5,000 ppm chlorine	Reduced microflora by <1 log and 2 logs, respectively	35
Salmonella Stanley, alfalfa seeds	Chlorine and hot water	No reduction at low levels; reduction of *S.* Stanley achieved with 2,040 ppm chlorine	27
Salmonella, alfalfa seeds	1,800 ppm $Ca(OCl)_2$ or 2,000 ppm NaOCl or 6% H_2O_2 or 80% ethanol	*Salmonella* populations reduced by >3 logs, but pathogen not eliminated	37
E. coli O157:H7, alfalfa seeds	500, 1,000, or >2,000 ppm $Ca(OCl)_2$; 500 ppm acidified ClO_2; >100 ppm and 500 ppm acidified ClO_2; 30% or 70% ethanol; >1% H_2O_2; 8% H_2O_2 for 10 min; dry storage	Populations reduced but not eliminated; germination decreased; pathogen unaffected by dry storage at 5ºC	38
E. coli O157:H7, alfalfa seeds at various stages of sprouting	2,000 ppm NaOCl; 200 and 2,000 ppm $Ca(OCl)_2$; 500 ppm acidified ClO_2	Populations substantially reduced but not eliminated	30
S. Stanley, alfalfa seeds	Heat, 54 to 71ºC	54ºC for 5 min reduced population from 260 to 6-9 cfu/g; treatment for 10 min reduced viability of seed	27
E. coli O157:H7, alfalfa seeds and sprouts	Irradiation at >1.0 kiloGray	Pathogen controlled without affecting germination	39

inoculated onto alfalfa seeds at populations of 10^2 to 10^3 cfu/g. Significant reduction ($p<0.05$) in population was observed when seeds were treated with 100 ppm chlorine for 5 or 10 minutes, and further reduction occurred after treatment with 290 ppm chlorine. Populations of 10^1 to 10^2 cfu of *S.* Stanley per g were reduced to undetectable levels (<1 cfu/g) after seeds were treated with 2,040 ppm chlorine solution. On the basis of these findings, in March 1996 the U.S. Food and Drug Administration recommended that sprout growers soak alfalfa seeds in 500 to 2,000 ppm chlorine solution for 30 minutes before sprouting. However, in none of the subsequent U.S. outbreaks listed in Table 1 was there documented evidence that this recommendation had been followed.

In another study, 10-minute treatment in solutions containing $Ca(OCl)_2$ or NaOCl at concentrations of 1,800 and 2,000 ppm chlorine, respectively, as well as 6% H_2O_2 or 80% ethanol, reduced *Salmonella* populations on alfalfa seeds by >3 logs (37) but did not eliminate the pathogen. Taormina and Beuchat (38) studied the efficacy of various chemical treatments in eliminating 2.0 to 3.2 $\log_{10}$ *E. coli* O157:H7 per g from alfalfa seeds and survivability of the pathogen on seeds during prolonged storage. Significant reductions ($p<0.05$) in population of *E. coli* O157:H7 on inoculated seeds were observed after treatments with 500 or 1,000 ppm chlorine [as $Ca(OCl)_2$] for 3 but not 10 minutes and with 2,000 ppm $Ca(OCl)_2$, regardless of pretreatment with a surfactant. Populations were reduced after treatment with 30% or 70% ethanol for 3 or 10 minutes, although germination percentage dramatically decreased. Treatment with 0.2% H_2O_2 for 3 or 10 minutes significantly ($p<0.05$) reduced populations of *E. coli* O157:H7 on alfalfa seeds, and the organism was not detected by direct plating after treatment with 1% H_2O_2. However, the pathogen

was detected by enrichment in seed treated with 8% H_2O_2 for 10 minutes. The initial populations of 3 $\log_{10}$ cfu of *E. coli* O157:H7/g of dry seeds stored at 5°C remained relatively constant for 20 weeks.

Taormina and Beuchat (30) investigated the growth of *E. coli* O157:H7 on alfalfa seeds at various stages during sprouting as affected by NaOCl, $Ca(OCl)_2$, acidified $NaClO_2$, acidified ClO_2, Na_3PO_4, or H_2O_2. Spray application of 2,000 ppm NaOCl, 200 and 2,000 ppm $Ca(OCl)_2$, or 500 ppm acidified ClO_2 to germinated seeds significantly ($p<0.05$) reduced the population of *E. coli* O157:H7. None of the chemical treatments evaluated eliminated *E. coli* O157:H7 on alfalfa seeds and sprouts.

Application of heat to kill pathogens on alfalfa seeds has been investigated. Treatment of seeds containing approximately 260 cfu of *S.* Stanley per g at temperatures from 54°C to 71°C for 5 or 10 minutes was studied by Jaquette et al. (27). Treatment at 54°C reduced the number to 6 to 9 cfu/g. Treatment at 57°C for 5 minutes reduced populations to <1 cfu/g. Heating seeds at 54°C, 57°C, or 60°C for 5 minutes did not substantially reduce the viability of seeds; however, treatment at these temperatures for 10 minutes reduced viability from 96% (control) to 88%, 84%, and 42%, respectively. Although heat treatment appears to be effective in killing *S.* Stanley on alfalfa seeds, the range of temperatures that can be used is narrow, i.e., 57°C to 60°C for 5 minutes. Lower temperatures may not kill the pathogens, and higher temperatures or longer exposure time (10 minutes) decreased germination. Heating (55°C) alfalfa seeds containing 2.2 to 2.3 $\log_{10}$ cfu of *E. coli* O157:H7 per g in solutions containing up to 20,000 ppm chlorine, 1,200 ppm acidified sodium chlorite, 500 ppm acidified ClO_2, 5% H_2O_2, or 8% Na_3PO_4 for 3 minutes did not eliminate the pathogen (38).

The use of gamma irradiation to eliminate *E. coli* O157:H7 on alfalfa seeds and sprouts has been investigated (39). Studies at the U.S. Department of Agriculture have shown that doses approved for irradiating meat (which are higher than the 1.0 kiloGray dose allowed for fruits and vegetables) control *Salmonella* and *E. coli* O157:H7 on alfalfa sprouts. Both pathogens are more resistant to irradiation on dry seeds than on sprouts. At doses required to eliminate *E. coli* O157:H7, germination of seeds was not affected. These preliminary results need to be confirmed by other studies.

Conclusions

Eating seed sprouts has been associated with numerous outbreaks in the United States and other countries, resulting in thousands of culture-confirmed illnesses; multiple pathogens have been involved, including *E. coli* O157, *B. cereus*, and many serotypes of *Salmonella*. Although most outbreaks have been associated with alfalfa sprouts, other raw seed sprouts have also been linked to illness.

Sprouts follow a complex path from farm to table that includes growing, harvesting, processing, and shipping of seeds, followed by sprouting and distribution of the finished product. Contamination can occur at any of these points in production and distribution. Measures that may help to reduce seed contamination include ensuring the use of properly treated manure as fertilizer on fields; using clean equipment to harvest, transport, and process seeds; and preventing contamination of seeds by rodents or other animals during processing, distribution, and storage. Some types of seeds used to produce sprouts for human consumption are also used to produce forage for animal feed, so these measures to reduce contamination may require changes in current agronomic, harvesting, and storage practices. At sprouting facilities, efforts must be made to ensure that good manufacturing practices are followed and that employees have access to adequate sanitary and handwashing facilities. Sprouters should be registered with the appropriate state and federal regulatory authorities to facilitate appropriate monitoring and inspection. To reduce the risk of sprout-associated foodborne disease, a comprehensive approach based on good manufacturing practices and principles of hazard analysis and critical control points needs to be implemented.

Compared with other fresh produce, sprouts pose a special risk because the sprouting process is a potent bacterial amplification step that occurs shortly before marketing and consumption. Pathogens can exceed 10^7 per gram of sprouts during sprout production without adversely affecting the appearance of the product. Thus, technical approaches to ensuring sprout safety may require several steps to remove pathogens from seeds both before they

are sprouted and during the sprouting process. The most effective chemical treatment currently available is soaking alfalfa seeds in 20,000 ppm active chlorine for at least 10 minutes before sprouting (38). However, this treatment may not be sufficient to eliminate the risk. Further research is needed to identify specific interventions, either applied alone or in combination with other chemical or physical treatments, to eliminate pathogens from contaminated seeds. The effort to address these research needs has resulted in an ongoing collaborative effort among industry, academia, and government, which provides a model example of interagency cooperation to prevent foodborne diseases (40,41). However, until effective measures to prevent sprout-associated illness are identified, persons who wish to reduce their risk for foodborne illness from raw sprouts are advised not to eat them; in particular, persons at high risk for severe complications of infections with *Salmonella* or *E. coli* O157:H7, such as the elderly, children, and those with compromised immune systems, should not eat raw sprouts (18,42).

Illness associated with eating sprouts and other fresh produce highlights the need for enhanced public health surveillance to detect foodborne outbreaks. Fresh produce such as lettuce, tomatoes, and seeds for sprouting may have complex and widely dispersed distribution patterns, as well as low or intermittent levels of contamination. Thus, outbreaks due to these items may be geographically diverse and have a low attack rate (1,43,44). Laboratory-based surveillance and subtyping of isolates from sprout- and produce-associated outbreaks are critical for recognition of these events and timely response. Subtyping of isolates, including serotyping and molecular typing such as PFGE, can help determine whether clusters of infections across several states are related (10), as well as to link geographically distinct outbreaks to a common source (18). At the national level, surveillance has been enhanced by a cluster-detection algorithm applied routinely to *Salmonella* serotype surveillance data to detect possible outbreaks (45). For *E. coli* O157:H7, a national electronic subtyping network has been established that will allow participating state public health laboratories and the Centers for Disease Control and Prevention to rapidly compare DNA PFGE patterns of *E. coli* O157:H7 strains with the patterns in a national database.

Some sprout-associated foodborne outbreaks have been international in scope, underscoring the importance of close communication and collaboration among nations to rapidly recognize and control such events (9-11). Successful response to international foodborne outbreaks has demonstrated the utility of a common language, such as *Salmonella* serotyping, for comparing strains from around the world (46). International surveillance networks such as Enternet (formerly Salm-Net) provide a forum for rapid exchange of surveillance data and notifications about outbreaks that may involve internationally distributed food products, including seeds intended for sprouts (10,47).

Peter Taormina is a graduate student in food science at the University of Georgia's Center for Food Safety and Quality Enhancement. His research interests include foodborne illness and the microbiologic hazards associated with fresh produce.

References

1. Tauxe R, Kruse H, Hedberg C, Potter M, Madden J, Wachsmuth K. Microbial hazards and emerging issues associated with produce: a preliminary report to the national advisory committee on microbiologic criteria for foods. J Food Protection 1997;60:1400-8.
2. Centers for Disease Control and Prevention. FoodNet population survey atlas of exposures, 7/96–6/97, Atlanta: 1997.
3. Portnoy BL, Goepfert JM, Harmon SM. An outbreak of *Bacillus cereus* food poisoning resulting from contaminated vegetable sprouts. Am J Epidemiol 1976;103:589-94.
4. Harmon SM, Kautter DA, Soloman HM. *Bacillus cereus* contamination of seeds and vegetable sprouts grown in a home sprouting kit. J Food Protection 1987;50:62-5.
5. O'Mahony M, Cowden J, Smyth B, Lynch D, Hall M, Rowe B, et al. An outbreak of *Salmonella* Saint-Paul infection associated with bean sprouts. Epidemiol Infect 1989;104:229-35.
6. Jerngklinchan J, Saitanu K. The occurrence of salmonellae in bean sprouts in Thailand. Southeast Asian J Trop Med Public Health 1993;24:114-8.
7. Joce R, O'Sullivan DG, Strong C, Rowe B, Hall MLM, Threlfall EJ. A national outbreak of *Salmonella* Gold-Coast. Commun Dis Rep CDR Rev 1990:4:3-4.
8. Puohiniemi R, Heiskanen T, Siitonen A. Molecular epidemiology of two international sprout-borne *Salmonella* outbreaks. J Clin Microbiol 1997;35:2487-91.
9. Ponka A, Anderson Y, Sitonen A, deJong B, Jahkola M, Haikala O, et al. *Salmonella* in alfalfa sprouts. Lancet 1995;345:462-3.

10. Mahon BE, Ponka A, Hall WN, Komatsu K, Dietrich SE, Sittonen A, et al. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. J Infect Dis 1997;175:876-82.
11. Van Beneden CA, Keene WE, Stang RA, Werker DH, King AS, Mahon B, et al. Multinational outbreak of *Salmonella enterica* serotype Newport infections due to contaminated alfalfa sprouts. JAMA 1999;281:158-62.
12. Aabo S, Baggesen DL. Growth of *Salmonella* Newport in naturally contaminated alfalfa sprouts and estimation of infectious dose in a Danish *Salmonella* Newport outbreak due to alfalfa sprouts. In: Program and abstracts of *Salmonella* and Salmonellosis '97; Nice, France; 1997;425-6.
13. Mouzin E, Werner SB, Bryant RG, Abbott S, Farrar J, Angulo F, et al. When a health food becomes a hazard: a large outbreak of salmonellosis associated with alfalfa sprouts. In: Program and abstracts of the 46th Annual Epidemic Intelligence Service Conference. Atlanta, GA:1997:15.
14. Glynn MK, Patrick S, Wuhib T, Neimann J, Flahert R, Boxrud D, et al. When health food isn't so healthy—an outbreak of *Salmonella* serotypes Anatum and Infantis infections associated with eating contaminated sprouts, Kansas and Missouri, 1997. In: Programs and abstracts of the 47th Annual Epidemic Intelligence Service Conference. Atlanta, GA:1998;16.
15. Buck P, Grimsrud K, Waters J, Cardinal R, Talbot J, Anand C, et al. Would you like a little *Salmonella* with your sandwich? In: Program and abstracts of the 47th Annual Epidemic Intelligence Service Conference, International Night. Atlanta, GA:1998.
16. Ministry of Health and Welfare of Japan. National Institute of Infectious Diseases and Infectious Disease Control Division. Verocytotoxin-producing *Escherichia coli* (enterohemorrhagic *E. coli*) infection, Japan, 1996–June 1997. Infectious Agents Surveillance Report 1997;18:153-4.
17. Gutierrez E. Japan prepares as O157 strikes again. Lancet 1997;349:1156.
18. Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts—Michigan and Virginia, June-July 1997. MMWR Morb Mortal Wkly Rep 1997;46:741-4.
19. Beuchat LR. Pathogenic microorganisms associated with fresh produce. J Food Protection 1996;59:204-16.
20. Beuchat LR, Ryu JH. Produce handling and processing practices. Emerg Infect Dis 1997;3:459-65.
21. Andrews WH, Mislivec PB, Wilson CR, Bruce VR, Poelma PL, Gibson R, et al. Microbial hazards associated with bean sprouting. Journal of the Association of Official Analytical Chemists 1982;65:241-8.
22. Prokopowich D, Blank G. Microbiological evaluation of vegetable sprouts and seeds. J Food Protection 1990;54:560-2.
23. Piernas V, Guirand JP. Microbial hazards related to rice sprouting. International Journal of Food Science and Technology 1997;32:33-9.
24. Potter AP, Ehrenfeld EE. Microbial loads of bean sprouts: coliform, *E. coli* and *Salmonella* [poster Q-243]. In the 98th General Meeting of the American Society for Microbiology; 1998 May 17-21; Atlanta, Georgia.
25. Patterson JE, Woodburn MJ. *Klebsiella* and other bacteria on alfalfa and bean sprouts at the retail level. Journal of Food Science 1980;45:492-5.
26. Sly T, Ross E. Chinese foods: relationship between hygiene and bacterial flora. J Food Protection 1982;45:115-8.
27. Jaquette CB, Beuchat LR, Mahon BE. Efficacy of chlorine and heat treatment in killing *Salmonella stanley* inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. Appl Environ Microbiol 1996;62:2212-5.
28. Itoh Y, Sugita-Konishi Y, Kusuga F, Iwaki M, Hara-Kudo Y, Saito N, et al. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. Appl Environ Microbiol 1998;4:1532-5.
29. Hara-Kudo Y, Konuma H, Iwaki M, Kasuga F, Sugita-Konishi Y, Ito Y, et al. Potential hazard of radish sprouts as a vehicle of *Escherichia coli* O157:H7. J Food Protection 1997;60:1125-7.
30. Taormina PJ, Beuchat LR. Behavior of enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa sprouts during the sprouting process as influenced by treatment with various chemicals. J Food Protection 1999;62:850-6.
31. Ingram DT, Kantor MA, Meng J. Survival of *E. coli* O157:H7 during sprouting of inoculated alfalfa seeds. Int Assoc Milk Food Environ Sanit Ann Mtg, Prog and Abst Book, August 16-19, 1998, Nashville, TN. p. 36.
32. Piernas V, Guiraud JP. Disinfection of rice seeds to sprouting. J Food Sci 1997;62:611-5.
33. Becker B, Holzapfel WH. Mikrobiologisches risiko von fertgverpackten keimlingen und maßnahmen zur reduzierung ihrer mikrobiellen belastung. Archiv für Lebensmittelhygiene 1997;48:73-96.
34. Naito S, Shiga I. Studies on utilization of ozone in food preservation. IX. Effect of ozone treatment on elongation of hypocotyl and microbial counts of bean sprouts. Journal of the Japanese Society of Food Science and Technology 1989;36:181-8.
35. Splittstoesser DF, Queale DT, Andaloro BW. The microbiology of vegetable sprouts during commercial production. Journal of Food Safety 1983;5:79-86.
36. Moline HE, Kulik MM. Contamination and deterioration of alfalfa sprouts caused by a seedborne isolate of *Erwinia herbicola*. Journal of Food Quality 1997;20:53-60.
37. Beuchat LR. Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds intended for sprout production. Int J Food Microbiol 1996;34:329-33.
38. Taormina PJ, Beuchat LR. Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. J Food Protection 1999;62:318-24.
39. Gordenker A. Better detection methods needed for pathogens on fresh sprouts. Inside Lab Management, Association of Official Analytical Chemists International 1999;3:21-3.
40. Public meeting on sprout safety. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Fresh Produce Subcommittee of the National Advisory Committee of Microbiological Criteria for Foods; 1998 Sep 28-29; Washington, D.C.
41. Pargas N. Sprout research to target 5-log reductions for *E. coli* O157:H7, *Salmonella*. Food Chemical News 1998; October 19:17.

42. Interim advisory on alfalfa sprouts. FDA. Washington, DC: August 31, 1998.
43. Tauxe RV. Emerging foodborne diseases: an evolving public health challenge. Emerg Infect Dis 1997;3:425-34.
44. Tauxe RV. New approaches to surveillance and control of emerging foodborne infectious diseases. Emerg Infect Dis 1998;4:455-6.
45. Hutwagner LC, Maloney EK, Bean NH, Slutsker L, Martin SM. Using laboratory-based surveillance data for prevention: an algorithm for detecting *Salmonella* outbreaks. Emerg Infect Dis 1997;3:395-400.
46. Tauxe RV, Hughes JM. International investigation of outbreaks of foodborne disease. BMJ 1996;313:1093-4.
47. Killalea D, Ward LR, Roberts D, de Louvois J, Sufi S, Stuart JM, et al. International epidemiological and microbiological study of outbreak of *Salmonella agona* infection from a ready to eat savoury snack – I: England, Wales, and the United States. BMJ 1996;313:1105-7.

The Human Ehrlichioses in the United States

Jennifer H. McQuiston, Christopher D. Paddock, Robert C. Holman, and James E. Childs
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

The emerging tick-borne zoonoses human monocytic ehrlichiosis (HME) and human granulocytic ehrlichiosis (HGE) are underreported in the United States. From 1986 through 1997, 1,223 cases (742 HME, 449 HGE, and 32 not ascribed to a specific ehrlichial agent) were reported by state health departments. HME was most commonly reported from southeastern and southcentral states, while HGE was most often reported from northeastern and upper midwestern states. The annual number of reported cases increased sharply, from 69 in 1994 to 364 in 1997, coincident with an increase in the number of states making these conditions notifiable. From 1986 through 1997, 827 probable and confirmed cases were diagnosed by serologic testing at the Centers for Disease Control and Prevention, although how many of these cases were also reported by states is not known. Improved national surveillance would provide a better assessment of the public health importance of ehrlichiosis.

First recognized in the United States in 1986, the human ehrlichioses are considered emerging zoonotic diseases. Two etiologically and epidemiologically distinct forms of illness are recognized: human monocytic ehrlichiosis (HME), caused by *Ehrlichia chaffeensis* (1), and human granulocytic ehrlichiosis (HGE), caused by an agent similar or identical to the veterinary pathogens *E. equi* and *E. phagocytophila* (2). A third species, *E. ewingii*, can also cause human illness (3). The bacteria that cause ehrlichiosis are transmitted to humans through the bite of infected ticks, which acquire the agents after feeding on infected animal reservoirs.

During infection, ehrlichiae form distinctive membrane-bound, intracytoplasmic bacterial aggregates (morulae) in white blood cells. HME is characterized by morulae in monocytes, HGE by morulae in granulocytes. Clinically, HME and HGE are nearly indistinguishable and are characterized by one or more of the following symptoms: fever, headache, myalgia, thrombocytopenia, leukopenia, and elevated liver enzyme levels (4-8). A rash occurs in approximately one third of patients with HME (8) but is less common in patients with HGE (4,9). Most cases of ehrlichiosis are characterized by mild illness. However, complications such as adult respiratory distress syndrome, renal failure, neurologic disorders, and disseminated intravascular coagulation can occur (6,10). Case-fatality ratios are as high as 5% for HME and 10% for HGE (10), although more serious cases are probably overrepresented in these estimates. Other studies have reported case-fatality ratios of <5% for these diseases (4,7).

HME and HGE are most often diagnosed by indirect immunofluorescence assay (IFA), although polymerase chain reaction (PCR) assays are increasingly used (11). A confirmed case is defined as a fourfold change in antibody titer by IFA in acute- and convalescent-phase serum samples, PCR amplification of ehrlichial DNA from a clinical sample, or detection of intraleukocytic morulae and a single IFA titer of ≥ 64. A probable case is defined as a single IFA titer of ≥ 64 or the presence of morulae within infected leukocytes. Laboratory data are only used to support clinical suspicion; the designation of a confirmed or probable case of ehrlichiosis is interpreted in the context of compatible illness (11).

The public health importance of the ehrlichioses has not been well defined, largely because these diseases are newly recognized. Because ehrlichiae are present in blood, concerns have been raised about the risk for perinatal and blood-transfusion transmission

Address for correspondence: J.E. Childs, Centers for Disease Control and Prevention, Mail Stop G13, 1600 Clifton Road, Atlanta, GA 30333, USA; fax: 404-639-2778; e-mail: jfc5@cdc.gov.

(12,13). Ehrlichiae are susceptible to tetracyclines, so rapid and effective treatment is possible (8). However, the nonspecific signs and symptoms of these diseases may interfere with timely clinical diagnosis. Ehrlichial infections can be life-threatening. Raising disease awareness and educating physicians and the public about clinical manifestations and proper treatment are indicated.

A national ehrlichiosis surveillance program does not exist, so national incidence rates have not been determined because of wide variability in state surveillance activities. The Council of State and Territorial Epidemiologists recommended that human ehrlichiosis be made nationally notifiable in 1998, but many states do not have a system for surveillance and do not test for ehrlichiosis in state diagnostic laboratories. We summarize the scope of state-supported surveillance efforts and present data on ehrlichiosis cases reported to state health departments from 1986 through 1997. In addition, we include data on ehrlichiosis cases diagnosed by serologic testing at the Centers for Disease Control and Prevention (CDC).

Reported Ehrlichiosis Cases in the United States

From 1986 through 1997, 1,223 ehrlichiosis cases were reported by 30 state health departments in the United States. Data were reported from 19 states that considered ehrlichiosis notifiable as of August 1998, five that routinely collected information on cases, and six that occasionally received reports of ehrlichiosis cases (Appendix I) (14-17). For states where ehrlichiosis was not notifiable, the designation routine reporting versus occasional reporting was based on the completeness of data provided. Because some states did not differentiate between probable and confirmed cases in their records, both categories were considered cases for the purposes of this report. Of the 1,223 reported ehrlichiosis cases, 742 (60.7%) were categorized as HME, 449 (36.7%) as HGE, and 32 (2.6%) as not ascribed to a specific ehrlichial agent. Using data from 20 states that reported information on deaths, we found case-fatality ratios of 2.7% (8 of 299) for HME and 0.7% (3 of 448) for HGE.

HME and HGE Incidence

Data provided through 1997 were used to calculate state-specific average annual incidence rates for 16 of the 19 states that considered ehrlichiosis notifiable and the five states that routinely collected surveillance data (Table). Although Missouri, South Carolina, and Tennessee considered ehrlichiosis notifiable, average annual incidence rates could not be calculated because these states did not differentiate between HME and HGE. Average annual incidence per one million population was calculated by dividing the number of reported cases by the number of years a state collected data (Table). When possible, average annual incidence by county was determined for HME and HGE (Figures 1-2) (15,17).

Table. Average annual ehrlichiosis incidence (per one million population) for reporting states[a] on the basis of 1995 census data (18)

	Incidence	
State	Human monocytic ehrlichiosis	Human granulocytic ehrlichiosis
Arkansas	5.53	0
Arizona	0.12	0
California	0.02	0.03
Connecticut	0.92	15.90
Florida	0.74	0
Illinois	0.11	0.03
Indiana	0.91	0
Kentucky	0.40	0
Maine	0	0
Minnesota	0.22	3.90
Missouri	3.05	0
North Carolina	4.72	0.05
New Hampshire	0	0
New Jersey	1.47	0.17
New York	0.38	2.68
Oklahoma	2.90	0
Pennsylvania	0.01	0.03
Rhode Island	0	0.67
Texas	0.20	0
Virginia	0.68	0
Wisconsin	0	8.79

[a]Includes states that consider ehrlichiosis notifiable, as well as five states where data are routinely collected. Michigan, South Carolina, and Tennessee did not differentiate between cases of human monocytic ehrlichiosis and human granulocytic ehrlichiosis and are not included in this table.

Most HME cases were reported from the southeastern and southcentral areas of the United States (Table, Figure 1). The highest reported average annual incidence rates of HME were in Arkansas (5.53 per million), North Carolina (4.72 per million), Missouri (3.05 per million), and Oklahoma (2.90 per million). In contrast, the highest reported average annual incidence rates

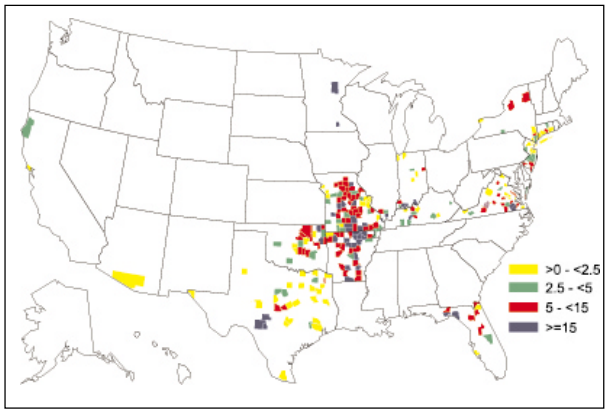


Figure 1. Average annual incidence of reported human monocytic ehrlichiosis (HME) by county, using 1995 population census data (29). Includes states that consider ehrlichiosis notifiable, as well as states that routinely collect information on ehrlichiosis cases. Michigan, South Carolina, and Tennessee are not included because cases of HME and human granulocytic ehrlichiosis were not distinguished by the state health departments. County-specific incidence could not be calculated for North Carolina or Pennsylvania because county of occurrence was not provided by the state health departments.

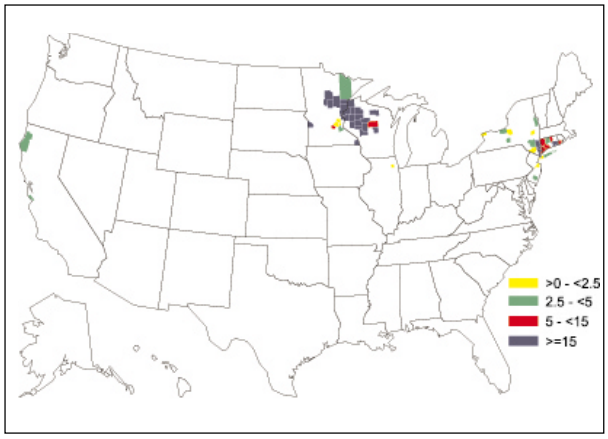


Figure 2. Average annual incidence of reported human granulocytic ehrlichiosis (HGE) by county, using 1995 population census data (29). Includes states that consider ehrlichiosis notifiable, as well as states that routinely collect information on ehrlichiosis cases. Michigan, South Carolina, and Tennessee are not included because cases of human monocytic ehrlichiosis and HGE were not distinguished by the state health departments. County-specific incidence could not be calculated for North Carolina or Pennsylvania because county of occurrence was not provided by the state health departments.

of HGE were in the northeastern and upper midwestern areas of the United States—Connecticut (15.90 per million), Wisconsin (8.79 per million), Minnesota (3.90 per million), and New York (2.68 per million) (Figure 2). The county reporting the highest average annual incidence of HME was Searcy, Arkansas (64.80 per million), and the county with the highest annual incidence of HGE was Jackson, Wisconsin (521.68 per million).

These incidence rates follow the expected geographic distribution of tick vectors for each type of ehrlichiosis. *E. chaffeensis* is primarily transmitted by the lone star tick *(Amblyomma americanum),* which is common in the southeastern United States (19). The black-legged tick (*Ixodes scapularis)* transmits the causative agent of HGE in the northeastern United States (20,21) and the western black-legged tick (*I. pacificus)* in the western coastal United States (22).

Reporting Trends

The annual number of ehrlichiosis cases reported by the state health departments was calculated with data from 18 states that considered ehrlichiosis notifiable as of August 1998 (yearly summaries were not available for Missouri) and the five additional states that routinely collected information on ehrlichiosis cases (Figure 3). The annual number of reported

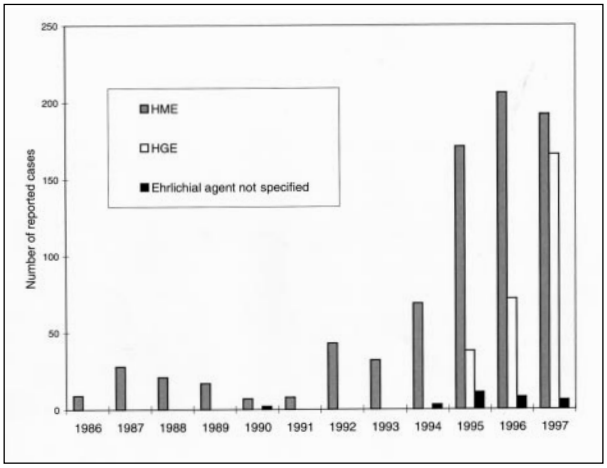


Figure 3. Reported cases of human monocytic ehrlichiosis (HME) and human granulocytic ehrlichiosis (HGE) in the United States, 1986-1997 (includes cases from states that consider ehrlichiosis notifiable, as well as states that routinely collect information). Because yearly summaries of reported cases were not available for Missouri, data from this state are not included. The number of states where ehrlichiosis was notifiable increased from 7 in 1994 to 17 in 1997.

ehrlichiosis cases increased sharply, from 69 in 1994 to 364 in 1997. This increase may be explained by the addition of ehrlichiosis as a notifiable disease in 10 states during this same 4-year interval, the discovery of HGE in 1994, increased availability of diagnostic tests, and increased awareness of ehrlichiosis.

Ehrlichioses Cases Diagnosed at CDC

At CDC, antigen from *E. chaffeensis,* Arkansas strain, is used to diagnose HME by IFA. Before *E. chaffeensis* was isolated in 1991, *E. canis* was used as a surrogate antigen (23). During 1995 to 1996, antigen from *E. equi* obtained from infected horse neutrophils was used, but cases submitted to CDC after 1996 were diagnosed by IFA using cell culture–derived antigen from the HGE agent (24). Antibody from patients with ehrlichial infection may cross-react with both *E. chaffeensis* and the HGE agent (24,25). For patients with significant antibody titers to both *Ehrlichia* species, the causative agent is assumed to be the one with a fourfold or greater change in antibody titer between paired serum samples. If both agents show a fourfold difference, the one with the highest titer is considered the causative agent. If neither shows a fourfold difference, the causative agent is usually not ascribed to a specific ehrlichial species (25).

Of 827 probable and confirmed ehrlichiosis cases diagnosed by IFA from serum or plasma specimens submitted to CDC through the end of 1997, 754 were HME, 44 were HGE, and 29 could not be differentiated because of antibody cross-reactivity. The geographic distribution was widespread (Figures 4, 5), and cases of ehrlichiosis were diagnosed from every state except North Dakota and South Dakota (Appendix 2). Imported disease acquired by travel to disease-endemic areas may explain cases reported from states without the recognized tick vectors, including Hawaii and Alaska. Because information about clinical manifestations was not always provided with specimens, whether all cases had compatible clinical illness is unknown. Of 754 HME cases, 423 (56.1%) were classified as probable and 331 (43.9%) as confirmed on the basis of serologic criteria established by CSTE and CDC (11). In contrast, of 44 HGE cases, 39 (88.6%) were classified as probable and 5 (11.4%) as confirmed.

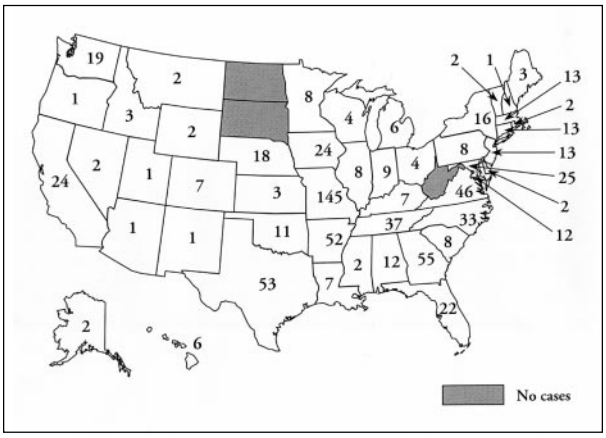


Figure 4. Human monocytic ehrlichiosis cases diagnosed by indirect immunofluorescence assay (IFA), Centers for Disease Control and Prevention, 1986 to 1997.

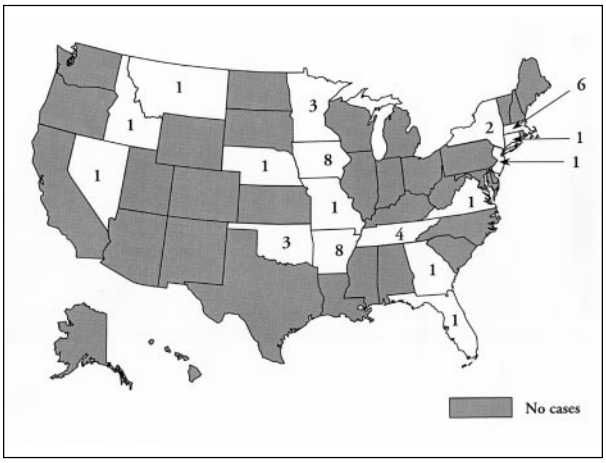


Figure 5. Human granulocytic ehrlichiosis cases diagnosed by indirect immunofluorescence assay (IFA), Centers for Disease Control and Prevention, 1995 to 1997.

Conclusions

Although a few state health departments have published information on local ehrlichiosis surveillance (14-17,26-28), comprehensive national surveillance data had not been collected until this review. This review further defines the public health problem posed by the ehrlichioses in the United States. These diseases have incidence rates comparable with or exceeding those of Rocky Mountain spotted fever in some states (29).

These state-reported data have several limitations. State health departments provided

information on ehrlichiosis cases in different ways. For example, some states provided only data compiled after ehrlichiosis became notifiable, while others provided information as far back as data were available. The ehrlichiosis cases in this article represent a compilation of existing (albeit incomplete) surveillance datasets and probably underestimate the true prevalence of the disease in the United States. Moreover, the accuracy of HME and HGE case-fatality ratios presented here is uncertain. The number of deaths may be underreported because diagnosis of ehrlichiosis requires laboratory confirmation. However, serious or complicated cases, more likely to end in death, are more likely to be investigated and reported to state health departments. The case-fatality ratios described in this article are compatible with findings from other studies (4,7). Finally, the state-reported data include some cases from areas where ehrlichiosis is not commonly diagnosed. For example, a single case of HME was reported from Arizona, although the recognized distribution of the lone star tick does not include this state. Ehrlichiosis cases are usually reported from the patient's county and state of residence at the time of diagnosis; however, ehrlichiosis may be acquired during travel to an area with *Ehrlichia*-infected ticks. Imported cases of ehrlichiosis in states where the disease is not common or tick vectors are absent underscores the need to consider this diagnosis even in areas of low risk.

Diagnostic serologic testing has been offered at CDC since 1986 for HME, and since 1995 for HGE. Records show that from 1986 through 1997 more than 800 ehrlichiosis cases were diagnosed from 48 states. This finding contrasts sharply with state-reported surveillance data, which identified specific geographic regions where ehrlichiosis was most likely to occur.

The number of cases diagnosed at CDC from each state may not accurately reflect expected regional incidence patterns; for example, states with public health laboratories that offer in-house diagnostic tests or states that frequently use commercial laboratories may be less likely to submit samples to CDC for testing. Some cases of ehrlichiosis diagnosed at CDC may also have been reported by state surveillance systems; these reporting systems cannot be regarded as mutually exclusive. The numbers of serologically diagnosed cases of ehrlichiosis reported here may differ from numbers published in other CDC reports because other reports include samples obtained for specific studies (7), whereas most of the cases in this report were submitted for routine diagnostic tests.

As of August 1998, only 19 states considered ehrlichiosis notifiable, and fewer than one fourth of state health departments offered in-house diagnostic assays for HME or HGE. Average annual incidence rates, an important indicator of disease prevalence, could be calculated for only 21 states. These data underscore the need for better nationwide surveillance of ehrlichiosis.

Acknowledgments

We thank the state health departments for sharing these data and gratefully acknowledge the contributions of Bob England, Thomas McChesney, Dennis Berry, Thomas Tsang, Mark Starr, David Schnurr, John Poppy, Jim Meek, Cathy Rebmann, Lisa Conti, Valerie Mock, Jeff Chapman, Mike Loeffelholz, Carl Langkop, Robert Teclaw, Pam Judson, Mike Auslander, Louise McFarland, Mike McGuill, David Blythe, Linda Traviti, Jeff Beckett, Mary Grace Stobierski, Sheril Arndt, Dave Neitzel, Mary Currier, Melissa Johnson, Faye Sorhage, Lorna Lynch, Paul Edistat, Philip Kurpiel, Susan Wong, Todd McPherson, Leslie Wolf, Mike Crutcher, Maria Moll, Ted Donnelly, Ted LeBlanc, Steve McLaughlin, Robert Taylor, Jerry Hindman, Julie Rawlings, Farah Babakhani, Les Branch, John Kobayashi, Jim Kazmierczak, and Ed Belongia.

We thank Joseph Singleton for help in collecting data on confirmed and probable ehrlichiosis cases diagnosed at CDC.

Dr. McQuiston, a veterinarian, is serving as an Officer in the Epidemic Intelligence Service, Centers for Disease Control and Prevention. Her research focuses on the epidemiologic investigation of several zoonotic pathogens, including rabies virus, ehrlichioses, and *Rickettsia rickettsii*.

References

1. Anderson BE, Dawson JE, Jones DC, Wilson KH. *Ehrlichia chaffeensis,* a new species associated with human ehrlichiosis. J Clin Microbiol 1991;29:2838-42.
2. Chen S, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. J Clin Microbiol 1994;32:589-95.
3. Hmiel SP, Buller R, Arens M, Gaudreault-Keener M, Storch GA. Human infection with *Ehrlichia ewingii*, the agent of Ozark canine granulocytic ehrlichiosis. Proceedings of the First International Conference on Emerging Infectious Diseases; 1998 Mar 8-11; Atlanta, Georgia; Addendum:4. [Abstract].
4. Bakken JS, Krueth J, Wilson-Nordskog C, Tilden RL, Asanovich K, Dumler JS. Clinical and laboratory characteristics of human granulocytic ehrlichiosis. JAMA 1996;275:199-205.
5. Dawson JE, Warner CK, Standaert S, Olson JG. The interface between research and the diagnosis of an emerging tick-borne disease, human ehrlichiosis due to *Ehrlichia chaffeensis.* Arch Intern Med 1996;156:137-42.

6. Eng TR, Harkess JR, Fishbein DB, Dawson JE, Greene CN, Redus MA, et al. Epidemiologic, clinical, and laboratory findings of human ehrlichiosis in the United States, 1988. JAMA 1990;264:2251-8.
7. Fishbein DB, Dawson JE, Robinson LE. Human ehrlichiosis in the United States, 1985 to 1990. Ann Intern Med 1994;120:736-43.
8. Fritz CL, Glaser CA. Ehrlichiosis. Infect Dis Clin North Am 1998;12:123-36.
9. Aguero-Rosenfeld ME, Horowitz HW, Wormser GP, McKenna DF, Nowakowski J, Munoz J, et al. Human granulocytic ehrlichiosis: a case series from a medical center in New York state. Ann Intern Med 1996;125:904-8.
10. Dumler JS, Bakken JS. Ehrlichial diseases of humans: emerging tick-borne infections. Clin Infect Dis 1995;20:1102-10.
11. Centers for Diseases Control and Prevention. Case definitions for infectious conditions under public health surveillance. MMWR Morb Mortal Wkly Rep 1997;46:46-7.
12. Arguin PM, Singleton J, Rotz LD, Marston E, Treadwell TA, Slater K, et al. An investigation into the possibility of transmission of tick-borne pathogens via blood transfusion. Transfusion. In press 1999.
13. Horowitz HW, Kilchevsky E, Haber S, Aguero-Rosenfeld M, Kranwinkel R, James EK, et al. Perinatal transmission of the agent of human granulocytic ehrlichiosis. N Engl J Med 1998;339:375-8.
14. Belongia E, Reed K, Mitchell P, Chyou P, Persing D, Finkel M, et al. Active surveillance for human granulocytic ehrlichiosis (HGE) in Northwestern Wisconsin: first year results. Proceedings of the First International Conference on Emerging Infectious Diseases; 1998 Mar 8-11; Atlanta, Georgia; p. 106 [abstract].
15. Hardin LE, Satalowich FT. Tick-borne disease summary—1997. Missouri Epidemiologist; 1998. p. 6-9.
16. Rawlings J. Human ehrlichiosis in Texas. Journal of Spirochetal and Tick-Borne Diseases 1996;3:94-7.
17. Satalowich FT. Tick-borne disease summary—1996. Missouri Epidemiologist; 1997. p. 10-2.
18. Bureau of Census. Intercensual estimates of the population of counties by age, sex, and race: 1995. Washington: The Bureau; 1996.
19. Anderson BE, Sims KG, Olson JG, Childs JE, Piesman JR, Happ CM, et al. *Amblyomma americanum*: a potential vector of human ehrlichiosis. Am J Trop Med Hyg 1993;49:239-44.
20. Magnarelli LA, Stafford KC, Mather TN, Yeh M, Horn KD, Dumler JS. Hemocytic rickettsia-like organisms in ticks: serologic reactivity with antisera to Ehrlichiae and detection of DNA of the agent of human granulocytic ehrlichiosis by PCR. J Clin Microbiol 1995;33:2710-4.
21. Pancholi P, Kolbert CP, Mitchell PD, Reed KD, Dumler JS, Bakken JS, et al. *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. J Infect Dis 1995;172:1007-12.
22. Richter PJ Jr, Kimsey RB, Madigan JE, Barlough JE, Dumler JS, Brooks DL. *Ixodes pacificus* (Acari: Ixodidae) as a vector of *Ehrlichia equi* (Rickettsiales: Ehrlichieae). J Med Entomol 1996;33:1-5.
23. Dawson JE, Rikihisa Y, Ewing SA, Fishbein DB. Serologic diagnosis of human ehrlichiosis using two *Ehrlichia canis* isolates. J Infect Dis 1991;163:564-7.
24. Nicholson WL, Comer JA, Sumner JW, Gingrich-Baker C, Coughlin RT, Magnarelli LA, et al. An indirect immunofluorescence assay using a cell culture-derived antigen for detection of antibodies to the agent of human granulocytic ehrlichiosis. J Clin Microbiol 1997;35:1510-6.
25. Comer JA, Nicholson WL, Olson JG, Childs JE. Serologic testing for human granulocytic ehrlichiosis at a national referral center. J Clin Microbiol 1999;37:558-64.
26. Centers for Disease Control and Prevention. Human ehrlichiosis—Maryland, 1994. MMWR Morb Mortal Wkly Rep 1996;45:798-802.
27. Centers for Disease Control and Prevention. Human granulocytic ehrlichiosis—New York, 1995. MMWR Morb Mortal Wkly Rep 1995;44:593-5.
28. Centers for Disease Control and Prevention. Statewide surveillance for ehrlichiosis—Connecticut and New York, 1994-1997. MMWR Morb Mortal Wkly Rep 1998;47:476-80.
29. Dalton MJ, Clarke MJ, Holman RC, Krebs JW, Fishbein DB, Olson JG, et al. National surveillance for Rocky Mountain spotted fever, 1981-1992: epidemiologic summary and evaluation of risk factors for fatal outcome. Am J Trop Med Hyg 1995;52:405-13.

Appendix I: Ehrlichiosis surveillance by state health departments as of August 1998 and total number of cases reported through 1997.

State	First year reportable	Laboratory Tests offered	Human monocytic ehrlichiosis cases	Human granulocytic ehrlichiosis cases	Ehrlichial agent not specified	Total cases
Alabama	Not reportable	None available	--	--	--	--
Alaska	Not reportable	None available	--	--	--	--
Arizona	1997	None available	1	0	0	1
Arkansas	1993	None available	55	0	0	55
California	1996	IFA for both; PCR for both	2	3	0	5
Colorado	Not reportable[a]	None available	--	--	3	3
Connecticut	1995	IFA for both; PCR for both	9	156	9	174
Delaware	Not reportable	None available	--	--	--	--
District of Columbia	Not reportable	None available	--	--	--	--
Florida	1996	IFA for HME only	21	0	0	21
Georgia	Not reportable	None available	--	--	--	--
Hawaii	Not reportable	None available	--	--	--	--
Idaho	Not reportable	None available	--	--	--	--
Illinois	Not reportable[b]	None available	5	1	2	8
Indiana	Not reportable[b]	IFA for HME only	21	0	0	21
Iowa	Not reportable	IFA for both; PCR for both	--	--	--	--
Kansas	Not reportable	None available	--	--	--	--
Kentucky	1989	None available	14	0	0	14
Louisiana	Not reportable[a]	None available	--	--	1	1
Maine	1996	None available	0	0	0	0
Maryland	Not reportable[a]	IFA for both	6	0	1	7
Massachusetts	Not reportable[a]	None available	0	5	0	5
Michigan	1993	None available	--	--	2	2
Minnesota	1996	None available	2	36	0	38
Mississippi	Not reportable[a]	None available	--	--	1	1
Missouri	Reportable, date unknown	None available	162	0	0	162
Montana	Not reportable	None available	--	--	--	--
Nebraska	Not reportable	None available	--	--	--	--
Nevada	Not reportable	None available	--	--	--	--
New Hampshire	1996	None available	0	0	0	0
New Jersey	1995	IFA for both; PCR for both	35	4	0	39
New Mexico	Not reportable[a]	None available	1	0	0	1
New York	1996	IFA for both; PCR for both	28	195	0	223
North Carolina	1998	IFA for HME only	204	1	0	205
North Dakota	Not reportable	None available	--	--	--	--
Ohio	Not reportable	None available	--	--	--	--
Oklahoma	Not reportable[b]	None available	76	0	0	76
Oregon	Not reportable	None available	--	--	--	--
Pennsylvania	1992	None available	1	1	1	3
Rhode Island	1996	None available	0	2	0	2
South Carolina	1990	None available	--	--	5	5
South Dakota	Not reportable	None available	--	--	--	--
Tennessee	1996	IFA for HME only	--	--	7	7
Texas	1996	IFA for HME only; PCR for both	45	0	0	45
Utah	Not reportable	None available	--	--	--	--
Vermont	Not reportable	None available	--	--	--	--
Virginia	Not reportable[b]	None available	54	0	0	54
Washington	Not reportable	None available	--	--	--	--
West Virginia	Not reportable	None available	--	--	--	--
Wisconsin	Not reportable[b]	IFA for both; PCR for HGE only	0	45	0	45
Wyoming	Not reportable	None available	--	--	--	--
Total	n/a	n/a	742	449	32	1,223

[a]Occasionally received reports of ehrlichiosis cases.
[b]Routinely collected information on ehrlichiosis cases.
HME, human monocytic ehrlichiosis; HGE, human granulocytic ehrlichiosis; —, not reported by states; IFA, indirect immunofluorescence assay; PCR, polymerase chain reaction; n/a, not applicable.

Appendix II: Probable and confirmed ehrlichiosis cases diagnosed by indirect immunfluorescence assay (IFA), Centers for Disease Control and Prevention, 1986 through 1997.

State	Human monocytic ehrlichiosis			Human granulocytic ehrlichiosis			Ehrlichial agent not determined[a]			Total cases
	Prob[b]	Conf[c]	Total	Prob[b]	Conf[c]	Total	Prob[b]	Conf[c]	Total	
Alabama	8	4	12	0	0	0	0	1	1	13
Alaska	2	0	2	0	0	0	0	0	0	2
Arizona	0	1	1	0	0	0	0	0	0	1
Arkansas	32	20	52	8	0	8	1	1	2	62
California	15	9	24	0	0	0	0	0	0	24
Colorado	6	1	7	0	0	0	0	0	0	7
Connecticut	9	4	13	0	1	1	0	0	0	14
Delaware	1	1	2	0	0	0	0	0	0	2
District of Columbia	4	8	12	0	0	0	1	0	1	13
Florida	15	7	22	0	1	1	2	1	3	26
Georgia	30	25	55	1	0	1	1	0	1	57
Hawaii	2	4	6	0	0	0	0	0	0	6
Idaho	3	0	3	1	0	1	0	0	0	4
Illinois	3	5	8	0	0	0	0	0	0	8
Indiana	9	0	9	0	0	0	0	0	0	9
Iowa	19	5	24	8	0	8	2	0	2	34
Kansas	1	2	3	0	0	0	0	0	0	3
Kentucky	5	2	7	0	0	0	0	0	0	7
Louisiana	5	2	7	0	0	0	0	0	0	7
Maine	2	1	3	0	0	0	0	0	0	3
Maryland	18	7	25	0	0	0	1	1	2	27
Massachusetts	10	3	13	5	1	6	1	1	2	21
Michigan	4	2	6	0	0	0	0	0	0	6
Minnesota	5	3	8	0	0	0	0	0	0	8
Mississippi	0	2	2	0	0	0	0	0	0	2
Missouri	61	84	145	0	1	1	2	2	4	150
Montana	2	0	2	1	0	1	0	0	0	3
Nebraska	17	1	18	1	0	1	0	1	1	20
Nevada	0	1	1	1	0	1	0	0	0	2
New Hampshire	1	0	1	0	0	0	0	0	0	1
New Jersey	4	9	13	1	0	1	0	0	0	14
New Mexico	1	0	1	0	0	0	0	0	0	1
New York	13	3	16	2	0	2	0	0	0	18
North Carolina	28	5	33	0	0	0	1	1	2	35
North Dakota	0	0	0	0	0	0	0	0	0	0
Ohio	4	0	4	0	0	0	0	0	0	4
Oklahoma	4	7	11	3	0	3	0	1	1	15
Oregon	0	1	1	0	0	0	0	0	0	1
Pennsylvania	2	6	8	0	0	0	0	0	0	8
Rhode Island	2	0	2	0	0	0	0	0	0	2
South Carolina	4	4	8	0	0	0	0	0	0	8
South Dakota	0	0	0	0	0	0	0	0	0	0
Tennessee	15	22	37	4	0	4	2	0	2	43
Texas	22	31	53	0	0	0	0	0	0	53
Utah	0	1	1	0	0	0	0	0	0	1
Vermont	2	0	2	0	0	0	0	0	2	4
Virginia	17	29	46	1	0	1	2	0	2	49
Washington	13	6	19	0	0	0	0	0	0	19
West Virginia	0	0	0	0	0	0	0	1	1	1
Wisconsin	3	1	4	2	1	3	1	1	2	9
Wyoming	0	2	2	0	0	0	0	0	0	2
Total	423	331	754	39	5	44	17	12	29	827

[a]Includes cases that could not be ascribed to a specific ehrlichial agent because of antibody cross-reactivity.
[b]Probable case (single antibody titer of ≥ 64 by IFA).
[c]Confirmed case (fourfold change in antibody titer in paired serum samples by IFA).

West Nile Fever—a Reemerging Mosquito-Borne Viral Disease in Europe

Zdenek Hubálek and Jirí Halouzka
Academy of Sciences, Brno, Czech Republic

West Nile virus causes sporadic cases and outbreaks of human and equine disease in Europe (western Mediterranean and southern Russia in 1962-64, Belarus and Ukraine in the 1970s and 1980s, Romania in 1996-97, Czechland in 1997, and Italy in 1998). Environmental factors, including human activities, that enhance population densities of vector mosquitoes (heavy rains followed by floods, irrigation, higher than usual temperature, or formation of ecologic niches that enable mass breeding of mosquitoes) could increase the incidence of West Nile fever.

The 1996-97 outbreak of West Nile fever in and near Bucharest, Romania, with more than 500 clinical cases and a case-fatality rate approaching 10% (1-3), was the largest outbreak of arboviral illness in Europe since the Ockelbo-Pogosta-Karelian fever epidemic caused by Sindbis virus in northern Europe in the 1980s. This latest outbreak reaffirmed that mosquito-borne viral diseases may occur on a mass scale, even in temperate climates.

West Nile virus is a member of the Japanese encephalitis antigenic complex of the genus *Flavivirus*, family *Flaviviridae* (4). All known members of this complex (Alfuy, Japanese encephalitis, Kokobera, Koutango, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, Stratford, Usutu, and West Nile viruses) are transmissible by mosquitoes and many of them can cause febrile, sometimes fatal, illnesses in humans.

West Nile virus was first isolated from the blood of a febrile woman in the West Nile district of Uganda in 1937 (5) and was subsequently isolated from patients, birds, and mosquitoes in Egypt in the early 1950s (6-7). The virus was soon recognized as the most widespread of the flaviviruses, with geographic distribution including Africa and Eurasia. Outside Europe (Figure), the virus has been reported from Algeria, Asian Russia, Azerbaijan, Botswana, Central African Republic, Côte d'Ivoire, Cyprus, Democratic Republic of Congo (former Zaire), Egypt, Ethiopia, India, Israel, Kazakhstan, Madagascar, Morocco, Mozambique, Nigeria, Pakistan, Senegal, South Africa, Tajikistan, Turkmenia, Uganda, and Uzbekistan. Furthermore, West Nile virus antibodies have been detected in human sera from Armenia, Borneo, China, Georgia, Iraq, Kenya, Lebanon, Malaysia, the Philippines, Sri Lanka, Sudan, Syria, Thailand, Tunisia, and Turkey (8-10). Kunjin virus is closely related to West Nile virus (11,12), representing a counterpart or subtype for Australia and Southeast Asia; some West Nile

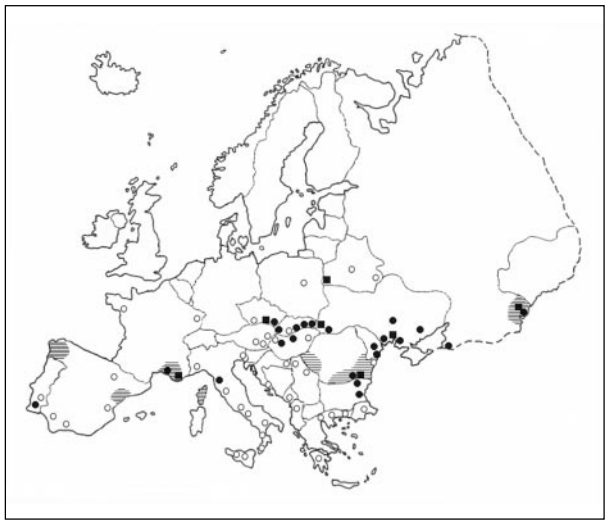


Figure. European distribution of West Nile virus, based on the virus isolation from mosquitoes or vertebrates, including humans (black dots), laboratory-confirmed human or equine cases of West Nile fever (black squares), and presence of antibodies in vertebrates (circles and hatched areas).

Address for correspondence: Z. Hubálek, Institute of Vertebrate Biology, Academy of Sciences, Klášterní 2, CZ-69142 Valtice, Czech Republic; fax: 420-627-352-387; e-mail: zhubalek@brno.cas.cz.

virus seroreactions in Southeast Asia may, in fact, represent antibodies to Kunjin virus.

West Nile Virus Ecology

Arthropod Vectors

Mosquitoes, largely bird-feeding species, are the principal vectors of West Nile virus. The virus has been isolated from 43 mosquito species, predominantly of the genus *Culex* (Table 1). In Africa and the Middle East, the main vector is *Cx. univittatus* (although *Cx. poicilipes, Cx. neavei, Cx. decens, Aedes albocephalus,* or *Mimomyia* spp. play an important role in certain areas). In Europe, the principal vectors are *Cx. pipiens*, *Cx. modestus,* and *Coquillettidia richiardii*, and in Asia, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus,* and *Cx. vishnui* predominate. Successful experimental transmission of the virus has been described in *Culiseta longiareolata*, *Cx. bitaeniorhynchus,* and *Ae. albopictus* (8,13). Transovarial transmission of the virus has been demonstrated in *Cx. tritaeniorhynchus*, *Ae. aegypti*, and *Ae. albopictus,* though at low rates.

Virus isolations have occasionally been reported from other hematophagous arthropods (e.g., bird-feeding argasid [soft] or amblyommine [hard] ticks) (Table 1), and experimental transmission has been observed in *Ornithodoros savignyi*, *O. moubata*, *O.maritimus*, *O. erraticus*, *Rhipicephalus sanguineus*, *R. rossicus*, *Dermacentor reticulatus,* and *Haemaphysalis leachii* (8,13).

Vertebrate Hosts

Wild birds are the principal hosts of West Nile virus. The virus has been isolated from a number of wetland and terrestrial avian species in diverse areas (7-10,14-16). High, long-term viremia, sufficient to infect vector mosquitoes, has been observed in infected birds (7,17,18). The virus persists in the organs of inoculated ducks and pigeons for 20 to 100 days (18). Migratory birds are therefore instrumental in the introduction of the virus to temperate areas of Eurasia during spring migrations (12,14-16,19).

Rarely, West Nile virus has been isolated from mammals (*Arvicanthis niloticus*, *Apodemus flavicollis*, *Clethrionomys glareolus*, sentinel mice and hamsters, *Lepus europaeus*, *Rousettus leschenaulti*, camels, cattle, horses, dogs, *Galago senegalensis*, humans) in enzootic foci (8-10). Mammals are less important than birds in maintaining transmission cycles of the virus in ecosystems. Only horses and lemurs (20) have moderate viremia and seem to support West Nile virus circulation locally. Frogs (*Rana ridibunda*) also can harbor the virus, and their donor ability for *Cx. pipiens* has been confirmed (21).

Transmission Cycles

Although Palearctic natural foci of West Nile virus infections are mainly situated in wetland ecosystems (river deltas or flood plains) and are characterized by the bird-mosquito cycle, argasid and amblyommine ticks may serve as substitute vectors and form a bird-tick cycle in certain dry and warm habitats lacking mosquitoes. Even a frog-mosquito cycle (21) may function under certain circumstances.

In Europe, West Nile virus circulation is confined to two basic types of cycles and ecosystems: rural (sylvatic) cycle (wild, usually wetland birds and ornithophilic mosquitoes) and urban cycle (synanthropic or domestic birds and mosquitoes feeding on both birds and humans, mainly *Cx. pipiens/molestus)*. The principal cycle is rural, but the urban cycle predominated in Bucharest during the 1996-97 outbreak (2,3). Circulation of West Nile fever in Europe is similar to that of St. Louis encephalitis in North America, where the rural cycle of exoanthropic birds—*Cx. tarsalis* alternates with the urban cycle of synanthropic birds—*Cx. pipiens/quinquefasciatus*.

West Nile Fever in Humans and Other Vertebrates

Humans

West Nile fever in humans usually is a febrile, influenzalike illness, characterized by an abrupt onset (incubation period is 3 to 6 days) of moderate to high fever (3 to 5 days, infrequently biphasic, sometimes with chills), headache (often frontal), sore throat, backache, myalgia, arthralgia, fatigue, conjunctivitis, retrobulbar pain, maculopapular or roseolar rash (in approximately half the cases, spreading from the trunk to the extremities and head), lymphadenopathy, anorexia, nausea, abdominal pain, diarrhea, and respiratory symptoms (9). Occasionally (<15% of cases), acute aseptic meningitis or encephalitis (associated with neck stiffness, vomiting, confusion, disturbed consciousness, somnolence, tremor of extremities, abnormal reflexes,

Table 1. Isolations of West Nile virus from hematophagous arthropods (7-10)

Species	No.	Countries
Mosquitoes		
Culex antennatus[a]	6	Egypt, Madagascar
decens group	8	Madagascar
ethiopicus	1	Ethiopia
guiarti	1	Côte d"Ivoire
modestus	3	France, Russia
neavei	4	Senegal, South Africa
nigripes	1	Central African Republic
perexiguus	1	Israel
perfuscus group	3	Central African Republic, Senegal
pipiens[a]	7	South Africa, Egypt, Israel, Romania, Czechland, Bulgaria[b]
poicilipes	29	Senegal
pruina	1	Central African Republic
quinquefasciatus[a]	7	India, Pakistan, Madagascar
scottii	1	Madagascar
theileri[a]	4	South Africa
tritaeniorhynchus[a]	3	Pakistan, India, Madagascar
univittatus[a]	51	Egypt, Israel, South Africa, Madagascar
vishnui[a] group	6	India, Pakistan
weschei	1	Central African Republic
sp.	3	Egypt, Algeria, Central African Republic
Coquillettidia metallica	1	Uganda
microannulata	1	South Africa
richiardii	5	South Russia, Bulgaria[b]
Mansonia uniformis	1	Ethiopia
Aedes aegypti[a]	1	Madagascar
africanus	1	Central African Republic
albocephalus	35	Madagascar
albothorax	1	Kenya
cantans	7	Slovakia, Ukraine, Bulgaria[b]
caspius[a]	1	Ukraine
circumluteolus	2	South Africa, Madagascar
excrucians	1	Ukraine
juppi+caballus	1	South Africa
madagascarensis	1	Madagascar
vexans	3	Senegal, Russia
Anopheles brunnipes	1	Madagascar
coustani	1	Israel
maculipalpis	1	Madagascar
maculipennis	3	Portugal, Ukraine
subpictus	1	India
sp.	1	Madagascar
Mimomyia hispida	8	Senegal
lacustris	4	Senegal
splendens	6	Senegal
sp.	2	Senegal
Aedeomyia africana	1	Senegal
Soft ticks		
Argas hermanni[a]	3	Egypt
Ornithodoros capensis[a]	5	Azerbaijan
Hard ticks		
Hyalomma marginatum	5	Astrakhan, Azerbaijan
detritum	1	Turkmenistan
Rhipicephalus turanicus	1	Azerbaijan
muhsamae	1	Central African Republic
Amblyomma variegatum	1	Central African Republic
Dermacentor marginatus[a]	1	Moldavia

[a]Experimental transmission of the virus also demonstrated.
[b]Detected in mosquitoes by immunofluorescence assay.

convulsions, pareses, and coma), anterior myelitis, hepatosplenomegaly, hepatitis, pancreatitis, and myocarditis occur. Laboratory findings involve a slightly increased sedimentation rate and a mild leukocytosis; cerebrospinal fluid in patients with central nervous system involvement is clear, with moderate pleocytosis and elevated protein. The virus can be recovered from the blood for up to 10 days in immunocompetent febrile patients, as late as 22 to 28 days after infection in immunocompromised patients; peak viremia occurs 4 to 8 days postinfection. Recovery is complete (less rapid in adults than in children, often accompanied by long-term myalgias and weakness), and permanent sequelae have not been reported. Most fatal cases have been recorded in patients older than 50 years. Many of the West Nile fever symptoms have been reproduced in volunteers with underlying neoplastic disease who had been inoculated with virus to achieve pyrexia and oncolysis (22).

Hundreds of West Nile fever cases have been described in Israel and South Africa. The largest African epidemic, with approximately 3,000 clinical cases, occurred in an arid region of the Cape Province after heavy rains in 1974 (23). An outbreak with approximately 50 patients, eight of whom died, was described in Algeria in 1994 (1). Other cases or outbreaks have been observed in Azerbaijan, Central African Republic, Democratic Republic of Congo (former Zaire), Egypt, Ethiopia, India, Madagascar, Nigeria, Pakistan, Senegal, Sudan, and in a few European countries.

Horses

Equine disease, called Near Eastern equine encephalitis in Egypt and lourdige in France, was observed and experimentally reproduced as fever and diffuse encephalomyelitis with a moderate to high fatality rate in Egypt (24), France (c. 50 cases in 1962-65) (25), Italy (14 cases in 1998, six died or were euthanised) (R. Lelli, G. Ferrari, pers. comm.), Portugal (26) and Morocco (42 of 94 affected horses died) (27). In the 1960s, the biphasic, encephalomyelitic form, which causes staggering gait and weakness to paralysis of the hind legs, was apparent among infected semiferal horses in Camargue (25).

Other Mammals

Inoculation of sheep with West Nile virus results in fever, abortion in pregnant ewes, and rare encephalitis, in contrast to the asymptomatic infection seen in pigs and dogs (9,28). Rabbits, adult albino rats, and guinea pigs are resistant to West Nile virus infection, but laboratory mice and Syrian hamsters are markedly susceptible; they often become ill with fatal encephalitis, even when inoculated peripherally (8). Adult rodents stressed or immunosuppressed by cold, isolation, cyclophosphamide, corticosterone, or bacterial endotoxin contract fatal encephalitis, even when an attenuated viral strain is given (29). Inoculation of rhesus and bonnet monkeys (but not cynomolgus monkeys or chimpanzees) causes fever, ataxia, and prostration with occasional encephalitis, tremor of extremities, pareses, or paralysis. Infection may be fatal or cause long-term virus persistence in survivors (5,6,30).

Birds

Birds usually do not show any symptoms when infected with West Nile virus. However, natural disease due to the virus has been observed in a pigeon in Egypt (7), and inoculation of certain avian species (e.g., pigeons, chickens, ducks, gulls, and corvids) causes occasional encephalitis and death or long-term virus persistence (7,10,17,18). Chick embryos may be killed by the virus (8).

West Nile Virus and Fever in Europe

In Europe, the presence of West Nile virus was indicated in 1958, when two Albanians had specific West Nile virus antibodies (31). The first European isolations of the virus were recorded in 1963 from patients and mosquitoes in the Rhône Delta (32) and from patients and *Hyalomma marginatum* ticks in the Volga Delta (33,34). West Nile virus was subsequently isolated in Portugal (35), Slovakia (36), Moldavia (37), Ukraine (38), Hungary (39), Romania (2), Czechland (40), and Italy (V. Deubel, G. Ferrari, pers. comm.).

The incidence of West Nile fever in Europe is largely unknown. In the 1960s, cases were observed in southern France (25), southern Russia (41), Spain (26), southwestern Romania (42), in the 1970s, 1980s, and 1990s in Belarus (43), western Ukraine (44), southeastern Romania (1,2), and Czechland (45). West Nile fever in Europe occurs during the period of maximum annual activity of mosquito vectors (July to September) (Table 2).

Table 2. West Nile Virus in Europe, 1960-1998

Country	Year	Species infected	HI[a] (%)	Neutralization (%)	Ref.
Portugal, southern	1967-1970	Cattle, sheep	15		26, 35
		Horses	29		
		Humans	3		
		Mosquitoes (1 isolate)			
		Wild birds	5		
Spain, northern	1979	Rodents	3		
Northwestern	1960s	Humans	17	17	26
Doñana National Park		Humans	+		
Ebro Delta	1979	Epidemic of influenza-like illness	8-30		
France, southern	1962	Humans, 10 severe cases (2 isolates)	19		25,32,46
		Horses, 50 encephalomyelitis cases (1 isolate)	9	30	
Camargue	1962-1965	Mosquitoes (2 isolates)			
		Wild birds	6		
	1975-1980	Humans	5		
		Horses	2		
Corsica	1965	Humans	30	55	
Italy, Tuscany	1998	Horses, 14 cases, 2 fatal (1 isolate)		40	47
Northeastern	1967-1969	Migrating birds	10		
		Humans	5		[b]
Northwestern		Humans	23		
Central		Humans	2-8		
		Domestic mammals	8		
		Rodents	8		
		Chickens	20		
Southern		Humans	2-5		
		Goats	2-13		
	1981	Rodents	1		
Former Yugoslavia					
Serbia		Humans	1-8		48
Croatia		Humans	1-3		
Montenegro		Humans	1		
Bosnia, Kosovo		Humans	1		
Albania	1958	Humans		2	31,49
		Domestic animals			
Greece	1970-1978	Humans	1-27	1	50,51
		Domestic animals	7		
		Rabbits	4		
		Birds	22		
Bulgaria	1960-1970	Humans	3		52,53
Eastern		Wetland birds	2	10	
		Domestic animals	1		
	1978	Mosquitoes (virus detected)			
Romania					1-3,42,54,55
Bucharest, SE lowlands	1996	Humans, 453 clin. cases, 9% fatality rate (1 isolate)	17		
	1997	Human, 14 cases, 2 fatal			
		Domestic & wild mammals	2-23		[c]
		Dogs	19-45		
		Wild birds	22		
	1966-70	Mosquitoes (1 isolate)			
Banat (SW)		Humans (cases)	17		
Southern	1980-1995	Humans	2-12		
Hungary	1970s	Rodents (2 isolates)			39,56
		Cattle	4-9		
		Humans	4-6		
Slovakia	1972	Mosquitoes (1 isolate)			16, 36, 57-60
	1970-1973	Migrating birds (4 isolates)		1-13	
		Game animals		1-8	
		Cattle, dogs	8		
		Sheep	1		
		Pigeons	5		
		Humans	1-4		
Austria	1964-1977	Wetland passerines	1-3		61,62
		Reptiles			
		Wild mammals			
		Domestic animals	7-33		
		Humans	1-6		
Czechland	1978	Domestic animals	2		40,45,63-67
Southern Bohemia	1978	Hares	5		
Southern Moravia	1980s	Game animals	8		
	1985	Wetland birds	4-10		
	1990	Cormorants	10		
	1997	Mosquitoes (1 isolate)			
		Humans (5 cases)		2	
Poland, near Warsaw	1996	Sparrows	3-12		68
Belarus	1977	Humans (cases in Brest area)	1		43
	1972-1973	Wild birds	3		
Ukraine					38,44,69
Southern, western		Birds (7 isolates), mosquitoes (3 isolates)			
Southern	1970s	Human cases (4 isolates)			
Western	1985	Humans, 38 cases, encephalitis in 16			
Moldavia	1970s	Ticks, mosquitoes (several isolates)			37, 70
		Humans	3		
Russia,					33,34,41,55
Volga Delta	1963-1968	Humans (>10 cases, 3 isolates)	7-31		
		Ticks (4 isolates)			
		Water birds (2 isolates)	4-59	2-11	
		Mosquitoes (2 isolates)			

[a]Hemagglutination inhibition.
[b]Q. Ferrari, R. Lelli, pers. comm.
[c] C. Ceianu, pers. comm.

The Future

West Nile virus can cause sporadic human cases, clusters, or outbreaks of West Nile fever, even in temperate Europe. Environmental factors, including human activities that enhance vector population densities (irrigation, heavy rains followed by floods, higher than usual temperatures, and formation of ecologic niches enabling the mass breeding of mosquitoes) allow the reemergence of this mosquito-borne disease. For instance, global warming scenarios hypothesize warmer, more humid weather that may produce an increase in the distribution and abundance of mosquito vectors (71). Surveillance for West Nile fever (monitoring population densities and infection rates of principal vectors, serosurveys on vertebrates and exposed human groups, and routine diagnosis of human infections) should therefore be carried out in affected areas.

The mechanism of West Nile virus persistence in disease-endemic foci of temperate Europe presents a challenge for further research. General hypotheses of how an arbovirus could overwinter under adverse climatic conditions have already been postulated (72). The virus could persist in hibernating female *Culex* spp.; transovarially infected *Culex* spp. progeny; or chronically infected vertebrate hosts, perhaps birds or frogs. Alternatively, the virus may be reintroduced by chronically infected migratory birds from tropical or subtropical foci at irregular intervals. These issues have to be addressed, because present data substantiate all particular mechanisms and their combinations. For instance, the hibernating vector idea has been supported by a few field and experimental data on female *Cx. univittatus* (7,73). On the other hand, if the reintroduction scheme is correct, a greatly increased activity of West Nile virus in Africa should be followed by an epidemic occurrence of West Nile fever in Europe in the next few years.

Zdenek Hubálek and Jirí Halouzka are scientists at the Academy of Sciences of the Czech Republic. They are interested in the ecology of arthropodborne human pathogenic viruses and bacteria.

References

1. Le Guenno B, Bougermouh A, Azzam T, Bouakaz R. West Nile: A deadly virus? Lancet 1996;348:1315.
2. Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI. West Nile encephalitis epidemic in southeastern Romania. Lancet 1998;352:767-71.
3. Savage HM, Ceianu C, Nicolescu G, Karabatsos N, Lanciotti R, Vladimirescu A, et al. Entomologic and avian investigations of an epidemic of West Nile fever in Romania, 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. Am J Trop Med Hyg. In press 1999.
4. Calisher CH, Karabatsos N, Dalrymple JM, Shope RE, Porterfield JS, Westaway EG, et al. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. J Gen Virol 1989;70:37-43.
5. Smithburn KC, Hughes TP, Burke AW, Paul JH. A neurotropic virus isolated from the blood of a native of Uganda. Am J Trop Med Hyg 1940;20:471-92.
6. Melnick JL, Paul JR, Riordan JT, Barnett VHH, Goldblum N, Zabin E. Isolation from human sera in Egypt of a virus apparently identical to West Nile virus. Proc Soc Exp Biol Med 1951;77:661-5.
7. Taylor RM, Work TH, Hurlbut HS, Rizk F. A study of the ecology of West Nile virus in Egypt. Am J Trop Med Hyg 1956;5:579-620.
8. Karabatsos N, editor. International catalogue of arboviruses, including certain other viruses of vertebrates. 3rd ed., and Supplements 1986-98. San Antonio: American Society of Tropical Medicine and Hygiene; 1985.
9. Peiris JSM, Amerasinghe FP. West Nile fever. In: Beran GW, Steele JH, editors. Handbook of zoonoses. Section B: Viral. 2nd ed. Boca Raton (FL): CRC Press; 1994. p. 139-48.
10. Hubálek Z, Halouzka J. Arthropod-borne viruses of vertebrates in Europe. Acta Scientiarum Naturalium Brno 1996;30(no.4-5):1-95.
11. Zanotto PM de A, Gould EA, Gao GF, Harvey PH, Holmes EC. Population dynamics of flaviviruses revealed by molecular phylogenies. Proc Natl Acad Sci U S A 1996;93:548-53.
12. Berthet FX, Zeller HG, Drouet MT, Rauzier J, Digoutte JP, Deubel V. Extensive nucleotide changes and deletions within the envelope glycoprotein gene of Euro-African West Nile viruses. J Gen Virol 1997;78:2293-7.
13. Hurlbut HS. West Nile virus infection in arthropods. Am J Trop Med Hyg 1956;5:76-85.
14. Nir Y, Goldwasser R, Lasowski Y, Avivi A. Isolation of arboviruses from wild birds in Israel. Am J Epidemiol 1967;86:372-8.
15. Watson GE, Shope RE, Kaiser MN. An ectoparasite and virus survey of migratory birds in the eastern Mediterranean. In: Cherepanov IA, editor. Transcontinental connections of migratory birds and their role in the distribution of arboviruses. Novosibirsk: Nauka; 1972 p.176-80.
16. Ernek E, Kozuch O, Nosek J, Teplan J, Folk C. Arboviruses in birds captured in Slovakia. Journal of Hygiene, Epidemioloigy, Microbiology and Immunology (Prague) 1977;21:353-9.
17. Work TH, Hurlbut HS, Taylor RM. Indigenous wild birds of the Nile Delta as potential West Nile virus circulating reservoirs. Am J Trop Med Hyg 1955;4:872-8.

18. Semenov BF, Chunikhin SP, Karmysheva VY, Yakovleva NI. Studies of chronic arbovirus infections in birds. 1. Experiments with West Nile, Sindbis, Bhanja and SFS viruses (in Russian). Vestnik Akademii Medicinskikh Nauk SSSR (Moskva) 1973;2:79-83.
19. Hannoun C, Corniou B, Mouchet J. Role of migrating birds in arbovirus transfer between Africa and Europe. In: Cherepanov AI, editor. Transcontinental connections of migratory birds and their role in the distribution of arboviruses. Novosibirsk: Nauka; 1972; p. 167-72.
20. Rodhain F, Petter JJ, Albignac R, Coulanges P, Hannoun C. Arboviruses and lemurs in Madagascar: experimental infection of *Lemur fulvus* with yellow fever and West Nile viruses. Am J Trop Med Hyg 1985;34:816.
21. Kostyukov MA, Alekseev AN, Bulychev VP, Gordeeva ZE. Experimental infection of *Culex pipiens* mosquitoes with West Nile virus by feeding on infected *Rana ridibunda* frogs and its subsequent transmission (in Russian). Med Parazitol (Mosk) 1986;6:76-8.
22. Southam CM, Moore AE. Induced virus infections in man by the Egypt isolates of West Nile virus. Am J Trop Med Hyg 1954;3:19-50.
23. McIntosh BM, Jupp PG, Dos Santos I, Meenehan GM. Epidemics of West Nile and Sindbis viruses in South Africa with *Culex (Culex) univittatus* Theobald as vector. South African Journal of Science 1976;72:295-300.
24. Schmidt JR, El Mansoury HK. Natural and experimental infection of Egyptian equines with West Nile virus. Am J Trop Med Hyg 1963;57:415-27.
25. Hannoun C, Panthier R, Corniou B. Epidemiology of West Nile infections in the South of France. In: Bárdoš V, editor. Arboviruses of the California complex and the Bunyamwera group. Bratislava: Publ House SAS; 1969. p. 379-87.
26. Filipe AR, De Andrade HR. Arboviruses in the Iberian Peninsula. Acta Virol 1990;34:582-91.
27. Abdelhaq AT. West Nile fever in horses in Morocco. Bulletin de l'Office International des Épizooties 1996;108:867-9.
28. Barnard BJH, Voges SF. Flaviviruses in South Africa: pathogenicity for sheep. Onderstepoort J Vet Res 1986;53:235-8.
29. Ben-Nathan D, Lustig S, Feuerstein G. The influence of cold or isolation stress on neuroinvasiveness and virulence of an attenuated variant of West Nile virus. Arch Virol 1989;109:1-10.
30. Pogodina VV, Frolova MP, Malenko GV, Fokina GI, Koreshkova GV, Kiseleva LL, et al. Study on West Nile virus persistence in monkeys. Arch Virol 1983;75:71-86.
31. Bárdoš V, Adamcová J, Dedei S, Gjini N, Rosický B, Šimková A. Neutralizing antibodies against some neurotropic viruses determined in human sera in Albania. Journal of Hygiene, Epidemiology, Microbiology and Immunology (Prague) 1959;3:277-82.
32. Hannoun C, Panthier R, Mouchet J, Eouzan JP. Isolement en France du virus West Nile à partir de malades et du vecteur *Culex molestus* Ficalbi. Compte Rendu de l'Académie des Sciences 1964;D259:4170-2.
33. Chumakov MP, Belyaeva AP, Butenko AM. Isolation and study of an original virus from *Hyalomma plumbeum plumbeum* ticks and from the blood of a febrile patient in the Astrakhan region (in Russian). Materialy XI Nauchnoi Sessii Instituta Poliomielita i Virusnykh Encefalitov (Moskva) 1964:5-7.
34. Butenko AM, Chumakov MP, Stolbov DN. Serological and virological examinations in a natural focus of West Nile fever in the Astrakhan region (in Russian). Voprosy Medicinskoi Virusologii 1967;1:208-11.
35. Filipe AR. Isolation in Portugal of West Nile virus from *Anopheles maculipennis* mosquitoes. Acta Virol 1972;16:361.
36. Labuda M, Kozuch O, Grešíková M. Isolation of West Nile virus from *Aedes cantans* mosquitoes in west Slovakia. Acta Virol 1974;18:429-33.
37. Chumakov MP, Bashkirtsev VN, Golger EI, Dzagurova TK, Zavodova TI, Konovalov YN, et al. Isolation and identification of Crimean hemorrhagic fever and West Nile fever viruses from ticks collected in Moldavia (in Russian). Medicinskaya Virusologia 1974;22:45-9.
38. Vinograd IA, Obukhova VR. Isolation of arboviruses from birds in western Ukraine (in Russian). Sbornik Trudov Instituta Virusologii (Moskva) 1975;3:84-7.
39. Molnár E, Gulyas MS, Kubinyi L, Nosek J, Kozuch O, Ernek E, et al. Studies on the occurrence of tick-borne encephalitis in Hungary. Acta Veterinaria Academiae Scientiarum Hungaricae 1976;26:419-38.
40. Hubálek Z, Halouzka J, Juricová Z, Šebesta O. First isolation of mosquito-borne West Nile virus in the Czech Republic. Acta Virol 1998;42:119-20.
41. Butenko AM, Chumakov MP, Bashkirtsev VN, Tkachenko EA, Rubin SG, Stolbov DN. New investigations of West Nile virus infections in the USSR—Astrakhan region (in Russian). Materialy XV Nauchnoi Sessii Instituta Poliomielita i Virusnykh Encefalitov (Moskva) 1968;3:175-6.
42. Topciu V, Rosiu N, Arcan P. Contribution to the study of arboviruses in Banat. Revue Roumaine d'Inframicrobiologie 1971;8:101-6.
43. Voinov IN, Rytik PG, Grigoriev AI. Arbovirus infections in Belarus (in Russian). In: Drozdov SG, editor. Virusy i virusnyje infektsii. Moskva: Inst Poliomiel Virus Enc; 1981. p. 86-7.
44. Buletsa BA, Turak JA, Korol MJ, Ignatovich II, Vitvitskyi AA. Neurological manifestations of West Nile fever in the Transcarpathian region, Ukrainian SSR (in Russian). Zhurnal Nevrologii Psikhiatrii 1989;89:29-30.
45. Hubálek Z, Halouzka J, Juricová Z. West Nile fever in Czechland. Emerg Infect Dis 1999;5:594-5.
46. Rollin PE, Rollin D, Martin P, Baylet R, Rodhain F, Hannoun C. Résultats d'enquetes séroépidemiologiques récentes sur les arboviroses en Camargue: populations humaines, équines, bovines et aviaires. Médecine et Maladies Infectieuses 1982;12:77-80.
47. Verani P, Balducci M, Lopes MC. Arboviruses in Italy. In: Kurstak E, editor. Arctic and tropical arboviruses. New York: Academic Press; 1979. p. 101-21.
48. Vesenjak-Hirjan J, Punda-Poli V, Dobec M. Geographical distribution of arboviruses in Yugoslavia. Journal of Hygiene, Epidemiology, Microbiology and Immunology (Prague) 1991;35:129-40.
49. Eltari E, Gina A, Bitri T, Sharofi F. Some data on arboviruses, especially tick-borne encephalitis, in Albania. Giornale de Malattie Infettive e Parassitaire 1993;45:404-11.
50. Koptopoulos G, Papadopoulos O. A serological survey for tick-borne encephalitis and West Nile viruses in Greece. Zentralblatt für Bakteriologie 1980;Suppl 9:185-8.

51. Antoniadis A, Alexiou-Daniel S, Malissiovas N, Doutsos J, Polyzoni T, LeDuc JW, et al. Seroepidemiological survey for antibodies to arboviruses in Greece. Arch Virol 1990;Suppl 1:277-85.
52. Rusakiev M. Studies on the distribution of arboviruses transmitted by mosquitoes in Bulgaria. In: Bárdoš V, editor. Arboviruses of the California complex and the Bunyamwera group. Bratislava: Publ House SAS; 1969. p. 389-92.
53. Katsarov G, Vasilenko S, Vargin V, Butenko S, Tkachenko E. Serological studies on the distribution of some arboviruses in Bulgaria. Problems of Infectious and Parasitic Diseases 1980;8:32-5.
54. Draganescu N, Gheorghiu V. On the presence of group B arbovirus infections in Romania. Investigations on the incidence of West Nile antibodies in humans and certain domestic animals. Revue Roumaine d'Inframicrobiologie 1968;5:255-8.
55. Antipa C, Girjabu E, Iftimovici R, Draganescu N. Serological investigations concerning the presence of antibodies to arboviruses in wild birds. Revue Roumaine de Médicine - Virologie 1984;35:5-9.
56. Molnár E, Grešíková M, Kubászová T, Kubínyi L, Szabó JB. Arboviruses in Hungary. Journal of Hygiene, Epidemiology, Microbiology and Immunology (Prague) 1973;17:1-10.
57. Kozuch O, Nosek J, Labuda M. Arboviruses occurring in western Slovakia and their hosts. In: Labuda M, Calisher CH, editors. New aspects in ecology of arboviruses. Bratislava: Inst Virol SAS; 1980. p. 323-33.
58. Kozuch O, Nosek J, Grešíková M, Ernek E. Surveillance on mosquito-borne natural focus in Záhorská lowland. In: Sixl W, Troger H, editors. Naturherde von Infektionskrankheiten in Zentraleuropa. Graz: Hyg Inst Univ; 1976. p. 115-8.
59. Sekeyová M, Grešíková M, Kozuch O. Haemagglutination-inhibition antibodies to some arboviruses in sera of pigeons trapped in Bratislava. In: Sixl W, Troger H, editors. Naturherde von Infektionskrankheiten in Zentraleuropa. Graz: Hyg Inst Univ; 1976. p. 187-9.
60. Grešíková M, Sekeyová M. Haemagglutination-inhibiting antibodies against arboviruses in the population of Slovakia. Journal of Hygiene, Epidemiology, Microbiology and Immunology (Prague) 1967;11:278-85.
61. Aspöck H, Kunz C, Picher O, Böck F. Virologische und serologische Untersuchungen über die Rolle von Vögeln als Wirte von Arboviren in Österreich. Zentralblatt für Bakteriologie 1973;A224:156-67.
62. Grešíková M, Thiel W, Batiková M, Stünzner D, Sekeyová M, Sixl W. Haemagglutination-inhibiting antibodies against arboviruses in human sera from different regions in Steiermark (Austria). Zentralblatt für Bakteriologie 1973;224:298-302.
63. Grešíková M, Sekeyová M, Vošta J, Hanák P. Haemagglutination-inhibiting antibodies to some arboviruses in human and animal sera from Ceské Budejovice. In: Sixl W, editor. Naturherde von Infektionskrankheiten in Zentraleuropa. Graz-Seggau: Hyg Inst Univ; 1979. p. 25-9.
64. Juricová Z. Antibodies to arboviruses in game animals in Moravia, Czechland (in Czech). Veterinární Medicina (Praha) 1992;37:633-6.
65. Juricová Z, Hubálek Z, Halouzka J, Machácek P. Virological examination of cormorants for arboviruses (in Czech). Veterinární Medicina (Praha) 1993;38:375-9.
66. Hubálek Z, Jur icová Z, Halouzka J, Pellantová J, Hudec K. Arboviruses associated with birds in southern Moravia, Czechoslovakia. Acta Scientiarum Naturalium Brno 1989;23(7):1-50.
67. Juricová Z, Halouzka J. Serological examination of domestic ducks in southern Moravia for antibodies against arboviruses of the groups A, B, California and Bunyamwera (in Czech). Biológia (Bratisl) 1993;48:481-4.
68. Juricová Z, Pinowski J, Literák I, Hahm KH, Romanowski J. Antibodies to Alphavirus, Flavivirus, and Bunyavirus arboviruses in house sparrows (*Passer domesticus*) and tree sparrows (*P. montanus*) in Poland. Avian Dis 1998;42:182-5.
69. Vinograd IA, Beletskaya GV, Chumachenko SS, Omelchenko GA, Lozinski IN, Yartys OS, et al. Ecological aspects of arbovirus studies in the Ukrainian SSR (in Russian). In: Lvov DK, Gaidamovich SY, editors. Ecology of viruses and diagnostics of arbovirus infections. Moscow: Acad Med Sci USSR; 1989. p. 21-7.
70. Chumakov MP, Spasskiy AA, Uspenskaya IG, Tikhon EI, Zaitsev NA, Konovalov YN, et al. Virological, serological, zoological, and ecological investigations of natural foci of arbovirus infections in Moldavian SSR (in Russian). Abstr Conf "Viruses and virus infections of humans." Moskva 1981, p. 101.
71. Reeves WC, Hardy JL, Reisen WK, Milby MM. Potential effect of global warming on mosquito-borne arboviruses. J Med Entomol 1994;310:323-32.
72. Reeves WC. Overwintering of arboviruses. Prog Med Virol 1974;17:193-220.
73. Cornel AJ, Jupp PG, Blackburn NK. Effect of environmental temperature on the vector competence of *Culex univittatus* (Diptera, Culicidae) for West Nile virus. J Med Entomol 1993;30:449-56.

Morphologic and Molecular Characterization of New *Cyclospora* Species from Ethiopian Monkeys: *C. cercopitheci* sp.n., *C. colobi* sp.n., and *C. papionis* sp.n.

Mark L. Eberhard,* Alexandre J. da Silva,* Bruce G. Lilley,† and Norman J. Pieniazek*
*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; and
†University of Alabama at Birmingham, Birmingham, Alabama, USA

In recent years, human cyclosporiasis has emerged as an important infection, with large outbreaks in the United States and Canada. Understanding the biology and epidemiology of *Cyclospora* has been difficult and slow and has been complicated by not knowing the pathogen's origins, animal reservoirs (if any), and relationship to other coccidian parasites. This report provides morphologic and molecular characterization of three parasites isolated from primates and names each isolate: *Cyclospora cercopitheci* sp.n. for a species recovered from green monkeys, *C. colobi* sp.n. for a parasite from colobus monkeys, and *C. papionis* sp.n. for a species infecting baboons. These species, plus *C. cayetanensis,* which infects humans, increase to four the recognized species of *Cyclospora* infecting primates. These four species group homogeneously as a single branch intermediate between avian and mammalian *Eimeria*. Results of our analysis contribute toward clarification of the taxonomic position of *Cyclospora* and its relationship to other coccidian parasites.

Cyclospora cayetanensis, a coccidian parasite recently described as a human pathogen causing prolonged watery diarrhea (1), has been identified as the cause of large, multistate outbreaks of diarrhea in the United States associated with imported produce, most notably raspberries (2,3). Molecular phylogenetic analysis showed that *Cyclospora* is closely related to *Eimeria* species (4), especially to mammalian *Eimeria* species (5). The parasite has been reported from many geographic regions but seems to be endemic in tropical countries. Recent foodborne outbreaks in the United States and Canada have generated considerable scientific interest and numerous questions about this organism; one of the most perplexing has to do with the possible role of other animals in harboring the infection and serving as a source of contamination.

Address for correspondence: Mark L. Eberhard, Division of Parasitic Diseases, Mail Stop F13, Centers for Disease Control and Prevention, 4770 Buford Hwy, NE, Atlanta, GA 30341-3724, USA; fax: 770-488-4253; e-mail: mle1@cdc.gov.

In 1996, Smith and colleagues reported the presence of *C. cayetanensis*-like oocysts in the feces of 37 of 37 baboons and 1 of 15 chimpanzees examined from the Gombe National Park, Tanzania. Other reports have documented *C. cayetanensis*-like oocysts in fecal samples from chickens in Mexico (6), a duck in Peru (7), and dogs in Brazil (8). However, only the Smith report (9) suggests a true natural host.

During spring 1997, we collected stool samples from free-ranging baboons (*Papio anubis*) and colobus monkeys (*Colobus guereza*) in Wollega Province in western Ethiopia. A high percentage of samples were positive for *Cyclospora* oocysts, but the organism, including sporulated oocysts, could not be completely described because the samples were fixed in formalin. In spring 1998, we returned to Wollega Province, collected additional stool samples from three species of primates (baboons, colobus, and African green monkeys [*Cercopithecus aethiops*]), and placed these samples in potassium dichromate

for subsequent biologic and molecular studies. This report describes the results of those collections, provides molecular phylogenetic analysis, and names the newly identified parasites.

The Study

We collected stool samples from troops of baboons and green monkeys by following them as they foraged and from colobus monkeys by quietly waiting under trees in which monkeys were sitting. Only fresh stool samples were collected, and in neither situation was it possible to determine the age or sex of the animal that produced the sample. On several occasions, samples from more than one animal of the same species were pooled; these are referred to as composite samples.

In the collections from 1997, each stool sample was placed directly in 10% formalin. In the collections from 1998, all stool samples were suspended in water and allowed to settle. The sediment was sieved and resuspended in clean water. The resulting sediment was mixed with a 2.5% aqueous (w/v) potassium dichromate ($K_2Cr_2O_7$) solution in a 3:1 ratio and allowed to settle. The supernatant was discarded, and fresh potassium dichromate solution was added in a 3:1 ratio. The potassium dichromate-stool mixture was kept at room temperature in 50-ml conical centrifuge tubes and returned to Atlanta.

The *C. cayetanensis* oocysts used in comparative studies were collected from stools from a 1997 Florida outbreak linked to consumption of Guatemalan raspberries and from stools collected in Leogane, Haiti.

DNA Extraction

DNA was extracted from 500-µl aliquots of stool samples, following the protocol of da Silva et al. (10). Extracted DNA was stored at 4°C until polymerase chain reaction (PCR) amplification was performed on the small subunit ribosomal RNA (SSU-rRNA) coding region of the genome. Both strands of PCR products were sequenced directly by using an automated DNA sequencer. We used a nested PCR protocol with primers CYCF1E and CYCR2B for the first step of the amplification and primers CYCF3E and CYCR4B for the second (11).

Results

Examination of stools collected in 1997 showed that 15 (68%) of 22 baboons and 9 (60%) of 15 colobus monkeys had detectable *Cyclospora* infections. In individual stool samples collected in 1998, 10 (50%) of 20 baboons, 0 of 11 colobus monkeys, and 1 (6%) of 16 green monkeys had detectable infections with *Cyclospora*. In composite stool samples collected in 1998, 2 (100%) of 2 baboon, 1 (50%) of 2 colobus monkey, and 0 of 3 green monkey samples tested positive for *Cyclospora*.

Sequencing of the SSU-rRNA Coding Region and Phylogenetic Analysis

SSU-rRNA sequences amplified from the *C. cayetanensis* isolates from Haiti and Florida were identical and showed seven differences from the sequence described by Relman et al. (4). Three of these differences correct previously unresolved bases: A at positions 400 and 549, and G at position 1694. Two other differences most probably correct a sequencing error, as they constitute an inversion next to an unresolved position (T at position 1695 and G at position 1696). The significance of the two remaining differences at positions 696 and 1360 is unknown at this time. The new sequence for *C. cayetanensis* SSU-rRNA coding region was deposited in GenBank and assigned accession number AF111183 (Table).

SSU-rRNA sequences obtained for two baboon isolates were identical. The colobus and baboon *Cyclospora* isolates were assigned GenBank accession numbers AF111186 and AF111187, respectively. Sequencing of SSU-rRNA coding region of *C. cercopitheci* from a single African green monkey specimen showed a heterozygotic position, T/A at position #280. This may reflect mixed infection with two closely related isolates or may represent polymorphism among several copies of this gene in a single isolate. Thus, both green monkey SSU-rRNA sequences were submitted separately to GenBank and were assigned accession numbers AF111184 for the green monkey *Cyclospora* sequence #1 and AF111185 for sequence #2 (Table).

The phylogenetic trees generated by the PUZZLE and PAUP programs displayed similar topologic features and demonstrated that on the basis of the SSU-rRNA the *Cyclospora* isolates from monkeys are distinct from each other and from *C. cayetanensis* of humans (Figure 1). The sequence identities between the human isolate with the baboon, colobus monkey, and green monkey isolates 1 and 2 were 98.6%, 98.7%, and

Table. New *Cyclospora* species from Ethiopian monkeys

Characteristics	*Cyclospora cercopitheci*	*C. colobi*	*C. papionis*
Host	*Cercopithecus aethiops* Linnaeus, 1758, African green or vervet monkey	*Colobus guereza* Ruppell, 1835, colobus monkey	*Papio anubis* Lesson, 1827, olive baboon
Oocysts	Spherical; 8 - 10 µm (mean 9.2) in diameter. Outer wall smooth. Wall autofluoresces in UV wavelength.	Small, spherical, 8 - 9 µm (mean 8.3) in diameter. Outer wall smooth. Wall autofluoresces in UV wavelength.	Spherical, 8 - 10 µm (mean 8.8) in diameter. Outer wall smooth. Wall autofluoresces in UV wavelength.
Sporocysts	Two per mature oocyst Lemon-shaped, 6-7 by 4-5 µm, with L/W ratio 1.5	Two per mature oocyst Lemon-shaped, 7-8 by 4-5 µm, with L/W ratio 1.66	Two per mature oocyst Lemon-shaped, 7-8 by 4-5 µm, with L/W ratio ratio 1.66
Stieda bodies	A prominent stieda body present; sub- and parastieda bodies absent	A prominent stieda body present; sub- and parastieda bodies absent	A prominent stieda body present; sub- and parastieda bodies absent
Sporocyst residuum	Prominent; made up of clumped globules	Prominent, irregularly shaped; 2-4 µm in diameter	Prominent, irregularly shaped; 2-3 by 3-4 µm in diameter
Micropyle	Absent	Absent	Absent
Sporozoites	Two per sporocyst 10-13 by 1.5 µm; tapered at both ends	Two per sporocyst 10-13 by 2 µm; tapered at both ends	Two per sporocyst 10-13 by 1.5 µm; tapered at both ends
Remarks	Marginally larger than other two species Heterozygotic position, T or A at position #280; therefore, SSU-rRNA sequences submitted separately. Assigned accession nos. AF111184 and AF111185	Marginally smaller than the two other species Sequence of SSU-rRNA assigned accession no. AF111186. Poorest sporulation rate of three species	The most commonly encountered of the three species. Sequence of SSU-rRNA assigned accession no. AF111187

98.4%, respectively. The phylogenetic relationship observed between *Cyclospora* and *Eimeria* species confirmed previous findings (4,5) with three distinct clades: avian *Eimeria*, mammalian *Eimeria*, and *Cyclospora*.

Conclusions

The genus *Cyclospora* was formed by Schneider in 1881 for organisms recovered from myriapods (terrestrial arthropods in the subphylum Mandibulata, Class Diplopoda [millipedes] and Class Chilopoda [centipedes]). Most knowledge about the genus *Cyclospora* is based on recently recognized species described from insectivores (moles) (12), heteromyid rodents in the southwestern United States (13), and humans (1).

In 1994, Ortega and colleagues described *C. cayetanensis* from human fecal material in Peru. In 1997, they described the parasite's intracellular life cycle in the duodenum and jejunum (14). *C. cayetanensis* differs significantly from all other described species not only in its host but also in its oocyst stage, which is much smaller and spherical rather than oblong. The recovery from nonhuman primates of other species of *Cyclospora* that produce small, spherical oocysts seems to suggest two distinct groupings: species that infect insectivores and rodents and produce large, oblong oocysts and those that infect primates (including humans) and produce small, spherical oocysts.

The geographic and host range for *C. papionis*, *C. colobi*, and *C. cercopitheci* needs to be defined. These primate species of *Cyclospora* are easily distinguished at the molecular level, but not at the light-microscope level. That *C. papionis*,

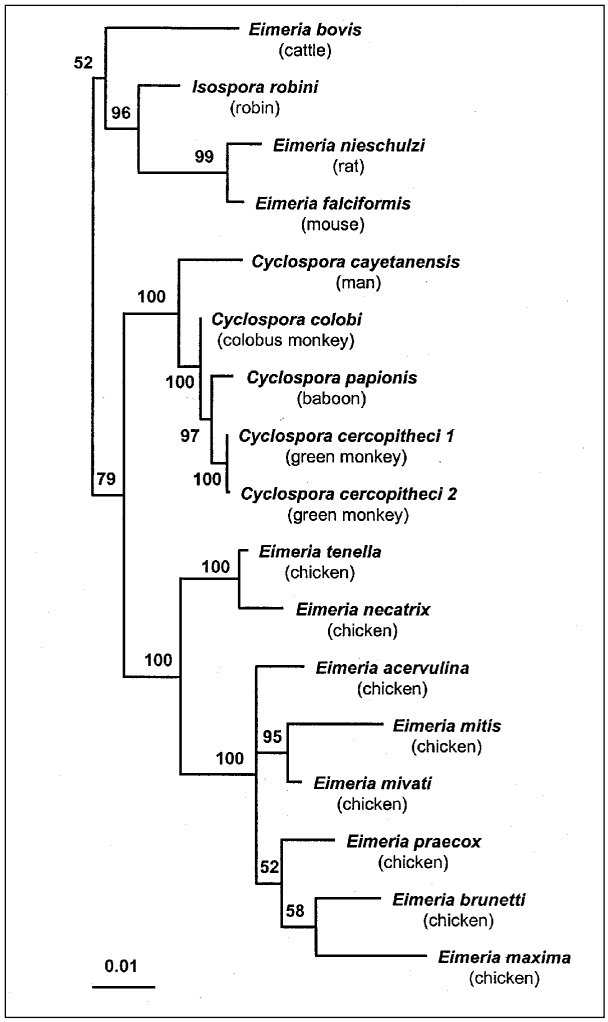


Figure 1.[1] Phylogenetic tree for small subunit ribosomal RNA sequences of *Cyclospora* and *Eimeria* species. Quartet puzzling maximum likelihood results are shown, with *Toxoplasma gondii* as the outgroup. After analysis, the outgroup branch was removed for clarity. Numbers to the left of the nodes indicate the quartet puzzling support for each internal branch. The scale bar indicates an evolutionary distance of 0.01 nucleotides per position in the sequence. Vertical distances are for clarity only. GenBank accession numbers of the sequences used for analysis: *Cyclospora cayetanensis,* AF111183; *C. cercopitheci* 1, AF111184; *C. cercopitheci* 2, AF111185; *C. colobi,* AF111186; *C. papionis,* AF111187; *Eimeria acervulina,* U67115; *E. bovis,* U77084; *E. brunetti,* U67116; *E. falciformis,* AF080614; *E. maxima,* U67117; *E. mitis,* U40262; *E. mivati,* U76748; *E. necatrix,* U67119; *E. nieschulzi,* U40263; *E. praecox,* U67120; *E. tenella,* U40264; *Isospora robini,* AF080612; and *Toxoplasma gondii,* U12138. The sequences were aligned with the program CLUSTALW (20). Phylogenetic analysis was done with the maximum likelihood method-based PUZZLE program (21), as well as with the parsimony method-based PAUP program (22). Unreliably aligned regions were removed, and the final length of the alignment was 1692 columns. Aligned sequences are available from the authors upon request.

C. colobi, and *C. cercopitheci* are species distinct from each other and from *C. cayetanensis* of humans is well substantiated, considering the molecular phylogeny based on the SSU-rRNA sequence data. This assumes that the differences in SSU-RNA reflect distinct species. For the time being, this method is probably the best for defining morphologically similar species. The separation of these parasite species is further supported by the distinct separation of the primate species on biologic grounds. Baboons are omnivorous, spend most of their time on the ground foraging for food, and have relatively large home ranges. Green monkeys spend a greater amount of time in trees but do forage on the ground. The canopy-dwelling colobus monkeys, on the other hand, have the smallest home range and are strict herbivores, predominantly eating leaves of certain trees.

The *Cyclospora* observed in baboons from Tanzania (9) is likely the same species as *C. papionis* from Ethiopia. A high percentage of baboons in the Gombe Stream Preserve are infected with a *Cyclospora* species indistinguishable from *C. papionis* (pers. obs.; Whittier, pers. comm.). Moreover, three sequences from Gombe baboon isolates submitted recently to GenBank (15) show only one base change from our sequence with each isolate (C to T at #1360 with

[1]Initially, we used a nested PCR protocol using primers CYCF1E and CYCR2B for the first step of the amplification and primers CYCF3E and CYCR4B for the second step (11). Samples were also amplified by using sets of PCR primers designed on the basis of the primers described above, but with the restriction sites removed. The primer CYCF1 (5'-ATTACCCAATGAA AACAGTTT- 3') was used in pairs with the primer CYCR4 (5'-TCGTCTTCAAACCCCCTACTG-3') to generate a DNA fragment of 577 bp. The other pair of primers, CYCF3 (5'-GCCTTCCGCGCTTCGCTGCGT-3') and CYCR2 (5'-TGC AGGAGAAGCCAAGGTAGG-3') was used to generate a fragment of 283 bp. To generate fragments spanning the full length of the SSU-rRNA coding region, we used generic apicomplexan PCR primer CRYPTOF (5'-AACCTG GTTGATCCTGCCAGT-3'), specific for the 5' end of the SSU-rRNA molecule and the apicomplexan generic PCR primer CRYPTOR (5'-GCTTGATCCTTCTGCAGGTTCACC TAC-3'), specific for the 3' end of this molecule. These generic primers were combined with the *Cyclospora*-specific primers (CYC-series, see above) to amplify overlapping fragments spanning the whole SSU-rRNA molecule. PCR products were analyzed by electrophoresis on 2% SeaKem GTG agarose (Cat. No. 50074, FMC Bioproducts, Rockland, ME), stained with ethidium bromide and visualized on a UV transilluminator.

sequences AF065566 and AF065567; C to T at #184 with AF065568), if unresolved base positions in their sequences are disregarded (three positions in AF065566 and AF065567 and six positions in AF065568).

The topology of the tree (Figure 1) displays the distinct *Cyclospora* species as a monophyletic branch with phylogenetic proximity to the genus *Eimeria*. The proximity between these coccidian genera has been demonstrated (4,5); we included in the tree an additional SSU-rRNA sequence of *E. falciformis*, a parasite of mice. The addition of this species clarified the resolution of the tree into three distinct clades: mammalian *Eimeria*, avian *Eimeria*, and *Cyclospora*. With the addition of molecular data for more species, especially the species of *Cyclospora* described from mammals other than primates, it may be reasonable to consider reclassifying the *Cyclospora* of primates (including humans) and either the bird or mammalian *Eimeria* to a new genus. However, morphologic and molecular taxonomists continue to struggle with the relationships within the coccidia. Morphologic criteria for naming the genera have provided a stable basis for many years. On the other hand, molecular data, based on the genetic information of these same organisms, suggest affiliations that do not always coincide with the existing associations based on morphologic features. Sterling and Ortega (16) suggest that small subunit rRNA sequences of *Isospora* should be compared with those of *Cyclospora* to help clarify taxonomic issues. They also point out the role of molecular taxonomy in establishing the validity of species and taxonomic groupings. Carreno and Barta (17) provided sequencing data for several species of *Isospora* and demonstrated the phylogenetic separation of various clades of *Isospora,* both with and without Stieda bodies. They propose separating mammalian species with Stieda bodies into the family Eimeriidae and retaining those without Stieda bodies in the family Sarcocystidae. We included sequences of *I. robini* in our analysis, and *Cyclospora* remains as a clearly separate grouping.

On the basis of the topology of the tree and the distance values obtained, the simian isolates are more closely related to each other than to *C. cayetanensis* of humans. This undoubtedly reflects host differences as well as other biologic features of each species. However, further molecular studies are needed to demonstrate whether these *Cyclospora* species described from lower primates occur in humans, or conversely, whether *C. cayetanensis* can occur in monkeys. At least in East Africa, researchers should continue to evaluate material collected from humans and nonhuman primates with care. We are continuing our efforts to determine whether other primate species are infected with these or distinct species of *Cyclospora*. Studies of human isolates of *C. cayetanensis* from different geographic regions have, thus far, not demonstrated any molecular differences. This further substantiates the taxonomic significance of the molecular differences detected between the *Cyclospora* from humans and lower primates.

Appendix I

Stool Processing Procedures

Stool samples collected in 1997 were processed by a conventional formalin-ethyl acetate sedimentation concentration procedure. A portion of the sediment was examined by UV fluorescent microscopy (18). Some positive samples were also stained by the acid-fast or hot safranin techniques (19). For stools collected in 1998, an aliquot of each sample was washed because potassium dichromate suppresses the autofluorescence of the oocysts. Any oocysts observed in the samples examined from the collection of 1998 were graded as either sporulated or unsporulated. Part of the remaining specimen in potassium dichromate was processed over sucrose gradient to harvest oocysts. Purified oocysts were returned to clean 2.5% potassium dichromate solution for storage, and portions of the purified oocysts were used for morphologic studies.

To excyst sporocysts and sporozoites, one of two procedures was used. If the intent was to obtain free sporocysts, but not sporozoites, a small drop of solution containing oocysts was placed on a glass slide and covered. To induce rupture of the oocyst wall, the coverslip was tapped with a blunt glass rod and then rotated on the slide. To obtain free sporozoites, one of two excysting fluids were used: either a solution made up in DMEM containing 0.25% trypsin plus 0.75% sodium taurocholate or a solution made up in PBS containing 0.25% trypsin, 0.75% sodium taurocholate, and 20 mM cystine HCl. Both solutions worked equally well. The oocysts were incubated in the excysting fluid for 2 hours in a heat block at 37°C.

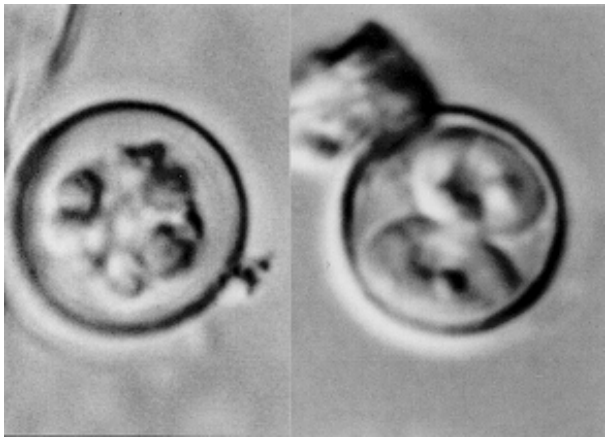
Appendix II

Taxonomic Description of the Parasites

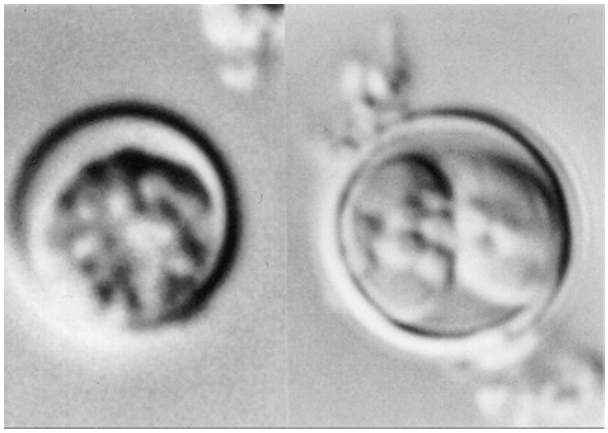
***Cyclospora cercopitheci* sp.n.** (Figures 2–3, 9)
Type host: *Cercopithecus aethiops* Linnaeus, 1758, African green or vervet monkey.
Type locality: Gimbie, Wollega Province, Ethiopia.
Prevalence: found in 6% of green monkeys sampled.
Site of infection: Unknown, oocysts collected from feces.
Material deposited: Phototypes and syntypes, U.S. National Parasite Collection, accession number 088837.
Etymology: The species name was derived from the genus name for the primate host from which this parasite was recovered.
Remarks: Sequencing of SSU-rRNA coding region of *C. cercopitheci* from a single African green monkey specimen revealed that there was a heterozygotic position, T or A at position #280. Thus, SSU-rRNA sequences for these two isolates were submitted separately to GenBank and were assigned accession numbers AF111184 for *C. cercopitheci* sequence #1 and AF111185 for *C. cercopitheci* sequence #2.

***Cyclospora colobi* sp.n.** (Figures 4–5, 10)
Type host: *Colobus guereza* Ruppell, 1835, colobus monkey.
Type locality: Gimbie, Wollega Province, Ethiopia.
Prevalence: Up to 60% of colobus monkeys sampled.
Site of infection: Unknown, oocysts collected from feces.
Material deposited: Phototypes and syntypes, U.S. National Parasite Collection, accession number 088838.
Etymology: The species name was derived from the genus name of the primate host from which this parasite was recovered.
Remarks: This species is marginally smaller than the two other species described from monkeys, but the overlap in sizes between the species does not allow a clear distinction on the basis of size. Sporulation of material collected from colobus monkeys was poor in comparison with *C. papionis* from baboons, despite the fact that material was collected and handled in a similar fashion. Sequence of the SSU-rRNA coding region for this species was deposited in GenBank and was assigned accession number AF111186.

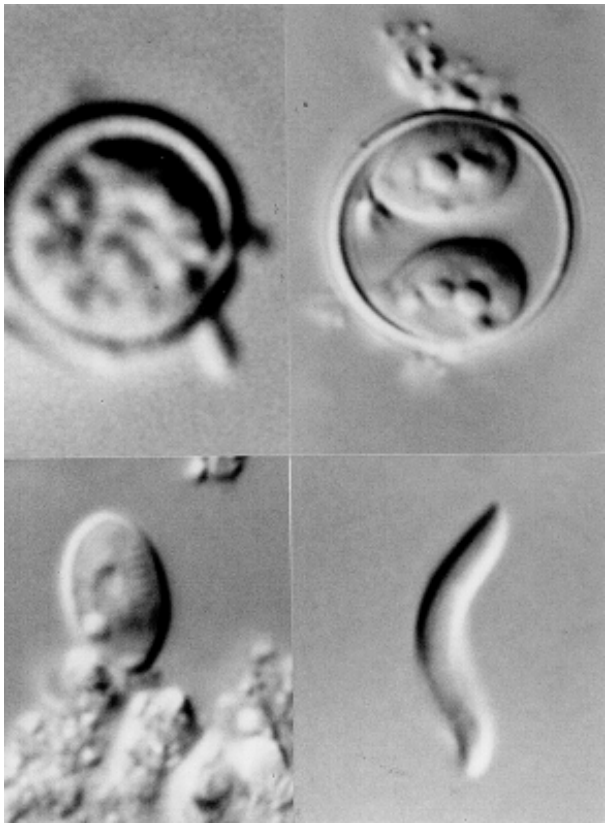
***Cyclospora papionis* sp.n.** (Figures 6–9, 11)
Type host: *Papio anubis* Lesson, 1827, olive baboon.
Type locality: Gimbie, Wollega Province, Ethiopia.
Prevalence: Found in >50% of baboons sampled.
Site of infection: Unknown, oocysts collected from feces.
Material deposited: Phototypes and syntypes, U.S. National Parasite Collection, accession number 088839.
Etymology: The species name was derived from the genus name for the primate host from which this parasite was recovered.
Remarks: More than 90% of the oocysts collected from baboons underwent sporulation in virtually all of the positive samples. Sequence of the SSU-rRNA coding region for this species was deposited in GenBank and was assigned accession number AF111187.



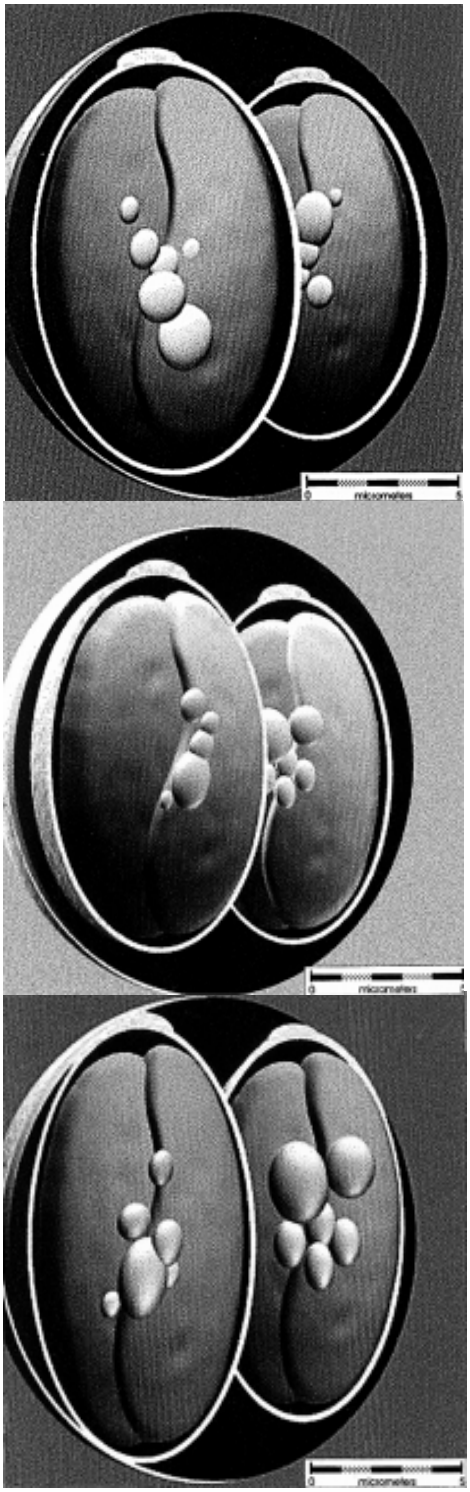
Figures 2–3. Photomicrographs of *Cyclospora cercopitheci* sp. n. from feces of African green monkeys (*Cercopithecus aethiops*) in Ethiopia, Africa. x 3300. 2. Unsporulated oocyst from feces. 3. Sporulated oocyst after 1 month of incubation.



Figures 4–5. Photomicrographs of *Cyclospora colobi* sp. n. from feces of colobus monkeys (*Colobus guereza*) in Ethiopia, Africa. x 3300. 4. Unsporulated oocysts from feces. 5. Sporulated oocyst after 1 month of incubation.



Figures 6–9. Photomicrographs of *Cyclospora papionis* sp. n. oocysts from feces of baboons (*Papio anubis*) in Ethiopia, Africa. x 3300. 6. Unsporulated oocysts from feces. 7. Sporulated oocyst after 1 month of incubation. 8. Free sporocyst from ruptured oocyst. 9. Free sporozoite from ruptured sporocyst.



Figures 10–12. Line drawings of sporulated oocysts of *Cyclospora* from feces of primates in Ethiopia, Africa. Bar = 5 µm. 10. *Cyclospora cercopitheci* sp. n. from African green monkeys, *Cercopithecus aethiops*. 11. *Cyclospora colobi* sp. n. from colobus monkeys, *Colobus guereza*. 12. *Cyclospora papionis* sp. n. from baboons, *Papio anubis*.

Acknowledgments

The authors thank Oleg Ditrich for providing useful hints on the Latin grammar usage and Robert Hobbs, Jr., for the computer images of the oocyst composite drawings of each species.

Dr. Eberhard is head of the Biology and Diagnostics Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, CDC. Trained in classical parasitology, he has broad interests in the diagnosis and biology of parasitic infections. His research interests include the identification of unusual parasites and zoonotic infections.

References

1. Ortega YR, Gilman RH, Sterling CR. A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. J Parasitol 1994;80:625-9.
2. Koumans EH, Katz DJ, Malecki JM, Kumar S, Wahlquist SP, Arrowood MJ, et al. An outbreak of cyclosporiasis in Florida in 1995: a harbinger of multistate outbreaks in 1996 and 1997. Am J Trop Med Hyg 1998;59:235-42.
3. Herwaldt BL, Ackers ML, the Cyclospora Working Group. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. N Engl J Med 1997;336:1548-56.
4. Relman DA, Schmidt TM, Gajadhar A, Sogin M, Cross J, Yoder K, et al. Molecular phylogenetic analysis of *Cyclospora*, the human intestinal pathogen, suggests that it is closely related to *Eimeria* species. J Infect Dis 1996;173:440-5.
5. Pieniazek NJ, Herwaldt BL. Reevaluating the molecular taxonomy: is human-associated *Cyclospora* a mammalian *Eimeria* species? Emerg Infect Dis 1997;3:381-3.
6. García-López HL, Rodríguez-Tovar LE, Medina de la Garza CE. Identification of *Cyclospora* in poultry. Emerg Infect Dis 1996;2:356-7.
7. Zerpa R, Uchima N, Huicho L. *Cyclospora cayetanensis* associated with watery diarrhoea in Peruvian patients. Am J Trop Med Hyg 1995;98:325-9.
8. Yai LE, Bauab AR, Hirschfeld MP, de Oliveira ML, Damaceno JT. The first two cases of *Cyclospora* in dogs, Sao Paulo, Brazil. Rev Inst Med Trop Sao Paulo 1997;39:177-9.
9. Smith HV, Paton CA, Girdwood RWA, Mtambo MMA. *Cyclospora* in non-human primates. Vet Rec 1996;138:528.
10. da Silva AJ, Bornay-Llinares FJ, Moura INS, Slemenda SB, Tuttle JL, Pieniazek NJ. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. Molecular Diagnosis 1999;4:57-64.
11. Pieniazek NJ, Slemenda SB, da Silva AJ, Alfano EM, Arrowood AJ. PCR confirmation of infections with *Cyclospora cayetanensis*. Emerg Infect Dis 1996;2:357-9.
12. Ford PL, Duszynski DW, McAllister CT. Coccidia (Apicomplexa) from heteromyid rodents in the southwestern United States, Baja California, and northern Mexico with three new species from *Chaetodipus hispidus*. J Parasitol 1990;76:325-31.
13. Ford PL, Duszynski DW. Coccidian parasites (Apicomplexa: Eimeriidae) from insectivores. VII. Six new species from the hairy-tailed mole, *Parascalops breweri*. J Parasitol 1989;75:508-13.
14. Ortega YR, Nagle R, Gilman RH, Watanabe J, Miyagui J, Quispe H, et al. Pathologic and clinical findings in patients with cyclosporiasis and a description of intracellular parasite life-cycle stages. J Infect Dis 1997;176:1584-9.
15. Lopez FA, Manglicmot JS, Schmidt TM, Yeh C, Smith HV, Relman DA. Molecular organization of *Cyclospora*-like organisms from baboons. J Infect Dis 1999;179:670-6.
16. Sterling CR, Ortega YR. *Cyclospora*: an enigma worth unraveling. Emerg Infect Dis 1999;5:48-53.
17. Carreno RA, Barta JR. An eimeriid origin of isosporoid coccidia with stieda bodies as shown by phylogenetic analysis of small subunit ribosomal RNA gene sequences. J Parasitol 1999;85:77-83.
18. Eberhard ML, Pieniazek NJ, Arrowood MJ. Laboratory diagnosis of *Cyclospora* infections. Arch Pathol Lab Med 1997;121:792-7.
19. Visvesvara GS, Moura H, Kovacs-Nace E, Wallace S, Eberhard ML. Uniform staining of *Cyclospora* oocysts in fecal smears by a modified safranin technique with microwave heating. J Clin Microbiol 1997;35:730-3.
20. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research 1994;22:4673-80.
21. Strimmer K, von Haeseler A. Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. Mol Biol Evol 1996;13:964-9.
22. Swofford DL. PAUP*: Phylogenetic analysis using parsimony (and other methods). 1998. Ver 4.0b1. Sunderland (MA): Sinauer Associates.

The Economic Impact of Pandemic Influenza in the United States: Priorities for Intervention

Martin I. Meltzer, Nancy J. Cox, and Keiji Fukuda
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

We estimated the possible effects of the next influenza pandemic in the United States and analyzed the economic impact of vaccine-based interventions. Using death rates, hospitalization data, and outpatient visits, we estimated 89,000 to 207,000 deaths; 314,000 to 734,000 hospitalizations; 18 to 42 million outpatient visits; and 20 to 47 million additional illnesses. Patients at high risk (15% of the population) would account for approximately 84% of all deaths. The estimated economic impact would be US$71.3 to $166.5 billion, excluding disruptions to commerce and society. At $21 per vaccinee, we project a net savings to society if persons in all age groups are vaccinated. At $62 per vaccinee and at gross attack rates of 25%, we project net losses if persons not at high risk for complications are vaccinated. Vaccinating 60% of the population would generate the highest economic returns but may not be possible within the time required for vaccine effectiveness, especially if two doses of vaccine are required.

Influenza pandemics have occurred for centuries, three times (1918, 1957, and 1968) in the 20th century alone. Another pandemic is highly likely, if not inevitable (1). In the 1918 influenza pandemic, more than 20 million people died (2). Improvements in medical care and technology since the last pandemic may reduce the impact of the next. When planning for the next pandemic, however, decision makers need to examine the following questions: Would it make economic sense to vaccinate the entire U.S. population if 15% were to become clinically ill? What if 25% were to become ill? To answer such questions, we conducted economic analyses of potential intervention scenarios.

Although many studies have examined or reviewed the economics of influenza vaccination (3-10), only one study (11), published in 1976, examined the economics of a vaccine-based intervention aimed at reducing the impact of an influenza epidemic in the United States. Our study examines the possible economic effects of the next influenza pandemic in the United States, analyzes these effects, and uses the results to estimate the costs, benefits, and policy implications of several possible vaccine-based interventions. These estimates can be used in developing national and state plans to respond to an influenza pandemic.[1] Unlike the 1976 study, ours examined the effect of varying the values of a number of key input variables. Specific objectives were to provide a range of estimates regarding the number of deaths, hospitalizations, outpatient visits, and those ill persons not seeking medical care in the next influenza pandemic; provide a cost estimate of health outcomes; estimate the potential net value of possible vaccination strategies;[2] evaluate the effect of using different criteria (e.g., death rates, economic returns due to vaccination) to set vaccination priorities; assess the economic impact of administering various doses of vaccine

Address for correspondence: Martin Meltzer, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Clifton Road, Mail Stop C12, Atlanta, GA 30333, USA; fax: 404-639-3039; e-mail: qzm4@cdc.gov.

[1]A complete plan detailing a response to an influenza pandemic should include definition of a pandemic, points that will initiate various steps in the response plan, and details about deploying the intervention. While a U.S. federal influenza pandemic plan is being developed, a guide to aid state and territorial health officials in developing plans for their jurisdictions is available at http://www.cdc.gov/od/nvpo/pandemicflu.htm. Printed copies can be obtained from the author.

[2]We limited our examination of possible interventions to those involving influenza vaccines. We did not consider the use of antiviral drugs for influenza prophylaxis because there may not be adequate supplies; first priority for such drugs may be for treatment; and the side-effects from the drugs, particularly amantadine, make them unsuitable for long-term prophylaxis for many workers, such as drivers, or heavy construction operators.

and of administering vaccine to different age groups and groups at risk; and calculate an insurance premium that could reasonably be spent each year for planning, preparedness, and practice.

Methods

The Model

Building a mathematical model of the spread of influenza is difficult largely because of differences in virus transmission and virulence, lack of understanding of the primary factors affecting the spread of influenza, and shortage of population-based data (12). Because of the difficulties in calculating realistic estimates of the numbers of cases in the next influenza pandemic, we used a Monte Carlo mathematical simulation model (13-15), which uses predefined probability distributions of key input variables to calculate the number of illnesses and deaths that could result from an influenza pandemic. Some of the most important probability distributions we used describe the population-based rates of illness and death. These rates are based on illness and death rates reported in earlier influenza pandemics and epidemics. The model produces a range of estimated effects rather than a single point estimate. The model is not epidemiologic and thus does not describe the spread of the disease through a population.

Many details of the model are presented below and in Appendix I; a more detailed explanation and a complete list of all the variables used and the values assigned to the variables are available at Appendix II.

For interventions to contain and reduce the impact of an influenza pandemic, we used a societal perspective, which takes into account all benefits and all costs regardless of who receives and who pays.

Age Distribution and Persons at High Risk

Since the age distribution of patients in the next pandemic is unknown, we assumed a distribution (Table 1) among the three age groups (0 to 19 years, 20 to 64 years, and 65 years and older).[3] Further, each age group was divided into those at high risk (persons with a preexisting medical condition making them more susceptible to complications from influenza) and those not at high risk (Table 1).[4] Age by itself was not considered a risk factor; persons 65 years and older were assumed to have higher rates of illness and death than the rest of the population (Table 2).

Table 1. Estimate of age distribution of cases and percentage of population at high risk used to examine the impact of pandemic influenza in the United States

Age group (yrs)	Percentage of all cases[a]
0-19	40.0
20-64	53.1
65 +	6.8
Totals[b]	100.0
	Percentage at high risk[c]
0-19	6.4
20-64	14.4
65 +	40.0
U.S. average[d]	15.4

[a]The actual number of cases will depend upon the assumed gross attack rate. The distribution of cases was based on lower and upper estimates of age-specific attack rates from the 1918, 1928-29, and 1957 epidemics and pandemics (19).
[b]Totals do not add to exactly 100% because of rounding.
[c]Persons are categorized at high risk if they have a preexisting medical condition that makes them more susceptible to influenza-related complications. The percentages of age groups at high risk were obtained from the Working Group on Influenza Pandemic Preparedness and Emergency Response (GrIPPE, unpub. data). The Advisory Committee on Immunization Practices estimates that 27 to 31 million persons aged <65 years are at high risk for influenza-associated complications (17).
[d]Average is an age-weighted average, using each age group's proportion of the total U.S. population.

[3]This article presents the results for one distribution of cases by age and risk group. The background paper in Appendix II, however, contains additional results obtained by using a different distribution.
[4]The Advisory Committee on Immunization Practices estimates that 27 to 31 million people ages <65 years are at high risk for influenza-associated complications (17). ACIP also classifies all 32 million people ≥65 years as being at elevated risk for influenza-related complications (17). Further, the working group on influenza pandemic preparedness and emergency response has assumed that approximately 19 million household members of persons at high risk should also be vaccinated to reduce the probability of transmission to those at high risk (GrIPPE, unpub. data, 1997).

Gross Attack Rates

In the model, we used gross attack rates (percentage of clinical influenza illness cases per population) of 15% to 35%, in steps of 5%. Infected persons who continued to work were not considered to have a clinical case of influenza, and were not included.

Illnesses and Deaths

The rates of adverse effects (outpatient visits, hospitalizations, deaths, and illnesses for which no medical care was sought), by age and risk group, were used to determine the number of persons in each category (Table 2) (Appendix II).

Table 2. Variables used to define distributions of disease outcomes of those with clinical cases[a] of influenza

	Rates per 1,000 persons[b]		
Variable	Lower	Most likely	Upper
Outpatient visits			
Not at high risk			
0-19 yrs old	165		230
20-64 yrs old	40		85
65 + yrs old	45		74
High risk			
0-19 yrs old	289		403
20-64 yrs old	70		149
65 + yrs old	79		130
Hospitalizations			
Not at high risk			
0-19 yrs old	0.2	0.5	2.9
20-64 yrs old	0.18		2.75
65 + yrs old	1.5		3.0
High risk			
0-19 yrs old	2.1	2.9	9.0
20-64 yrs old	0.83		5.14
65 + yrs old	4.0		13
Deaths			
Not at high risk			
0-19 yrs old	0.014	0.024	0.125
20-64 yrs old	0.025	0.037	0.09
65 + yrs old	0.28	0.42	0.54
High risk			
0-19 yrs old	0.126	0.22	7.65
20-64 yrs old	0.1		5.72
65 + yrs old	2.76		5.63

[a]Clinical cases are defined as cases in persons with illness sufficient to cause an economic impact. The number of persons who will be ill but will not seek medical care, are calculated as follows: Number ill_{age} = ($Population_{age}$ x gross attack rate) - ($deaths_{age}$ + $hospitalizations_{age}$ + $outpatients_{age}$). The number of deaths, hospitalizations, and outpatients are calculated by using the rates presented in this table.

[b]For Monte Carlo simulations, rates are presented as lower and upper for uniform distributions, and lower, most likely, and upper for triangular distributions (18).

Sources: 3,6,11,19-29, and Appendix II.

Net Returns of Vaccinating against an Influenza Pandemic

Vaccinating predefined segments of the population will be one of the major strategies for reducing the impact of pandemic influenza, and the net return, in dollars, from vaccination is an important economic measure of the costs and benefits associated with vaccination. We calculated the net return by using the following formula for each age and risk group:

$$\text{Net returns}_{\text{age, risk group}} = \text{Savings from outcomes averted in population}_{\text{age, risk group}} - \text{cost of vaccination of population}_{\text{age, risk group}}$$

The savings from illnesses and deaths averted and the cost of vaccinations are described in Appendix I. Some input variables are described below and in Appendix II.

Input Variables

The direct medical costs (i.e., those reimbursed by third-party payers such as health insurance companies) associated with hospitalizations, outpatient visits, and drug purchases were obtained from a proprietary database containing health insurance claims data from approximately 4 million insured persons (The MEDSTAT Group, Ann Arbor, MI) (Table 3). Following the methods used by McBean et al. (28), we extracted the data for outpatient visits from the database with codes from the International Classification of Diseases, Ninth Revision (ICD-9) for pneumonia and bronchitis (ICD-9: 480-487.8), acute bronchitis (ICD-9: 466-466.1), and chronic respiratory disease (ICD-9: 490-496). Costs for inpatient care were extracted with the same codes, when recorded as the principal diagnosis and when recorded as any of the diagnoses in a patient's chart. Further, because influenza can cause patients with preexisting medical conditions to seek inpatient care, data were extracted for the inpatient costs of treating heart-related conditions (common preexisting conditions that place a person at high

Table 3. Input variables used to calculate the economic impact (direct and indirect costs) of health outcomes due to an influenza pandemic in the United States (in 1995 US$)

Outcome category item	Type of cost	Age group (yrs) 0-19	20-64	65+	Sources
Deaths					
Average age (years)		9	35	74	Assumed
PV earnings lost ($)[a]	Indirect	1,016,101	1,037,673	65,837	16, 30
Most likely ± min or max hospital costs ($)[b]	Direct	3,435±2,632	7,605±3,888	8,309+3,692	Marketscan Database; 31.
Subtotal ($)[c]		1,019,536	1,045,278	74,146	
Hospitalizations					
Most likely ± min or max hospital costs ($)[b]	Direct	2,936±2,099	6,016±2,086	6,856±3,200	Marketscan Database; 31.
Most likely ± min or max net pay for outpatient visits ($)[d]	Direct	74 ± 40	94 ± 70	102 ± 60	Marketscan Database; 31.
Avg. copayment for outpatients visit ($)	Direct	5	4	4	Marketscan Database
Most likely ± min or max net payment for drug claims($)[e]	Direct	26 ± 9	42 ± 30	41 ± 10	Marketscan Database
Most likely ± min or max days lost[f]	Indirect	5 ± 2.7	8 ± 4.8	10 ±5.4	Marketscan Database; 31.
Value 1 day lost ($)[g]	Indirect	65	100 or 65	65	30
Subtotal ($)[c]		3,366	6,842	7,653	
Outpatient visits					
Avg. no. visits[h]	Direct	1.52	1.52	1.52	Marketscan Database
Most likely ± min or max net payment per visit($)[i]	Direct	49 ±13	38 ± 12	50 ± 16	Marketscan Database
Avg. copayment for outpatient visit ($)	Direct	5	4	4	Marketscan Database
Most likely ± min or max net payment per prescription($)[j]	Direct	25 ± 18	36 ± 27	36 ± 22	Marketscan Database
Avg. prescriptions per visit	Direct	0.9	1.8	1.4	Marketscan Database
Avg. copayment per prescription ($)	Direct	3	3	3	Marketscan Database
Days lost	Indirect	3	2	5	4,5
Value 1 day lost ($)[g]	Indirect	65	100	65	30
Subtotal ($)[c]		300	330	458	
Ill, no medical care sought					
Days lost	Indirect	3	2	5	4,5
Value 1 day lost ($)[g]	Indirect	65	100	65	30
Over-the-counter drugs ($)	Direct	2	2	2	Assumed
Subtotal ($)[c]		197	202	327	

[a]Average present value (PV), using a 3% discount rate, of expected future lifetime earnings and housekeeping services, weighted by age and gender (30) and adjusted to 1995 dollars (by multiplying by a factor of 1.07) (16).
[b]Most likely, with ± defining the minimum and maximum costs for a triangular distribution (18) for Monte Carlo analysis (13-15). The values were calculated by using cost data from Marketscan Database (The MEDSTAT Group, Ann Arbor, MI) and multiplying it by a hospital cost-to-charge ratio of 0.53. The latter ratio is a weighted average of the urban and rural (urban = 0.80, rural = 0.20) cost-to-charge ratios calculated by the Health Care Finance Administration for August 1996 (31).
[c]Subtotals are the totals for each category of outcome, using the most likely estimates.
[d]Most likely, with minimum and maximum values of net payments for outpatient visits up to 14 days before admission date and up to 30 days after discharge date.
[e]Net payment for drug claims associated with outpatient visits up to 14 days before admission and up to 30 days after discharge.
[f]Most likely, with ± defining the minimum and maximum days lost due to hospitalization for a triangular distribution (18) for Monte Carlo analysis (13-15). Calculated using length of stay in hospital data from Marketscan Database (The MEDSTAT Group, Ann Arbor, MI) and adding a total of one additional day for convalescence and pre- and posthospitalization outpatient visits for 0-19 and 20-64 years of age. For 65 + years, two additional days were added to length of stay in hospital for convalescence and pre- and posthospitalization outpatient visits.
[g]For 0-19 and 65+ years age groups, a day lost to influenza was valued as equivalent to an unspecified day (30), denoting a value for time lost by care givers and family members related to taking care of a patient in these age groups. For 20-64 years of age, 60% of days lost due to hospitalizations and related convalescence and pre- and posthospitalization outpatient visits were valued as day off work ($100/day). The remaining 40% of days lost were valued as unspecified days ($65/day). For 20-64 years of age, when patients were not hospitalized at any point during their illness (i.e., outpatient status), all days lost were assumed days off work ($100/day).
[h]The number of visits per episode of influenza is an average across all age groups. From the database, it was found that 85% of all patients had less than three outpatient visits, with an average of 1.52 visits (Appendix II).
[i]Most likely, with minimum and maximum values of net payments for outpatient visits without any specified association to hospitalizations.
[j]Most likely, with ± defining the minimum and maximum cost per prescription, with the number of prescriptions per visit.

risk for influenza-related illness or death). Hospital costs attributed to pneumonia and bronchitis, acute bronchitis, chronic respiratory disease, and the identified heart conditions were then estimated as weighted averages (Appendix II).

The principal indirect cost was lost productivity, which was valued by using an age- and gender-weighted average wage (Table 3) (30). The economic cost of a death was valued at the present net value of the average expected future lifetime earnings, weighted for gender and age (30). All costs were standardized to 1995 US$ values.

The cost of fully vaccinating a person (i.e., administering the number of doses necessary to protect against disease) was modeled with two assumed values, approximately $21 and $62 per person fully vaccinated (Table 4). These costs include the cost of the vaccine, as well as its distribution and administration (health-care worker time, supplies); patient travel; time lost from work and other activities; and cost of side effects (including Guillain-Barré syndrome) (Table 4) (Appendix II).

Table 4. Cost of vaccination[a] during an influenza pandemic, with specific costs assigned to side effects of vaccination

Item	Probability of effect[b]	Cost of case of side effect ($)[b]	Lower-cost scenario ($/patient)	Upper-cost scenario ($/patient)
Assumed cost of vaccination[a] (excluding side effects)			18	59
Side effects				
Mild[c]	0.0325	94	3.05	3.05
GBS[d]	0.000002	100,800	0.20	0.20
Anaphylaxis	0.000000157	2,490	0.01	0.01
Total cost per patient			21.26	62.26

[a]The cost of vaccination includes the cost of the vaccine, the cost of administering the vaccine, value of time spent by a person traveling to and from the place of vaccination, and patient-associated travel costs. Included in the costs of the vaccine are any costs associated with the rapid production of a larger-than-usual number of doses and the rapid delivery and correct storage of doses at vaccination sites around the country. For $18, the costs were assumed to be $10 for vaccine + administration, $4 patient time (half hour), $4 patient travel costs. For $59, the costs were assumed to be $20 for vaccine + administration (this could include the cost of two doses), $32 patient time (two trips at 2 hours per trip), and $7 patient travel costs. For comparison, a review of 10 published articles found a range of $5 to $22 per dose of vaccine, with a medium [sic] cost of $14 per dose (10). Additional details are provided in the background paper (see Appendix II). These breakdowns are illustrations only of what might be deemed reasonable estimates of time and cost. Actual costs might vary substantially and will depend on the number of doses needed to achieve a satisfactory protective response, as well as the efficiency of giving vaccinations to millions of persons.
[b]Probabilities and average cost of treating each category of side effect were derived from (3).
[c]Mild side effects include sore arms due to vaccination, headaches, and other minor side effects that may require a visit to a physician or may cause the patient to miss 1 to 2 days of work.
[d]GBS = Guillain Barré syndrome.

Vaccine Effectiveness

The assumed levels of vaccine effectiveness used to estimate the savings gained due to a vaccine-based intervention are described in Appendix I; the equation defining savings from outcomes averted contains the rate of compliance multiplied by the assumed vaccine effectiveness. In cases requiring two doses of vaccine to satisfactorily protect against influenza-related illness and death, a person was considered compliant only after both doses.

Net Returns of Vaccination: Sensitivity Analyses

To illustrate the importance of the death rate in determining economic outcomes, we conducted further sensitivity analyses in which the death rates for persons not at high risk were one quarter or half of those used in the main analyses (Table 2).

Insurance Premiums

To determine how much should be spent each year to plan, prepare, and practice to ensure that mass vaccinations can take place if needed, we considered the funding of those activities as an annual insurance premium (32). The premium would be used to pay for improving surveillance systems, ensuring sufficient supply of vaccine for high-priority groups (and possibly the entire U.S. population), conducting research to improve detection of new influenza subtypes, and developing emergency preparedness plans to ensure adequate medical care and maintenance of essential community services (32). We calculated the premium as follows (33): annual insurance premium = net returns from an intervention x the annual probability of a pandemic.

Vaccination Priorities and Distribution

During the early stages of a pandemic, the supply of influenza vaccine will likely be limited. Even if sufficient vaccine is produced to vaccinate the entire U.S. population, it will take time to administer the vaccine to all, especially if two doses are required. Because a pandemic will be caused by a new subtype of influenza, two doses of vaccine may be required. Who should receive priority for vaccination until vaccine supplies are more plentiful? To illustrate the use of the model in estimating the impact of different priorities, we created sample priority lists by using three different criteria: total deaths, risk for death, and maximizing net returns due to vaccination. In choosing the criteria for priorities, society must debate the main goal of a pandemic vaccination plan: prevent deaths, regardless of age and position in society; prevent deaths among those at greatest risk (i.e., 65 years of age); or minimize the social disruption. If the last is the goal of society, the net return due to vaccination should be used to set priorities.

The model can also be used to compare the economic consequences of plans that specify which target populations are vaccinated. To illustrate this capability, we constructed four options for prioritizing vaccine distribution. For Option A, the target population is similar to current Advisory Committee on Immunization Practices (ACIP) recommendations, with production and use of vaccine similar to current, intrapandemic recommendations (17). We assumed 77.4 million vaccinees.[4] Option B targets the number of vaccinees as outlined in Option A plus approximately 20 million essential service providers (5 million health-care workers and 15 million providers of other service) (99.2 million vaccinees). Option C aims to achieve a 40% effective coverage of the entire U.S. population (106.1 million vaccinees), and Option D, 60% effective coverage of the entire U.S. population (159.2 million vaccinees).

The number of vaccine doses required to meet each option will depend on the number of doses per person needed to obtain an immune response. If two are needed, lack of compliance with a two-dose regimen will mean that the actual number of doses needed will be higher than double the target population for each option (i.e., >40% or >60% of the population will have to receive the first dose to ensure that 40% or 60% are fully vaccinated). If two doses are required, the cost per person vaccinated will increase (Table 4).

Findings

Illnesses and Deaths

The number of hospitalizations due to an influenza epidemic ranged from approximately 314,000 (5th percentile = 210,000; 95th percentile = 417,000) at a gross attack rate of 15% to approximately 734,000 (5th percentile = 441,000; 95th percentile = 973,000) at a gross attack rate of 35% (Figure 1). The mean numbers of persons requiring outpatient-based care ranged from approximately 18 million (gross attack rate of 15%) to 42 million (gross attack rate of 35%) (Figure 1). The mean numbers of those clinically ill not seeking medical care but still sustaining economic loss ranged from approximately 20 million (gross attack rate of 15%) to 47 million (gross attack rate of 35%) (Figure 1). The estimated number of deaths ranged from approximately 89,000 (5th percentile = 55,000; 95th percentile = 122,000) at a gross attack rate of 15%, which increased to approximately 207,000 deaths (5th percentile = 127,000; 95th percentile = 285,000) at a gross attack rate of 35% (Figure 1).

Groups at high risk (approximately 15% of the total U.S. population) (Table 1) would likely be disproportionately affected by an influenza pandemic. These groups accounted for approximately 85% of all deaths, with groups at high risk in the 20- to 64-year-old age group accounting for approximately 41% of total deaths (Table 5). Groups at high risk also accounted for 38% of all hospitalizations and 20% of all outpatient visits (Table 5).

Economic Impact of an Influenza Pandemic

Without large-scale immunization, the estimates of the total economic impact in the United States of an influenza pandemic ranged from $71.3 billion (5th percentile = $35.4 billion; 95th percentile = $107.0 billion) (gross attack rate of 15%) to $166.5 billion (5th percentile = $82.6 billion; 95th percentile = $249.6 billion) (gross attack rate of 35%) (Table 6). At any given attack rate, loss of life accounted for approximately 83% of all economic losses. Outpatients, persons ill but not seeking medical care, and inpatients accounted for approximately 8%, 6%, and 3%, respectively, of all economic losses (Table 6) (Appendix II).

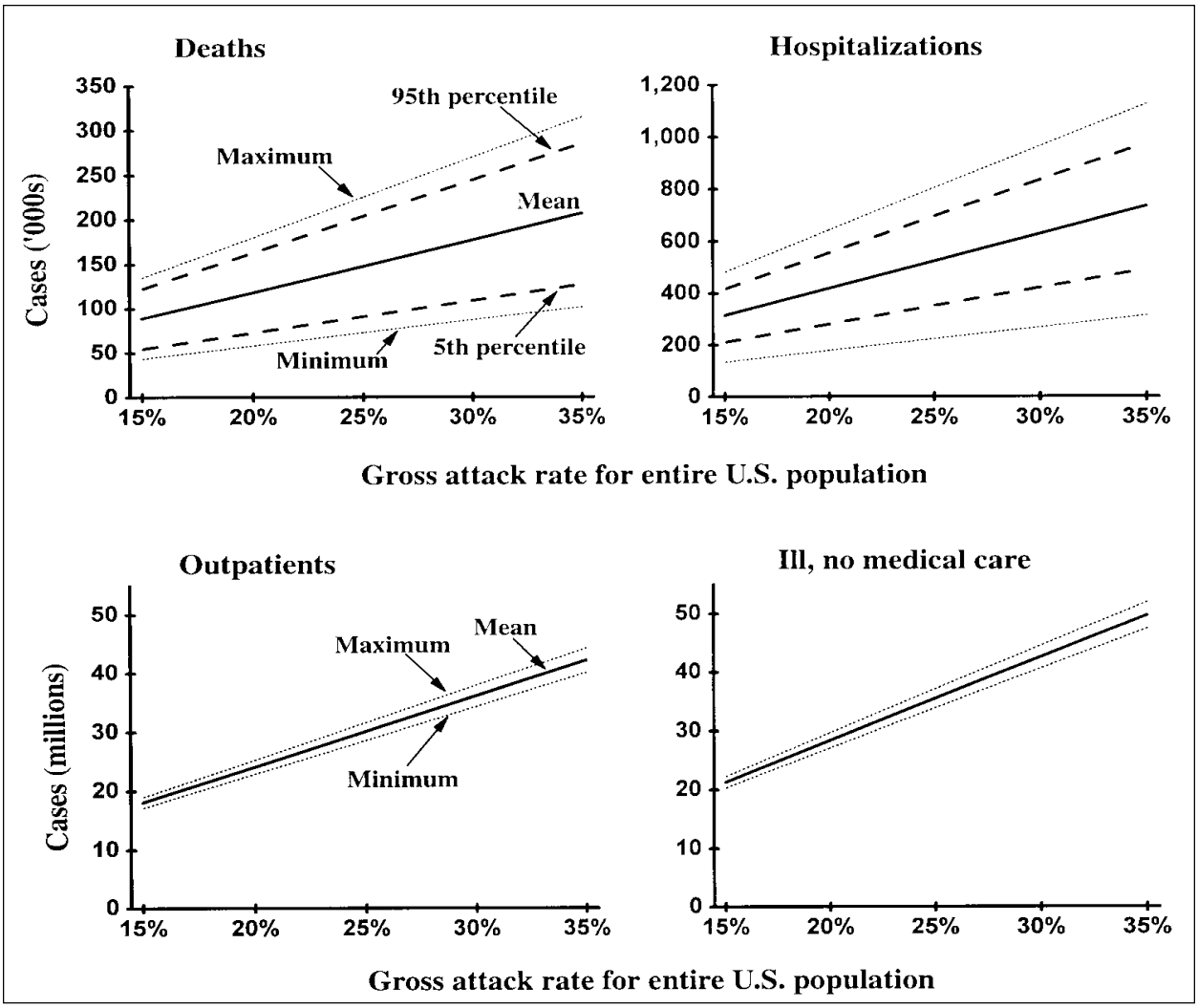


Figure 1: Impact of influenza pandemic in the United States: mean, minimum, maximum, and 5th and 95th percentiles of total death, hospitalizations, outpatients, and those ill (but not seeking medical care) for different gross attack rates. Note that for each gross attack rate, data are totals for all age groups and risk categories.

Table 5. Impact, by age group, death, hospitalizations, and outpatients accounted for by groups at high risk during an influenza pandemic[a]

Category	Age group (yrs)	Total cases at high risk (%) Mean	5th	95th
Death	0-19	9.0	1.4	20.2
	20-64	40.9	11.1	60.9
	65 +	34.4	22.7	52.1
	Total	84.3		
Hospitalizations	0-19	4.6	2.1	7.9
	20-64	14.7	7.4	23.4
	65 +	18.3	11.0	27.6
	Total	37.6		
Outpatients	0-19	5.0	4.7	5.4
	20-64	10.4	9.8	11.0
	65 +	4.0	3.9	4.2
	Total	19.5		

[a]See Table 1 for distribution of groups at high and not at high risk within the U.S. population.

Net Value of Vaccination

If it cost $21 to vaccinate a person and the effective coverage were 40%, net savings to society would result from vaccinating all age and risk groups (Figure 2). However, vaccinating certain age and risk groups rather than others would produce higher net returns. For example, vaccinating patients ages 20 to 64 years of age not at high risk would produce higher net returns than vaccinating patients ages 65 years of age and older who are at high risk (Figure 2). At a cost of $62 per vaccinee and gross attack rates of less than 25%, vaccinating populations at high risk would still generate positive returns (Figure 2). However, vaccinating populations not at high risk would result in a net loss (Figure 2).

Table 6. Costs (direct and indirect) of influenza pandemic per gross attack rate:[a] deaths, hospitalizations, outpatients, illnesses, and total costs (in 1995 US$)

	Cost per gross attack rate ($ millions)				
	15%	20%	25%	30%	35%
Deaths					
Mean	59,288	79,051	98,814	118,577	138,340
5th percentile	23,800	31,733	39,666	47,599	55,532
95th percentile	94,907	126,543	158,179	189,815	221,451
Hospitalizations					
Mean	1,928	2,571	3,214	3,856	4,499
5th percentile	1,250	1,667	2,084	2,501	2,917
95th percentile	2,683	3,579	4,472	5,367	6,261
Outpatients					
Mean	5,708	7,611	9,513	11,416	13,318
5th percentile	4,871	6,495	8,119	9,742	11,366
95th percentile	6,557	8,742	10,928	13,113	15,299
Ill, no medical care sought[b]					
Mean	4,422	5,896	7,370	8,844	10,317
5th percentile	3,270	4,360	5,450	6,540	7,629
95th percentile	5,557	7,409	9,262	11,114	12,967
Grand totals					
Mean	71,346	95,128	118,910	142,692	166,474
5th percentile	35,405	47,206	59,008	70,810	82,611
95th percentile	106,988	142,650	178,313	213,975	249,638

[a]Gross attack rate = percentage of clinical influenza illness per population.
[b]Persons who become clinically ill due to influenza but do not seek medical care; illness has an economic impact (e.g., half day off work).

Sensitivity Analyses

At a vaccination cost of $21.26 per vaccinee, reducing the death rates to half and one quarter of the initial values (Table 2) left positive mean net returns for all age groups not at high risk. However, at a vaccination cost of $62.26 per vaccinee, reducing death rates to half and one quarter of the initial values resulted in negative mean net returns for all age groups not at high risk. The results are much less sensitive to increases in gross attack rate than to increases in death rate. For example, assuming a cost of $62.26 per vaccinee and death rates that are half the initial rates, increasing the gross attack rate from 15% to 25% still resulted in negative net returns for all age groups, regardless of assumed level of vaccine effectiveness.

Implications for Policy

The amount of the insurance premium to spend on planning, preparedness, and practice for responding to the next influenza pandemic ranged from $48 million to $2,184 million per year (Table 7). The amount was sensitive to the probability of the pandemic, the cost of vaccinating a person, and the gross attack rate. Because higher costs of vaccination reduce net returns from an intervention, increased vaccination costs reduced the premiums. Conversely, increases in gross attack rates (all other inputs held constant) increased the potential returns from an intervention and thus the amount of premiums.

When risk for death is used as the criterion for who will be vaccinated first, persons ages 65 years and older receive top priority (Table 8); however, when mean net returns due to vaccination are used as the criterion, that group receives the lowest priority (Table 8). Regardless of criteria used, persons at high risk ages 0 to 19 and 20 to 64 years would always receive priority over persons not at high risk from the same age groups (Table 8).

While Option A would ensure positive mean net returns, Option B would result in greater mean net returns (Figure 3). Changing the strategy from vaccinating specific groups (Option B) to vaccinating 40% of the population decreased mean net returns (Figure 3). Only Option D resulted in higher mean net returns

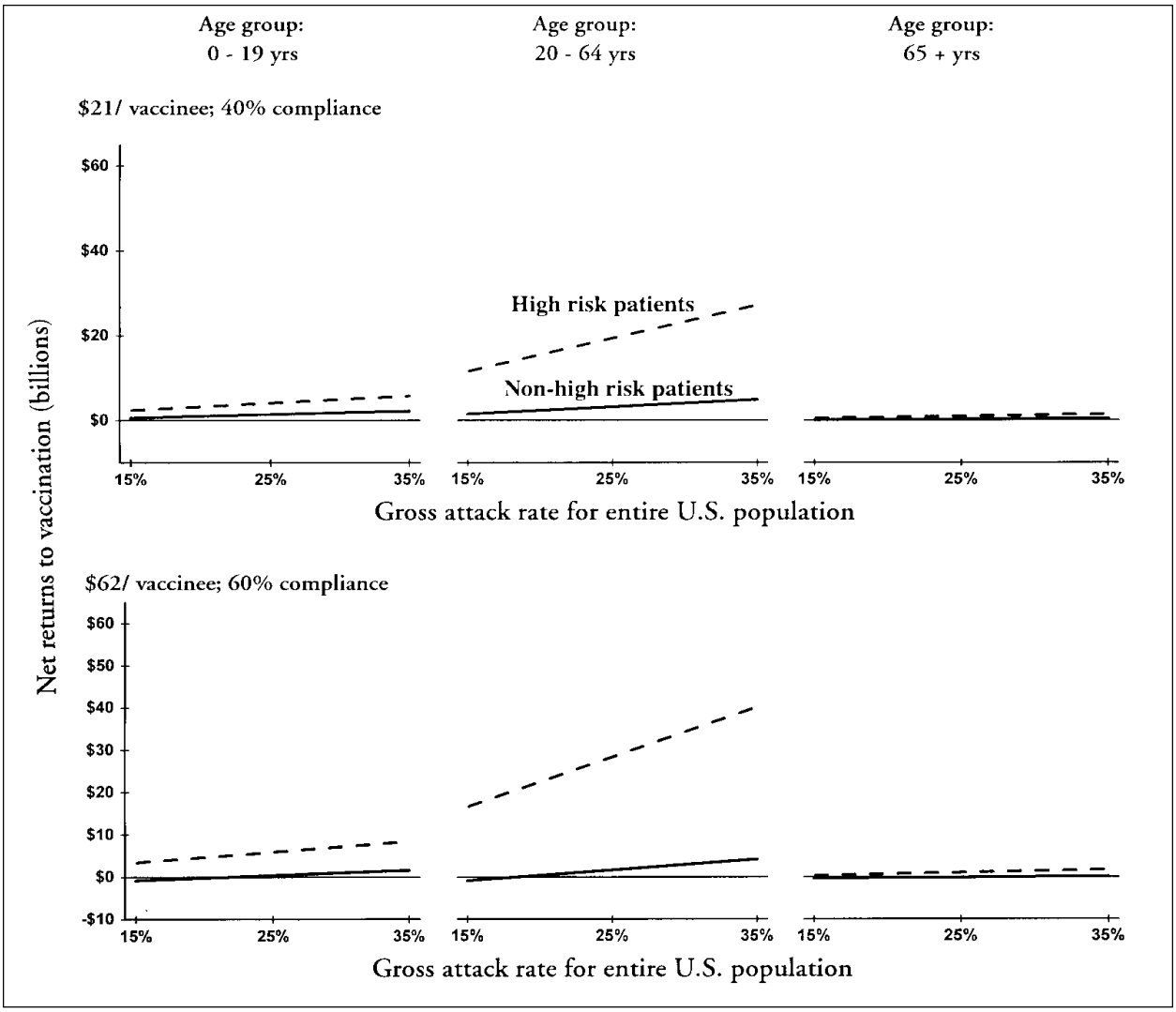


Figure 2: Mean net returns due to vaccination, by age group, for different gross attack rates and percentages of compliance. Case-age distributions are given in Table 1. Assumed vaccine effectiveness is the same as the high vaccine effectiveness defined in Appendix I.

Table 7. The mean annual insurance premium[a] for planning, preparing, and practicing to respond to the next influenza pandemic

		Mean (s.d.) insurance premium ($ millions)					
		Low vaccine effectiveness[b] x 40% compliance Probability of pandemic			High vaccine effectiveness[b] x 60% compliance Probability of pandemic		
Gross attack rate	Cost of vaccination per vaccinee($)	1 in 30 years	1 in 60 years	1 in 100 years	1 in 30 years	1 in 60 years	1 in 100 years
15%	21	306 (122)	153 (61)	92 (37)	872 (341)	435 (170)	262 (103)
	62	162 (122)	81 (61)	48 (37)	654 (341)	326 (170)	196 (103)
25%	21	561 (204)	280 (102)	168 (61)	1,528 (569)	762 (284)	459 (171)
	62	416 (204)	207 (102)	125 (61)	1,311 (569)	653 (284)	394 (171)
35%	21	815 (286)	406 (142)	245 (86)	2,184 (796)	1,089 (397)	656 (239)
	62	670 (286)	334 (142)	201 (86)	1,967 (796)	980 (397)	591 (239)

[a]Defined here as the amount of money to be spent each year to plan, prepare, and practice to ensure that such mass vaccinations can take place if needed. See text for description of calculating premiums. The mathematically optimal allocation of such funds for each activity requires a separate set of calculations.
[b]Low and high levels of vaccine effectiveness are defined in Appendix I.

than Option B. Note, however, that the 5th and 95th percentiles for each option overlapped with those of other options. Thus, the differences in mean values between the options may not occur in practice.

Conclusions

Impact of an Influenza Pandemic

Although the next influenza pandemic in the United States may cause considerable illness

Table 8. Setting vaccination priorities: Which age group or group at risk should be vaccinated first?

	Criteria for prioritization		
Priority	Risk for death[a]	Total deaths[b]	Returns due to vaccination
1 (top)	High risk 65 + yrs	High risk 20 - 64 yrs	High risk 20 - 64 yrs
2	Not at high risk 65 + yrs	High risk 65 + yrs	High risk 0 - 19 yrs
3	High risk 0 - 19 yrs	High risk 0 - 19 yrs	Not at high risk 20 - 64 yrs
4	High risk 20 - 64 yrs	Not at high risk, 65 + yrs	Not at high risk 0 - 19 yrs
5	Not at high risk 20 - 64 yrs	Not at high risk 20 - 64 yrs	High risk 65 + yrs
6 (bottom)	Not at high risk 0 - 19 yrs	Not at high risk 0 - 19 yrs	Not at high risk 65 + yrs

[a]Priorities set by risk for death are set according to lower-limit estimates of deaths per 1,000 population for each age and risk group.
[b]The priority list using the total deaths criteria was set by examining the percentage of total deaths that each age and risk group contributed to the total deaths estimated due to a pandemic. The group with the highest percentage (i.e, contributes the largest number of deaths) is listed as having the highest priority.

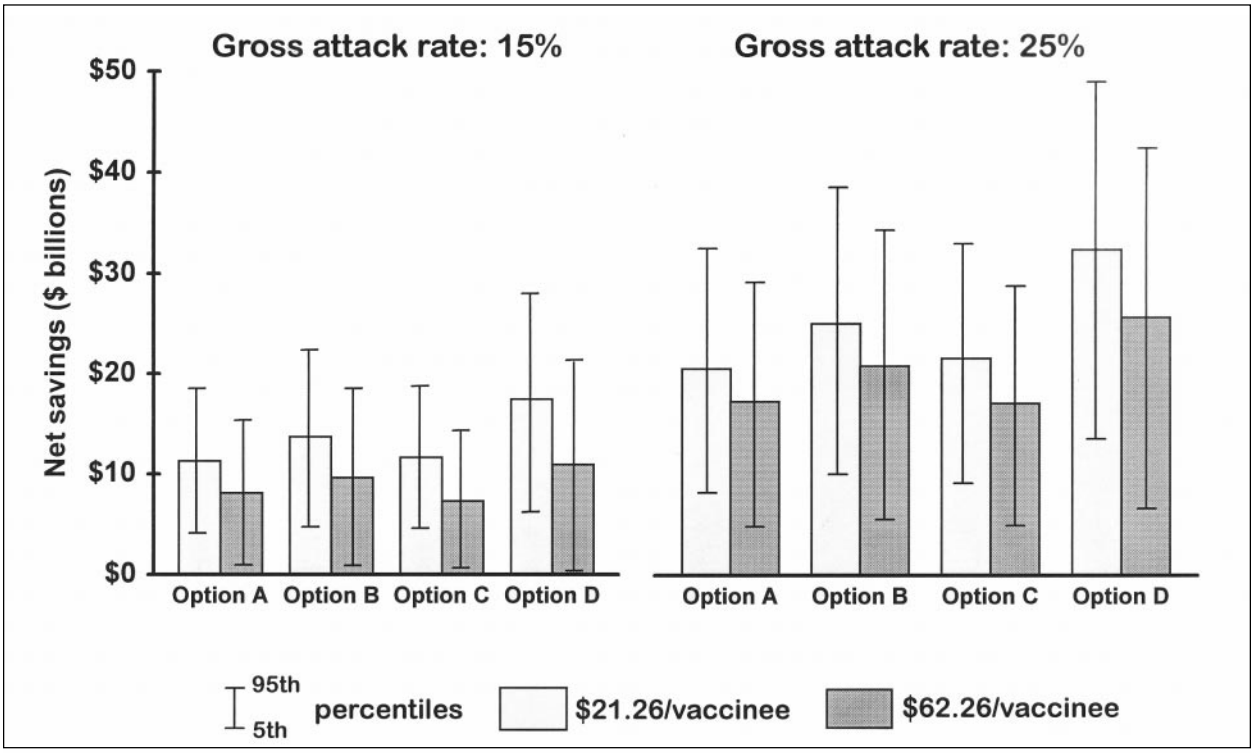


Figure 3: Four options for responding to an influenza pandemic: mean net economic returns. Notes: a) Bars show mean net returns for each option and assumed cost of vaccination. b) Option A: Similar to current Advisory Committee on Immunization Practices recommendations, with production and use similar to current, intra-pandemic recommendations (17). Assumed approximately 77 million vaccinees. Option B: Number of vaccinees as outlined in Scenario A plus 20 million essential service providers (5 million health-care workers + 15 million other service providers). Option C: Aim to achieve a 40% coverage of total U.S. population. Option D: Aim to achieve 60% coverage of total U.S. population (Appendix II).

and death (Figure 1), great uncertainty is associated with any estimate of the pandemic's potential impact. While the results can describe potential impact at gross attack rates from 15% to 35%, no existing data can predict the probability of any of those attack rates actually occurring. In addition, the groups at high risk are likely to incur a disproportionate number of deaths (Table 5); 50% or more of the deaths will likely occur among persons age 65 years and older (Appendix II), a distribution also found in the influenza pandemics of 1918, 1957, and 1968 (2).

Our results illustrate that the greatest economic cost is due to death (Table 6). Therefore, all other things being equal, the largest economic returns will come from the intervention(s) that prevents the largest number of deaths. A limitation of the model is that, beyond the value of a lost day of work (Table 3), the model does not include any valuation for disruptions in commerce and society. For example, if many long-distance truck drivers were unavailable to drive for 1 or 2 weeks, there might be disruptions in the distribution of perishable items, especially food. These multiplier effects are not accounted for in this model, mainly because an estimate of an appropriate multiplier will depend on who becomes ill, how many become ill, when they become ill, and for how long they are ill.

All other factors being held constant, the net returns due to vaccination are sensitive to the combination of price and gross attack rate, with some scenarios generating negative mean returns (Figure 2). Further, some scenarios with a positive mean net return had a negative 5th percentile (Appendix II). The fact that negative results can be generated should serve as a warning that many interventions may not guarantee a net positive economic return.

Implications for Policy

The premium that could be spent each year for influenza pandemic response (planning, preparedness, and practice) depends most on the assumed probability of the pandemic (Table 7). The wide range in premiums presents a cautionary tale of the difference between possibility and probability of an influenza pandemic. What cannot be stated with any certainty are the probability of a pandemic and the number of persons who will become ill and die. Deciding the difference between possibility and probability was a key decision point in the swine flu incident of 1976-77 (34).

Vaccination priorities depend on the objectives. If preventing the greatest number of deaths is the most important goal, society should ensure that those in the groups at high risk become vaccinated first, followed by those age 65 years or older who have no preexisting medical conditions making them more susceptible to complications from influenza (Table 8). However, if maximizing economic returns is the highest priority, persons 0 to 64 years of age, regardless of risk, should be vaccinated first (Table 8). Results also illustrate the need to be precise in defining the criterion used for setting priorities. For example, stating that preventing death will be the criteria used is not sufficiently precise because different priority lists can be drawn up using death rates versus total deaths (Table 8).

The criteria used to generate the results in Table 8 do not define the entire set of possible methods of setting priorities. Society may decide to use another criterion or set of criteria. Priorities for vaccination may also depend on the epidemiology of the pandemic. For example, if the strain causing the pandemic were particularly virulent among those ages 20 to 40 years, that age group may receive highest priority. Since the epidemiology of the next pandemic is unknown, any plan must allow flexibility in determining criteria for setting priorities. Table 8 provides a starting point for debate regarding who should be vaccinated first.

The net returns for the four scenarios modeled (Figure 3) further illustrate the need to clearly set criteria, goals, and objectives for a vaccine-based intervention for the next influenza pandemic. Some may state that Options C and D represent a more egalitarian means of distributing vaccine. However, egalitarianism would cost society more since the mean net returns from Options C are lower than those from Option B (Figure 3). Option D produces higher returns than Option B (Figure 3), but vaccinating 60% of the U.S. population in a short time would be difficult, especially if two doses of vaccine are required. If two doses were required, Option D would mean producing, delivering, and administering approximately 320 million doses of vaccine in a 2- to 3-month period, which has never been accomplished in the United States.

Acknowledgments

We thank Nancy Arden, Rob Breiman, Bill Jordan, Marty Meyers, Alicia Postema, Steve Schoenbaum, Larry Schonberger, Larry Sparks, and Ray Strikas for their help and encouragement.

Dr. Meltzer is senior health economist, Office of the Director, National Center for Infectious Diseases, Centers for Disease Control and Prevention. His research interests focus on assessing the economics of public health interventions such as oral raccoon rabies vaccine, Lyme disease vaccine, and hepatitis A vaccine, as well as estimating the economic burden of bioterrorism, dengue, pandemic influenza, and other infectious diseases. His research uses various methods, including Monte Carlo modeling, willingness-to-pay surveys (contingent valuation), and the use of nonmonetary units of valuation, such as Disability Adjusted Life Years.

References

1. Patriarca PA, Cox NJ. Influenza pandemic preparedness plan for the United States. J Infect Dis 1997;176 Suppl 1:S4-7.
2. Simonsen L, Clarke MJ, Schonberger LB, Arden NH, Cox NJ, Fukuda K. Pandemic versus epidemic influenza mortality: a pattern of changing age distribution. J Infect Dis 1998;178:53-60.
3. Office of Technology Assessment, U.S. Congress. Cost effectiveness of influenza vaccination. Washington: Government Printing Office; 1981.
4. Kavet J. A perspective on the significance of pandemic influenza. Am J Public Health 1977;67:1063-70.
5. Campbell DS, Rumley MA. Cost-effectiveness of the influenza vaccine in a healthy, working-age population. J Occup Environ Med 1997;39:408-14.

Appendix I

For the equation in the main text defining net returns due to vaccinations, savings from outcomes averted and the costs of vaccination are calculated as follows:

$$\text{Savings from outcomes averted}_{\substack{\text{age,}\\\text{risk}\\\text{group}}} = \sum_{\text{Outcomes}} \left(\text{Number with outcome}_{\substack{\text{death,}\\\text{hospitalization,}\\\text{outpatient, ill,}\\\text{no medical care}}} \text{ before intervention}_{\substack{\text{age,}\\\text{risk}\\\text{group}}} \times \text{compliance}_{\substack{\text{age,}\\\text{risk}\\\text{group}}} \times \text{vaccine effectiveness}_{\text{Outcomes}} \times \$\text{value of outcome}_{\substack{\text{death,}\\\text{hospitalization,}\\\text{outpatient, ill,}\\\text{no medical care}}} \text{ prevented}\right)$$

and;

$$\text{Cost of vaccination}_{\substack{\text{age,}\\\text{risk}\\\text{group}}} = \$\text{cost/vaccinee} \times \text{population}_{\substack{\text{age}\\\text{risk}\\\text{group}}} \times \text{compliance}_{\substack{\text{age,}\\\text{risk}\\\text{group}}}$$

Table: High and low levels of assumed vaccine effectiveness

	Vaccine effectiveness in preventing disease outcomes[ab]					
	High[c]			Low[c]		
Disease outcomes	0-19 yrs	20-64 yrs	65+ yrs	0-19 yrs	20-64 yrs	65+ yrs
Death	0.70	0.70	0.60	0.40	0.40	0.30
Hospitalization	0.55	0.55	0.50	0.55	0.55	0.50
Outpatient visits	0.40	0.40	0.40	0.40	0.40	0.40
Ill, no medical care sought	0.40	0.40	0.40	0.40	0.40	0.40

[a]Vaccine effectiveness is defined as the reduction in the number of cases in each of the age and disease categories.
[b]Within a defined age group, it was assumed that there was no difference in vaccine effectiveness between subgroups at high risk and not at high risk.
[c]The terms high and low level of effectiveness are subjective and reflect only a judgment of the levels of effectiveness in the two scenarios relative to each other.

Appendix II

A background paper, containing additional methodological details and results, is available electronically at the following URL: http.www.cdc.gov/ncidod/EID/vol5no5/meltzerback.htm.

6. Carrat F, Valleron A-J. Influenza mortality among the elderly in France, 1980-90: how many deaths may have been avoided through vaccination? J Epidemiol Community Health 1995;49:419-25.
7. Riddiough MA, Sisk JE, Bell JC. Influenza vaccination: cost-effectiveness and public policy. JAMA 1983;249:3189-95.
8. Patriarca PA, Arden NH, Koplan JP, Goodman RA. Prevention and control of type A influenza infections in nursing homes: benefits and costs of four approaches using vaccination and amantadine. Ann Intern Med 1987;107:732-40.
9. Schoenbaum SC. Economic impact of influenza: the individuals perspective. Am J Med 1987;82 Supp 6A:26-30.
10. Jefferson T, Demicheli V. Socioeconomics of influenza. In: Nicholson KG, Webster RG, Hay AJ, editors. Textbook of influenza. London (UK): Blackwell Science; 1998. p. 541-7.
11. Schoenbaum SC, McNeil BJ, Kavet J. The swine-influenza decision. N Eng J Med 1976;295:759-65.
12. Cliff AD, Haggett P. Statistical modelling of measles and influenza outbreaks. Stat Methods Med Res 1993;2:43-73.
13. Critchfield GC, Willard KE. Probabilistic analysis of decision trees using Monte Carlo simulation. Med Decis Making 1986;6:85-92.
14. Dobilet P, Begg CB, Weinstein MC, Braun P, McNeil BJ. Probabilistic sensitivity analysis using Monte Carlo simulation: a practical approach. Med Decis Making 1985;5:157-77.
15. Dittus RS, Roberts SD, Wilson JR. Quantifying uncertainty in medical decisions. J Am Coll Cardiol 1989;14:23A-8.
16. U.S. Bureau of the Census. Statistical abstract of the United States: 1997. 117th ed. Washington: The Bureau; 1997.
17. Centers for Disease Control and Prevention. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep 1998;47(RR-6):1-26.
18. Evans M, Hastings N, Peacock B. Statistical distributions. 2nd ed. New York: John Wiley; 1993.
19. Glezen PW. Emerging infections: pandemic influenza. Epidemiol Rev 1996;18:64-76.
20. Mullooly JP, Barker WH. Impact of type A influenza on children: a retrospective study. Am J Public Health 1982;72:1008-16.
21. Barker WH, Mullooly JP. Impact of epidemic type A influenza in a defined adult population. Am J Epidemiol 1980;112:798-813.
22. Simonsen L, Clarke MJ, Williamson GD, Stroup DF, Arden NH, Schonberger LB. The impact of influenza epidemics on mortality: introducing a severity index. Am J Public Health 1997;87:1944-50.
23. Fox JP, Hall CE, Cooney MK, Foy HM. Influenzavirus infections in Seattle families, 1975-1979. I. Study design, methods and the occurrence of infections by time and age. Am J Epidemiol 1982;116:212-27.
24. Glezen WP, Decker M, Joseph SW, Mercready RG. Acute respiratory disease associated with influenza epidemics in Houston, 1981-1983. J Infect Dis 1987;155:1119-26.
25. Serfling RE, Sherman Il, Houseworth WJ. Excess pneumonia-influenza mortality by age and sex in three major influenza A2 epidemics, United States, 1957-58, 1960 and 1963. Am J Epidemiol 1967;86:433-41.
26. Barker WH, Mullooly JP. Pneumonia and influenza deaths during epidemics: implications for prevention. Arch Intern Med 1982;142:85-9.
27. Glezen WP, Payne AA, Snyder DN, Downs TD. Mortality and influenza. J Infect Dis 1982;146:313-21.
28. McBean AM, Babish JD, Warren JL. The impact and cost of influenza in the elderly. Arch Intern Med 1993;153:2105-11.
29. Barker WH. Excess pneumonia and influenza associated hospitalization during influenza epidemics in the United States, 1970-78. Am J Public Health 1986;76:761-5.
30. Haddix AC, Teutsch SM, Shaffer PA, Dunet DO. Prevention effectiveness. New York: Oxford University Press; 1996.
31. The Federal Register. Vol 61, No. 170; 1996 Aug 30; p. 46301-2.
32. Kaufmann AF, Meltzer MI, Schmid GP. The economic impact of a bioterrorist attack: are prevention and postattack intervention programs justifiable? Emerg Infect Dis 1997;3:83-94.
33. Robinson LJ, Barry PJ. The competitive firm's response to risk. New York: Macmillian; 1987.
34. Neustadt RE, Fineberg HV. The swine flu affair: decision making on a slippery disease. Washington: U.S. Department of Health Education, and Welfare; 1978.

Influence of Host Genetics on the Severity of Coccidioidomycosis

Leslie Louie,* Susanna Ng,* Rana Hajjeh,† Royce Johnson,‡ Duc Vugia,§ S. Benson Werner,§ Ronald Talbot,¶ and William Klitz*
*University of California, Berkeley, California, USA; †Centers for Disease Control and Prevention, Atlanta, Georgia, USA; ‡Kern Medical Center, Bakersfield, California, USA; §California Department of Health Services, Berkeley, California, USA; ¶Kern County Public Health Department, Bakersfield, California, USA

Coccidioidomycosis, a mild flulike illness in approximately 40% of infected persons, progresses to severe pulmonary or disseminated disease in 1% to 10% of symptomatic cases. We examined host genetic influences on disease severity among class II HLA loci and the ABO blood group. Participants included African-American, Caucasian, and Hispanic persons with mild or severe disseminated coccidioidomycosis from Kern County, California. Among Hispanics, predisposition to symptomatic disease and severe disseminated disease is associated with blood types A and B, respectively. The HLA class II DRB1*1301 allele marks a pre-disposition to severe disseminated disease in each of the three groups. Reduced risk for severe disease is associated with DRB1*0301-DQB1*0201 among Caucasians and Hispanics and with DRB1*1501-DQB1*0602 among African-Americans. These data support the hypothesis that host genes, in particular HLA class II and the ABO blood group, influence susceptibility to severe coccidioidomycosis.

Coccidioidomycosis (CM), also known as Valley fever, is caused by the dimorphic fungus *Coccidioides immitis*, endemic to the southwestern United States, Mexico, and Central and South America. In California before 1991, approximately 400 to 500 new cases were reported annually. During 1991 to 1994, the number of cases increased dramatically, with 1,200 new cases reported in 1991, 4,541 in 1992, and 4,137 in 1993 (1-4).

C. immitis is a soil-dwelling organism that blooms after the rainy season. Infection usually occurs in the dry season, when arthroconidia become airborne and can be inhaled by the host. In California in 1991, increased rainfall following a drought allowed increased fungal proliferation, resulting in an increased rate of infection. The 1994 Northridge earthquake led to an increase in infections associated with landslides and dust clouds that aerosolized arthroconidia. Increased risk for symptomatic CM was directly proportional to time spent in the dust cloud (5).

Of those infected, an estimated 60% are asymptomatic, with the only evidence of exposure being delayed-type hypersensitivity (DTH) reaction to a coccidioidal antigen skin test. Of the symptomatic patients, 90% to 99% experience only mild flulike symptoms. More severe chronic pulmonary disease or disseminated infection beyond the thoracic cavity occurs in 1% to 10% of symptomatic cases, depending on ethnicity (N. Ampel, pers. comm.). Disseminated CM is particularly devastating and usually requires lifelong antifungal treatment. Risk factors include male sex, compromised immune status, pregnancy, diabetes, advancing age, and smoking (6-9).

Risk for disseminated CM seems to differ according to ethnicity (9). For example, during the 1977 windborne outbreak of CM in the nonendemic-disease region of Sacramento County, California, the rate per 100,000 of acute

Address for correspondence: Leslie Louie, University of California School of Public Health, Public Health Biology and Epidemiology Department, 140 Warren Hall, Berkeley, CA 94720, USA; fax: 510-643-5144; e-mail: leslie@allele5.biol.berkeley.edu.

symptomatic pulmonary CM among African-American men compared with Caucasian men was 67 versus 19 (ratio 3.5:1) and of disseminated CM was 23.8 versus 2.5 (ratio 9.1:1). These differences could not be explained by differential exposure (11). More recently, in the endemic area of Kern County, California, African-American men had an adjusted odds ratio for disseminated CM of 28 (95% confidence interval [CI] 2-385), higher than that of any other ethnic group. No environmental or occupational exposures were associated with either severe pulmonary or disseminated disease (9). Both the range of response given *C. immitis* exposure and the apparent variation in susceptibility among ethnic groups suggest that genetic factors influence the development of symptomatic, severe, and disseminated CM.

T-cell-mediated immunity is important in the elimination of the fungus, but it eventually diminishes if the disease progresses (12,13). Although little is known about the role of T-cells in eliminating *C. immitis*, activated T-cells elicit a DTH inflammatory response, indicating a Th1-type response (14). While DTH reactivity is regulated by class II HLA interactions with T cells, the host immune response to intracellular pathogens is primarily regulated by class I HLA molecules. HLA genes are therefore prime candidates for the study of host genetic influences on the severity of CM. Some studies have shown disseminated CM to be associated with HLA antigens (A-9, B-5) and ABO blood group B (6,7,15,16). Both African-Americans and Filipinos have greater frequency of the B blood group and HLA-A9 than do Caucasians (16).

Kern County, where CM is highly endemic, contributed substantially to the 1991 to 1994 epidemic in California. We investigated the role of genetic factors in CM in a case-control study of persons from Kern County among patients with severe disseminated disease or mild disease. This study explored the possible association of HLA class II alleles and haplotypes and ABO phenotypes with severity of CM disease in three ethnic groups.

Methods and Study Design

Participants

Patients were recruited from the Kern County Health Department and Kern Medical Center. Severe cases (n = 109) were defined as extrapulmonary disseminated CM, with disease spreading beyond the thoracic cavity, including prevalent and incident cases seen or diagnosed from 1995 through 1997. Mild cases (n = 83) were diagnosed during 1995 to 1996, with uncomplicated disease limited to the lung (or lymph nodes draining the lung) and not requiring hospitalization (Table 1). Incident cases, which had laboratory evidence of acute infection, were detected during a population-based surveillance study of CM in Kern County (11). All diagnoses were laboratory confirmed by serologic testing, enzyme immunoassay ([EIA], immunodiffusion, and complement fixation), or culture at the Kern County Health Department Laboratory.

For controls, data from ethnically and geographically matched populations were obtained from the literature (Table 1). For ABO phenotype comparisons, populations from San Francisco, were selected (17). Molecular data for comparing HLA allele frequencies were selected from healthy Caucasians from California (18), Hispanics from Los Angeles (19), and African-Americans from New York (20).

Table 1. Coccidioidomycosis participants, by ethnicity and severity of disease

Cases	Caucasians	Hispanics	African Americans	Other	Total
Severe	27	50	25	7	109
Mild	50	32	1	0	83
Total	77	82	26	7	192
Outside controls					
ABO	8,962	335	3,146		
HLA	107	115 (109 for DPB1)	241		

Genetic Testing

Standard ABO antigen blood typing was performed at the Kern County Health Department laboratories. All persons were typed for four HLA class II loci (DRB1, DQA1, DQB1, and DPB1) by molecular methods (21-24).

Statistical Analyses

For the HLA data, Hardy-Weinberg genotypic proportions were calculated to examine the equilibrium in each locus (25). Methods of testing genotypic ratios in the ABO system with the

recessive O allele were followed (26). HLA haplotype frequencies were estimated for each risk group by ethnicity and disease severity by using standard maximum likelihood methods (27,28).

ABO phenotype and allele frequencies and HLA class II allele and haplotype frequencies were compared by using 2 x k contingency tables that give an overall G-statistic, where k is the total number of alleles present in the comparison groups for a particular locus. All analyses were stratified by ethnicity. Comparing severe cases with mild cases assessed risk for disease progression after symptomatic infection, and comparing severe or mild cases with controls examined risk for disease severity and infection relative to each ethnic population. In any comparison, alleles present in fewer than three persons were pooled into a combined class of rare alleles. If the overall comparison showed statistically significant heterogeneity, the underlying difference was examined among individual alleles, phenotypes, or haplotypes by using the chi-square statistic, with odds ratios (OR) and Yates' corrected or Fisher's p values reported. Since only one African-American with mild disease was enrolled, analyses for African-Americans were limited to comparing severe cases with controls.

Interaction effects of ABO and HLA alleles associated with disease were assessed with the Mantel-Haenszel chi-square test and weighted OR by using EpiInfo version 6.01.

Results

Hardy-Weinberg Tests

Severe and mild cases were examined for deviations from Hardy-Weinberg equilibrium to validate the source populations and genotyping and to detect alleles that might be associated with disease. In each instance, the HLA loci DRB1 and DQA1 genotypic ratios were in equilibrium (Table 2). However, the DPB1 locus for mild cases among Hispanics and mild and severe cases among Caucasians and the DQB1 locus for Hispanics with mild cases were not in equilibrium ($p < 0.05$, Table 2). For DPB1 among Caucasians with mild cases, DPB1*0301-*0401 was less common than expected (none observed vs. three expected). For DPB1 for mild cases among Caucasians and Hispanics, genotypes with the *1401, *1601, and *4601 alleles were observed more often than expected. Other loci not in Hardy-Weinberg equilibrium ($p < 0.05$) had no significant deviations at individual genotypes.

Table 2. Hardy-Weinberg test results (p values) for HLA class II loci, by ethnicity and severity of disease[a]

Cases	DRB1	DQA1	DQB1	DPB1	N
Hispanic, Severe	0.34	0.18	0.38	0.27	50
Hispanic, Mild	0.12	0.11	**0.002**	**0.045**	32
Caucasian, Severe	0.09	0.14	0.38	**0.008**	27
Caucasian, Mild	0.12	0.14	0.11	**0.022**	50
African-American, Severe	0.63	0.80	0.67	0.31	25

[a]Numbers in bold indicate alleles not in Hardy-Weinberg equilibrium for a particular group and locus.

ABO phenotype frequencies did not depart from Hardy-Weinberg equilibrium except for the large Caucasian control sample, which differed at $p = 0.04$, indicating a small deviation from equilibrium (data not shown).

Testing for Allelic Association

ABO blood types were analyzed by comparing the blood type phenotype to disease status (severe, mild, control) in contingency table tests. No differences between groups were seen for Caucasians ($p = 0.80$). This was also true for African-Americans when patients with severe disease were compared with controls ($p = 0.21$). Blood group phenotype frequencies among Hispanics, however, were highly heterogeneous with respect to disease status ($p < 0.001$) (Table 3). Among affected persons with either severe or mild disease, the A phenotypes were more frequent (OR = 1.71, $p = 0.18$ and OR = 5.53, $p < 10^{-4}$, respectively) and the B phenotypes less frequent (OR = 0.36, $p = 0.021$ and OR = 0, $p < 10^{-3}$, respectively) than among controls. Comparing severe versus mild cases among Hispanics, the A phenotypes were associated with decreased risk (OR = 0.31, $p = 0.024$) and the B

Table 3. Blood group antigen frequencies for coccidioidomycosis, by ethnicity and severity of disease

Ethnic group		Phenotype A	AB	B	O	N
Hispanic[a]	Severe	0.24	0.02	0.12	0.62	50
	Mild	0.53	0.00	0.00	0.47	32
	Controls[b]	0.13	0.04	0.27	0.56	335
Caucasian	Severe	0.48	0.04	0.04	0.44	27
	Mild	0.46	0.02	0.12	0.40	50
	Controls[b]	0.41	0.03	0.11	0.45	8,962
African-American	Severe	0.12	0.04	0.16	0.68	25
	Controls[b]	0.28	0.04	0.19	0.50	1,540

[a]ABO phenotype distribution for cases and controls among Hispanics, $p < 0.001$

[b]Population control frequencies from Mourant, 1976 (17)

phenotypes with increased risk (OR >5.2, Fisher's p = 0.039) of disseminated disease (Table 3).

Several of the HLA class II loci showed overall heterogeneity in the allele frequencies tested pairwise among the three disease status categories (Table 4). Effects occurred in all three ethnic groups. Significant effects occurred more extensively and with greater significance in comparisons of cases versus controls than in comparisons of severe cases versus mild cases for DQB1, DRB1, and DR-DQ haplotype. This result might be due to the higher sample size in the controls, allowing greater power in comparisons with this group.

Significant HLA allelic and haplotypic associations differed for each ethnic group (Table 5). However, Caucasians and Hispanics share a haplotype, DRB1*0301-DQB1*0201, associated with reduced risk for disease. Among Caucasians, both mild and severe cases had this effect when compared with controls (OR = 0.2, p = 0.012 and OR = 0.1, p = 0.007, respectively). Among Hispanics, the effect was observed only for mild cases versus controls (OR = 0.1, p = 0.007).

The DRB1 allele, *1301, was associated with increased risk for disease in all three ethnic groups compared with controls. For Caucasians, mild cases were associated with this allele (OR = 2.6, p = 0.028), whereas severe cases were associated among Hispanics (OR = 4.9, p = 0.01) and African-Americans (OR = 4.2, p = 0.008). The DRB1-DQB1 haplotypes containing DRB1*1301 in each group were also associated with increased risk, although the DQB1 alleles in the haplotypes differed (Table 5).

Table 4. Significance levels from contingency table analyses of allele distributions for coccidioidomycosis, by ethnicity and severity of disease[a]

Ethnic group	Locus	Severe vs. mild	Severe vs. control	Mild vs. control
Caucasians	DQA1	0.231	0.069	**0.048**
	DQB1	**0.024**	**0.02**	**0.002**
	DRB1	0.075	0.12	**<0.001**
	DR-DQ	**0.031**	**<0.001**	**<0.001**
	DPB1	0.781	0.215	0.355
Hispanics	DQA1	0.074	---	---
	DQB1	0.41	**0.003**	0.16
	DRB1	0.63	**<0.001**	0.16
	DR-DQ	0.29	**<0.001**	**0.02**
	DPB1	**0.024**	0.403	**0.026**
African-Americans	DQA1	---	0.89	---
	DQB1	---	0.34	---
	DRB1	---	**0.001**	---
	DR-DQ	---	**<0.001**	---
	DPB1	---	0.38	---

[a]Significant test results are in bold

Table 5. HLA alleles and haplotypes associated with severity of coccidioidomycosis, by ethnicity

Locus	Caucasian S v. M[a]	Caucasian S v. C	Caucasian M v. C	Hispanic S v. M	Hispanic S v. C	Hispanic M v. C	African-American S v. C
a. Alleles associated with decreased risk							
DQB1	*0402[b] *0504[b]	*0604[b]	*0604[b]		*0302[b]		
DRB1			*0301[c] *1104[b]		*0407[c] *0408[b]		*1501[c]
DRB1-DQB1	*0700-*0303[b]	***0301-*0201**[b] *0404-*0302[b] *0700-*0303[b] *1302-*0604[b]	***0301-*0201**[b] *1104-*0301[b]		*0407-*0302[c] *0700-*0201[b] *1101-*0301[c] *0101-*0501[b]	***0301-*0201**[c] *1402-*0301[c]	*1501-*0602[c]
DPB1						*0501[b]	
b. Alleles associated with increased risk							
DQB1	*0602[b]	rare[b]	*0504[d] rare[d]		*0602[c] *0603[b]		
DRB1			*0303[c] ***1301**[b] rare[d]		*1103[c] ***1301**[c] *1406[c] rare[b]		***1301**[c] *1503[c] rare[c]
DRB1-DQB1	***1301-*0602**[b]	***1301-*0602**[d] *0700-*0201[b] rare[d]	***1301-*0504**[c] *1302-*0201[c] *0303-*0201[c] rare[b]		*0700-*0301[c] ***1301-*0603**[b] *1302-*0301[c] *1406-*0301[c] rare[b]	rare[c]	***1301-*0501**[c] *1503-*0602[c]
DPB1				*0101[b] *0201[b]		rare[b]	

[a]S, severe cases; M, mild cases; C, outside controls
[b] $p < 0.05$
[c] $p < 0.01$
[d] $p < 0.001$

Among African-Americans, only DRB1 showed overall allelic heterogeneity (p = 0.001). DRB1*1301, *1501, and *1503 each contributed to the risk for severe disseminated disease (OR = 4.2, p = 0.008; OR = 0.1, p = 0.002; and OR = 10.2, p = 0.01, respectively). Comparing haplotype frequencies increased the significance of these differences (p <0.001). DRB1-DQA1-DQB1 haplotypes *1301-*0102-*0501 and *1503-*0102-*0602 were associated with higher risk (OR = 10.4, p = 0.003 and OR = 10.2, p = 0.01, respectively), and *1501-*0102-*0602 was associated with lower risk (OR = 0.1, p = 0.003) of severe disseminated disease. Rare alleles of DRB1 and DRB1-DQB1 haplotypes were consistently associated with increased risk in all three ethnic groups (Table 5).

The joint effects of HLA DRB1*1301 and blood group A or B phenotype on progression to disseminated disease were tested in Hispanics, which had substantial ABO effects. When severe cases were compared with mild cases, an effect persisted for the A phenotype (OR_{MH} = 0.30, p = 0.02, data not shown).

Discussion

Except for the Native American genetic component of the Hispanic (Mexican-American) population, the ethnic groups examined show no evidence of an evolutionary response to the pathogenic threat of *C. immitis*. Despite this fact, evidence is presented that for each ethnic group, allele and haplotype distributions at HLA and ABO genes vary according to disease outcome. Substantial differences at these loci were identified in the risk for symptomatic disease after infection with *C. immitis*. The differences in susceptibility associated with ethnic background can be attributed to genetic influences. In certain instances, identical HLA alleles were observed among Hispanic, Caucasian, and African-American persons. In contrast, only Hispanic participants demonstrated ABO effects.

Effects could be observed if controls inadequately represent cases. Controls were matched as closely as possible to cases by ethnicity and geographic proximity. Caucasians were defined as persons of "mixed European" descent, while Hispanics were defined as "Mexican-Americans." The African-Americans were persons with "European-African admixture." For ABO comparisons, all case patients were from California. For HLA, all Hispanic and Caucasian persons were from California.

The lack of cases of mild disease noted among African-Americans may be due to ascertainment bias or biologic cause. Severe cases, which require medical management, are much more likely to come to medical attention and be identified by the study. Because mild infections are self-limiting, some persons may not seek medical care and may not be identified. In a population-based surveillance study of CM in Kern County between 1995 and 1996 (11), African-Americans comprised 6% of all mild cases and 22% of all disseminated cases (Table 1). By comparison, Hispanics comprised 38% of mild cases and 39% of disseminated cases in the surveillance study versus 39% and 46%, respectively, in the present study. Thus mild cases among African-Americans are underrepresented in this study by approximately fourfold.

Patients with mild disease may respond differently, depending on ethnic background, in seeking treatment, use of medical care, or response rate to study participation. Statistics on use of health-care services in the United States during 1996-1997 showed that the time since last physician contact was nearly identical for Caucasians and African-Americans. Overall, African-Americans had similar numbers of ambulatory health-care visits per person per year (3.3 for Caucasians versus 3.9 for African-Americans) (29). However, a greater proportion of visits by African-American patients was to emergency rooms or hospitals as outpatients (an average of one visit per person per year for African-Americans versus 0.6 for Caucasians) (29). If this pattern of health-care use is similar in Kern County, then the >10:1 difference in the frequency of mild cases among Caucasians versus those among African-Americans in this study cannot be explained by ascertainment bias.

An alternative explanation is that mild CM is intrinsically rare among African-Americans compared with Hispanics and that most exposures to *C. immitis* in African-Americans result in severe disseminated disease. The surveillance study indicates that mild cases are more rare among African-Americans.

ABO Effects

ABO blood group is one of the first human genetic polymorphisms identified (30). Numerous

studies (17,31) have examined the relationship between ABO and disease, with inconclusive results. The distribution of A and B antigens in the body, the differences between the two alleles (32), and the existence of A, B, and O alleles in aboriginal human populations worldwide (except the New World) point to the evolutionarily demonstrated functional importance of this system. The association of CM with blood group phenotypes between the mild and severe cases among Hispanics, as well as between these cases and controls, again suggests a direct role for blood group antigens in disease susceptibility.

Previous studies reported that the B blood group was more frequent among persons with severe disseminated cases than among those with mild cases (7,16). These analyses did not consider ethnicity. Our results among Hispanics support earlier findings. However, the frequency of the B phenotype among severe cases (14%) and mild cases (0%) is lower than the frequency (31%) reported in the Hispanic population from San Francisco (17). Assuming the controls were adequately matched to cases, the lack of blood group B in mild cases suggests perceived risk when compared with severe cases. For Caucasians, an opposite (nonsignificant) trend is observed: the frequency of blood group B phenotypes in the population is approximately 14%, the same as among persons with mild cases in this study, and relatively fewer persons with severe cases (8%) have this antigen. The frequency of B phenotypes among African-Americans with severe cases (20%) is similar to that observed in the control population (23.4%) (17).

The A blood group phenotypes are more frequent among Hispanics with cases than among controls, but less frequent among those with severe cases than mild cases. This antigen appears to have the opposite effect on disease expression and severity to that of the B antigen.

Culture filtrates of *C. immitis* were reported to contain blood group A activity (33). If this is true, *C. immitis* A-like polysaccharides may be adsorbed to the surface of host cells and cross-react with A antibodies to give false positivity. Certain instances of acquired B expression have been documented in persons with advanced age or disease (30). Also noted is cross-reactivity of anti-B antibodies with polysaccharides from *Escherichia coli* O_{86}, which can induce false B reactivity in infected persons (30). Hardy-Weinberg genotypic frequencies for the ABO system were in equilibrium among Hispanics, an observation that does not support these explanations.

Alternatively, the immune systems of persons with A phenotypes may not recognize the A-like coccidioidal antigens as foreign. This may allow infection to be established without immunologic challenge to these antigens, leading to increased risk for symptomatic disease. The lower frequencies of B phenotypes may reflect the increased frequency of A phenotypes in this study.

HLA Effects

When analyzing HLA allele and haplotype distributions across ethnic groups, one looks for unifying themes that might implicate a particular allele or haplotype in a universal role for susceptibility in all groups. Such a finding would suggest that the set of molecules themselves is functionally responsible for the observed associations. The specificity of class II molecules depends on the alleles of the heterodimer subunits, which differ in their combined ability to recognize antigenic peptides. HLA polymorphisms defining allelic variation generally occur in the amino acid sites found in or near the peptide binding groove, thus affecting the interaction of the HLA molecule with the peptide or with the T-cell receptor. Some HLA alleles bind and present particular antigens to T cells better than other alleles. In this context, persons who present coccidioidal antigens more effectively to T cells may be able to eliminate the fungus before the disease progresses to more severe forms.

We found that the DRB1*1301 allele was associated with increased risk for disease in comparisons of mild or severe cases versus controls among all three ethnic groups. However, the DQA1-DQB1 portions of the haplotypes associated with this allele and with risk for each ethnic group were not the same. This suggests that the DRB1*1301 allele itself might be responsible for increased risk for symptomatic disease, regardless of ethnic background. DRB1*1301 has previously been associated with reduced risk for perinatal HIV infection (34,35) and decreased risk for progression of perinatally acquired HIV disease (36).

For both Caucasians and African-Americans, the strength of the DRB1*1301 association increases when more specifically defined by the

DRB1-DQA1-DQB1 extended haplotype. For African-Americans with severe cases, this haplotype is *1301-*0102-*0501 (OR = 10.4, p = 0.003 vs. controls). Among Caucasians with severe cases, the extended haplotype is *1301-*0103-*0602 (OR = 35.0, p = 0.0003 vs. controls) and for mild cases is *1301-*0103-*0504 (OR = 18.1, p = 0.002 vs. controls). DQA1*0103 alone is associated with increased risk for disease. However, the haplotype *1301-*0103-*0603 is common among both cases and controls and neutral for CM risk. Among Hispanics with severe cases, the marker for severe disease rishk is the extended haplotype *1301-*0103-*0603 (OR = 3.6, p = 0.05 vs. controls). These data suggest that DRB1*1301-DQA1*0103 may predispose Caucasians to symptomatic infection, but this risk is modified by DQB1 alleles. DQB1*0603 may negate this risk in Caucasians, while *0504 decreases or *0602 increases the risk for progression to severe disseminated disease. This stepwise mechanism is not evident from results for the other ethnic groups.

For Caucasians and Hispanics with cases, DRB1*0301-DQB1*0201 was associated with lower risk for disease than among controls, consistent with a protective effect of this haplotype. The DRB1*0301 allele has been associated with autoimmune disease risk in Caucasians (37). Among African-Americans, the *1501-*0602 haplotype was associated with lower risk for severe disease.

Another common theme observed in these data is that differences can be attributed in several comparisons to rare alleles or haplotypes present in a higher proportion of cases (either mild or severe) than controls. Since they otherwise share no particular molecular features, this may represent absence of alleles that specifically protect from infection by *C. immitis*.

Lack of consistency for HLA associations across ethnic groups does not preclude involvement of these alleles in specific populations, as HLA effects may produce disease susceptibility across ethnic groups as well as within one ethnic group. For example, HLA class II haplotypes predispose to insulin-dependent diabetes mellitus among Caucasian and Japanese (38). Among Caucasians, DR3 haplotypes are highly significantly associated with disease. This is not true among the Japanese, because DR3 haplotypes are extremely rare in the Japanese population, in which the associated haplotypes are DR4 and DR9. Thus, HLA alleles and haplotypes may be associated with disease in only one ethnic group.

Future studies could better address the risk for symptomatic CM by recruiting controls who are resistant to disease, i.e., persons with positive coccidioidal antigen skin tests who were not hospitalized for their infections. This selection would identify a more suitable control group for comparison with patients who have either mild or severe forms of CM in investigating risk factors for disease progression.

Our data support the hypothesis that host genes, in particular HLA class II and ABO blood group, play a complex role in susceptibility to severity of CM. Identifying genes that influence risk for developing severe disseminated CM may aid prevention efforts by targeting persons at high risk for disseminated disease who should be monitored closely after potential exposures, and may also aid in study design to test potential vaccines for efficacy, once candidate vaccines become available (39-41).

Acknowledgments

We thank Arthur Reingold for academic support and critical review of the manuscript and Gretchen Rothrock for administrative support, Kirt Emery and Denise Rogers for local epidemiologic support, Kern Medical Center staff for clinical support, and David Lee, Kathy Schultz, and Thomas Featherston for technical support.

This work was done with support from the Centers for Disease Control and Prevention (CDC#584).

Dr. Louie is an epidemiologist and research scientist at the University of California, Berkeley, School of Public Health. Her areas of expertise are in genetic epidemiology and infectious diseases. Dr. Louie's current research focuses on genetic risk factors for AIDS, tuberculosis, and coccidioidomycosis, including aspects of host-pathogen genetic interaction.

References

1. Pappagianis D, Sun RK, Werner SB, Rutherford GW, Elsea RW, Miller GB, et al. Coccidioidomycosis—United States, 1991-1992. MMWR Morb Mortal Wkly Rep 1993;42:21-4.
2. Pappagianis D. Marked increase in cases of coccidioidomycosis in California: 1991, 1992, and 1993. Clin Infect Dis 1994;19 Suppl 1:S14-8.
3. Pappagianis D, Feldman G, Billimek M, Mascola L, Werner SB, Jackson RJ, et al. Coccidioidomycosis following the Northridge earthquake—California, 1994. MMWR Morb Mortal Wkly Rep 1994;43:194-5.
4. Jinadu BA, Welch G, Talbot R, Caldwell J, Johnson R, Blume D, et al. Coccidioidomycosis—California, 1991-1993. MMWR Morb Mortal Wkly Rep 1994;43:421-3.

5. Schneider E, Hajjeh RA, Spiegel RA, Jibson RW, Harp EL, Marshall GA, et al. A coccidioidomycosis outbreak following the Northridge, Calif, earthquake. JAMA 1997;277:904-8.
6. Galgiani JN, Cohen IM. Coccidioidomycosis in renal transplant recipients: blood group, diabetes, and tissue type as risk factors. Current chemotherapy and infectious disease. Proceedings of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy. Boston (MA): American Society for Microbiology; 1980. p. 992-4.
7. Cohen IM, Galgiani JN, Potter D, Ogden DA. Coccidioidomycosis in renal replacement therapy. Arch Intern Med 1982;142:489-94.
8. Galgiani JN. Coccidioidomycosis. West J Med 1993;159:153-71.
9. Pappagianis D. Epidemiology of coccidioidomycosis. In: McGinnis M, editor. Current topics in medical mycology. New York: Springer Verlag; 1988. p. 199-238.
10. Flynn NM, Hoeprich PD, Kawachi MM, Lee KK, Lawrence RM, Goldstein E, et al. An unusual outbreak of windborne coccidioidomycosis. N Engl J Med 1979;301:358-61.
11. Rosenstein N, Werner SB, Kao A, Emery K, Rogers D, Vugia D, et al. Coccidioidomycosis: risk factors for severe pulmonary and disseminated disease. Kern County, California. Proceedings of the 35th Annual Meeting of the Infectious Diseases Society of America; 1997 Sept 13-17**;** San Francisco, California; 1997.
12. Catanzaro A, Spitler LE, Moser KM. Cellular immune response in coccidioidomycosis. Cell Immunol 1975;15:360-71.
13. Cox RA, Vivas JR, Gross A, Lecara G, Miller E, Brummer E. In vivo and in vitro cell-mediated responses in coccidioidomycosis. I. Immunologic responses of persons with primary, asymptomatic infections. American Review of Respiratory Disease 1976;114:937-43.
14. Mosmann TR, Coffman RL. Heterogeneity of cytokine secretion patterns and functions of helper T cells. Adv Immunol 1989;46:111-47.
15. Scheer M, Opelz G, Terasaki P, Hewitt W. The association of disseminated coccidioidomycosis and histocompatibility type. Proceedings of the 13th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington: American Society for Microbiology; 1973.
16. Deresinski SC, Pappagianis D, Stevens DA. Association of ABO blood group and outcome of coccidioidal infection. Sabouraudia 1979;17:261-4.
17. Mourant AE, Kopec AC, Domaniewska-Sobczak K. The distribution of the human blood groups, and other polymorphisms. London: Oxford University Press; 1976. p. 230.
18. Barcellos LF. Genetic analysis of multiple sclerosis in Caucasian and Northern Chinese populations: An investigation of HLA and non-HLA loci. School of Public Health [dissertation]. Berkeley (CA): University of California; 1996.
19. Erlich HA, Zeidler A, Chang J, Shaw S, Raffel LJ, Klitz W, et al. HLA class II alleles and susceptibility and resistance to insulin dependent diabetes mellitus in Mexican-American families. Nat Genet 1993;3:358-64.
20. Just JJ, King MC, Thomson G, Klitz W. African-American HLA class II allele and haplotype diversity. Tissue Antigens 1997;49:547-55.
21. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
22. Erlich H, Bugawan T, Begovich AB, Scharf S, Griffith R, Saiki R, et al. HLA-DR, DQ and DP typing using PCR amplification and immobilized probes. Eur J Immunogenet 1991;18:33-55.
23. Carrington M, Miller T, White M, Gerrard B, Stewart C, Dean M, et al. Typing of HLA-DQA1 and DQB1 using DNA single-strand conformation polymorphism. Hum Immunol 1992;33:208-12.
24. Olerup O, Aldener A, Fogdell A. HLA-DQB1 and -DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. Tissue Antigens 1993;41:119-34.
25. Guo SW, Thompson EA. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 1992;48:361-72.
26. Vogel F, Motulsky AG. Human Genetics. Berlin: Springer Verlag; 1986. p. 130-1.
27. Baur MP, Danilovs J. Population genetic analysis of HLA-A, B, C, DR, and other genetic markers. In: Terasaki PI, editor. Histocompatibility testing 1980. Los Angeles: UCLA Tissue Typing Laboratory; 1980. p. 955-93.
28. Long JC, Williams RC, Urbanek M. An E-M algorithm and testing strategy for multiple-locus haplotypes. Am J Hum Genet 1995;56:799-810.
29. U.S. Department of Health and Human Services. United States 1996-1997 health and injury chartbook. Hyattsville (MD): The Department; 1997. Pub. no. (PHS) 97-1232.
30. Race RR, Sanger R. Blood groups in man. Oxford (PA): Blackwell Scientific Publications distributed by J. B. Lippincott; 1975.
31. Reid ME, Bird GW. Associations between human red cell blood group antigens and disease. Transfus Med Rev 1990;4:47-55.
32. Yamamoto F, Clausen H, White T, Marken J, Hakomori S. Molecular genetic basis of the histo-blood group ABO system. Nature 1990;345:229-33.
33. Gale D, Lockhart EA, Kimbell E. Studies of *Coccidioides immitis.* 4. The effect of previous injection with human red cell stromata of the ABO groups on infection of mice with *C. immitis.* J Infect Dis 1968;118:54-64.
34. Greggio NA, Cameran M, Giaquinto C, Zacchello F, Koroliuk D, Colizzi V. DNA HLA-DRB1 analysis in children of positive mothers and estimated risk of vertical HIV transmission. Dis Markers 1993;11:29-35.
35. Winchester R, Chen Y, Rose S, Selby J, Borkowsky W. Major histocompatibility complex class II DR alleles DRB1*1501 and those encoding HLA-DR13 are preferentially associated with a diminution in maternally transmitted human immunodeficiency virus 1 infection in different ethnic groups: determination by an automated sequence-based typing method. Proc Natl Acad Sci U S A 1995;92:12374-8.
36. Chen Y, Winchester R, Korber B, Gagliano J, Bryson Y, Hutto C, et al. Influence of HLA alleles on the rate of progression of vertically transmitted HIV infection in children: association of several HLA-DR13 alleles with long-term survivorship and the potential association of HLA-A*2301 with rapid progression to AIDS. Long-term survivor study. Hum Immunol 1997;55:154-62.

37. Tiwari JL, Terasaki PI, editors. HLA and disease associations. New York: Springer Verlag; 1985.
38. Serjeantson SW, Easteal S. Class II histocompatibility genes and insulin-dependent diabetes mellitus. Molecular Biology and Medicine 1989;6:219-26.
39. Kirkland TN, Finley F, Orsborn KI, Galgiani JN. Evaluation of the proline-rich antigen of *Coccidioides immitis* as a vaccine candidate in mice. Infect Immun 1998;66:3519-22.
40. Kirkland TN, Thomas PW, Finley F, Cole GT. Immunogenicity of a 48-kilodalton recombinant T-cell-reactive protein of *Coccidioides immitis*. Infect Immun 1998;66:424-31.
41. Zimmermann CR, Johnson SM, Martens GW, White AG, Zimmer BL, Pappagianis D. Protection against lethal murine coccidioidomycosis by a soluble vaccine from spherules. Infect Immun 1998;66:2342-5.

Abscesses due to *Mycobacterium abscessus* Linked to Injection of Unapproved Alternative Medication

Karin Galil,* Lisa A. Miller,† Mitchell A. Yakrus,* Richard J. Wallace Jr.,‡ David G. Mosley,§ Bob England,§ Gwen Huitt,¶ Michael M. McNeil,* and Bradley A. Perkins*

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; †Colorado Department of Public Health and Environment, Denver, Colorado, USA; ‡University of Texas Health Center, Tyler, Texas, USA; §Arizona Department of Health Services, Phoenix, Arizona, USA; and ¶National Jewish Medical and Research Center, Denver, Colorado, USA

An unlicensed injectable medicine sold as adrenal cortex extract (ACE*) and distributed in the alternative medicine community led to the largest outbreak of *Mycobacterium abscessus* infections reported in the United States. Records from the implicated distributor from January 1, 1995, to August 18, 1996, were used to identify purchasers; purchasers and public health alerts were used to identify patients. Purchasers and patients were interviewed, and available medical records were reviewed. Vials of ACE* were tested for mycobacterial contamination, and the product was recalled by the U.S. Food and Drug Administration. ACE* had been distributed to 148 purchasers in 30 states; 87 persons with postinjection abscesses attributable to the product were identified. Patient and vial cultures contained *M. abscessus* identical by enzymatic and molecular typing methods. Unusual infectious agents and alternative health practices should be considered in the diagnosis of infections that do not respond to routine treatment.

Almost half of the U.S. population uses some form of unconventional therapy, most without the knowledge of their physician (1). Although many alternative therapies are not directly associated with adverse outcomes, unlicensed injectable preparations may pose a significant health risk. Outbreaks due to alternative therapies are particularly challenging to detect, investigate, and control. The difficulty is compounded when the adverse outcome is an unusual infection with a prolonged incubation period.

We report on a multistate outbreak of postinjection abscesses associated with the use of an injectable product purported to contain adrenal cortex extract (ACE). The product was widely distributed in the alternative medicine community. ACE has been in use since 1895, when William Osler reported success with a glycerol extract of fresh adrenal tissue in the treatment of Addison disease (2). In the 1930s, ACE became commercially available for the diagnosis and treatment of Addison disease and other states of adrenal insufficiency. Synthetic formulations of adrenal hormones replaced ACE in general use. Although ACE never received U.S. Food and Drug Administration (FDA) approval, it remains in use by alternative medicine practitioners for such conditions as alcohol and drug withdrawal, allergies, inflammation, and stress management, as well as hypoglycemia and depression attributed to a state of "hypoadrenalism" (3,4).

The *Mycobacterium abscessus* outbreak was detected after two Denver-area physicians reported soft-tissue abscesses in patients who received injections of a product purported to contain ACE. We determined that the cause of the outbreaks was intrinsic contamination with *M. abscessus* of vials of the product from one distributor (this product is hereafter referred to as ACE*); ACE* was recalled by FDA. This article describes the investigation of the

Address for correspondence: Karin Galil, Centers for Disease Control and Prevention, 1600 Clifton Road, Mail Stop E61, Atlanta, Georgia 30333, USA; fax: 404-639-8616; e-mail: kcg7@cdc.gov.

distribution and purchasers of ACE*; the conditions treated with ACE*; and the occurrences of abscesses as well as the treatment and clinical course of affected patients.

Methods

Epidemiologic Investigation

Passive surveillance was enhanced, and patients and practitioners were alerted of health risks associated with ACE* (5). We requested invoices for purchases of ACE* from the implicated Arizona-based distributor for January 1, 1995, to August 18, 1996. Purchasers were contacted by telephone, informed of ACE*'s possible contamination, and asked to submit remaining unopened and opened vials for culture. A case-patient was defined as a person who had received an injection of ACE* between January 1, 1995, and August 18, 1996, and had developed an abscess at the site of injection.

Purchaser and Patient Questionnaires

A standardized questionnaire was used to collect information about the purchasers of ACE*, their use of the product, and any adverse outcomes. Purchasers who prescribed the product to others or were involved in further distribution were asked to identify persons who received ACE*.

Persons who received injections of ACE* were identified through the distribution list, health-care providers, and pharmacies that dispensed ACE* directly to patients. Other patients contacted health departments after learning of the outbreak through news reports. We completed a standardized questionnaire by reviewing the medical record (whenever possible) or by interviewing the patient on the telephone. When a practitioner declined to disclose the patient's identity, we interviewed the practitioner to complete the patient questionnaire. Data collected included demographic information; conditions treated with ACE*; dosage, route, and frequency of ACE* administration; location of ACE* injection, in a practitioner's office or at home; complications; dates of injections, abscess onset, and seeking of medical care; treatments given; and dates of abscess resolution. If dosage, frequency, or dates were not documented, the best estimate of the provider or patient was recorded. When only a range of doses, frequencies, or dates was given, the midpoint was selected.

Laboratory Investigation

All purchasers and patients contacted by CDC were asked to submit opened and unopened vials of ACE*. FDA also collected vials for analysis and submitted acid-fast isolates cultured from vials to CDC for identification and subtyping. Clinical laboratories also sampled vials for contamination and sent isolates from vials as well as patients to the National Jewish Medical and Research Center. Isolates identified as *M. abscessus* were forwarded to CDC for subtyping.

Mycobacterial cultures were performed by aspirating 5 ml of fluid from each vial of ACE*; 0.1 ml was plated onto Middlebrook and Cohn 7H10 agar with OADC enrichment (Difco Laboratories, Detroit, MI), and the remainder was used to inject 40 ml of Middlebrook 7H9 broth with ADC enrichment (Difco). Mycobacterial isolates were grown and identified by methods previously described (6,7).

Isolates were typed by multilocus enzyme electrophoresis (MEE) and pulsed-field gel electrophoresis (PFGE) (8,9). All isolates of mycobacteria and control strains were typed by MEE. PFGE was used to compare three randomly selected outbreak isolates of *M. abscessus* (two vial isolates, one patient isolate) and 27 random control isolates for their large restriction fragment patterns with the endonucleases *Xba I* and *Dra I* (9,10).

Selected isolates of *M. abscessus* were tested for susceptibility to amikacin, kanamycin, and tobramycin with disk diffusion; cefoxitin and minocycline with agar elution; and imipenem, ciprofloxacin, and clarithromycin with broth microdilution (11). Three outbreak isolates of *M. abscessus* and controls were tested for susceptibility to mercury (12).

The Manufacturing Process and the Product

An investigation of the manufacturing process of the product was undertaken by FDA (13). The product was analyzed at the FDA Forensic Chemistry Center in Cincinnati, Ohio.

Results

Abscesses Following ACE* Administration

We identified 140 persons (treated in 20 states) who received ACE* during the interval of interest. Persons who received ACE* injections were 15 to 77 years of age (median 43 years,

n = 131); 123 (88%) were women; 87 (62%) persons from 16 states had abscesses (Figure 1); 3% had other complications: fever and chills after injection (n = 2), acute reaction requiring intravenous fluids (n = 1), and a tender, swollen nodule at the site of a previous ACE* injection

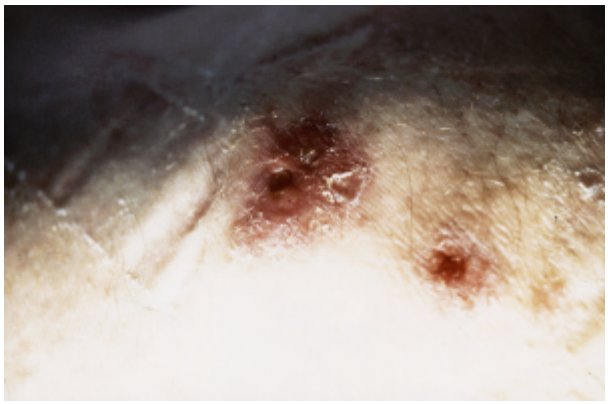


Figure 1. Abscesses due to *Mycobacterium abscessus* on the left hip of 64-year-old man who had injected (numerous times) a presumed adrenal cortex extract. The first lesion developed 9 weeks before this photograph was taken.

that did not meet our case definition (n = 1); 42 persons reported using ACE* without adverse effects (median follow-up 117 days, range 49-770 days); and data for 7 persons were lacking or insufficient. The Table compares case-patients with persons who reported that they did not develop an abscess after using ACE*. The dates of last injection of ACE* and onset of abscesses are shown in Figures 2 and 3, respectively. Most patients with abscesses had received intramuscular injections, though one had received an intravenous injection; most (n = 77, 89%) had received injections in a health-provider's office. Of the 35 case-patients who received only a single injection of ACE*, time to noticeable development of an abscess or documentation of the abscess in the medical record, whichever was earlier, was 4 to 149 days (median 32 days).

Treatment and Natural History of Abscesses

Sixty (69%) case-patients received medical care for the abscesses, and some received more than one type of therapy: 51 (59%) had incision

Table. Persons who received presumed adrenal cortex extract (ACE*) and reported an abscess and persons who received ACE* but did not develop an abscess, United States, January 1, 1995, to August 18, 1996

Patient and treatment characteristics	Persons with abscesses (n = 87)	Persons without abscesses (n = 42)
Median age (Range)	45 years (15-74)	46 years (20-77)
Sex		
Female	81 (93%)	35 (83%)
Male	6 (7%)	7 (17%)
Median dose (Range)	1 cc (1-2 cc)	1 cc (1-10 cc)
Dose frequency		
Single	35 (40%)	11 (26%)
Monthly	25 (29%)	10 (24%)
Source of injection		
Provider	77 (89%)	34 (81%)
Self/home	9 (10%)	8 (19%)
Both	1 (1%)	
Primary route of administration		
Intramuscular	86 (99%)	36 (86%)
Intravenous	1 (1%)	6 (14%)
Intramuscular site		
Gluteal	82 (95%)	29 (69%)
Other	4 (5%)	13 (31%)
Indication[a]		
Weight loss	61 (70%)	23 (55%)
Fatigue	11 (13%)	9 (21%)
Hypoadrenalism	9 (10%)	10 (24%)
Other	13 (15%)	8 (19%)

[a]Some persons received ACE* for more than one indication.

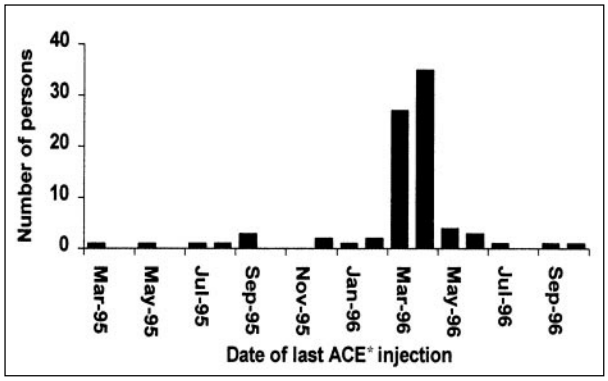


Figure 2. Dates of last injection of a presumed adrenal cortex extract among persons who developed postinjection *Mycobacterium abscessus* abscesses, United States, January 1995 to September 1996.

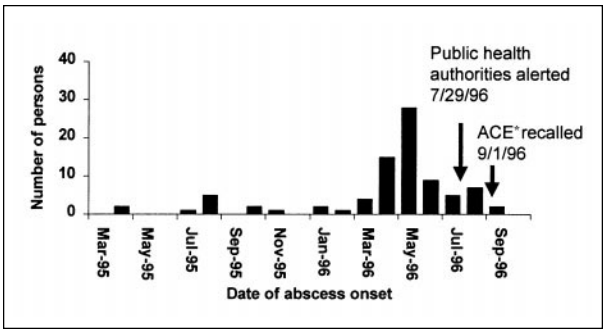


Figure 3. Dates of abscess onset in persons who had postinjection *Mycobacterium abscessus* abscesses after using a presumed adrenal cortex extract, United States, January 1995 to September 1996.

and drainage (14 more than once), 41 (47%) were prescribed an antibiotic, and 11 (13%) required surgical excision or plastic surgery. Abscesses were cultured for mycobacteria in 21 (24%) case-patients, 12 of whom were patients of the same practitioner. Treatment was delayed in many cases. Thirty-four (39%) case-patients received either an incision and drainage procedure or a prescription for an antibiotic active against *M. abscessus* within 6 weeks of developing a noticeable abscess. Treatment courses of clarithromycin, in this outbreak the most commonly prescribed antibiotic having activity against *M. abscessus* (14), lasted a median of 30 days, (range 4-210 days, n = 19). Data were not sufficient to allow a comparison of treatment regimens.

Time to abscess resolution was estimated by using the earliest reported or documented date of the complication and the date on which the abscess had resolved (on the basis of the medical record or the patient's interpretation of resolution). Follow-up data were available on 42 (48%) case-patients. Eleven (13%) patients reported complete resolution of abscesses during the study period. Abscesses lasted 31 to 428 days (median 167 days) in the 10 persons for whom we have dates of onset and resolution. Abscesses persisted in 31 (36%) case-patients (median follow-up interval 217 days, range 22-672 days, n = 30). The outcome of the remaining 45 case-patients could not be ascertained.

Administration of ACE* by Health-Care Providers

Of 103 health-care providers who purchased ACE*, 58 (56%) were medical doctors, 19 (18%) doctors of osteopathy, 17 (17%) alternative practitioners (9 naturopaths, 6 chiropractors, 1 practitioner of homeopathic medicine, 1 holistic practitioner); the qualifications of 9 (9%) providers could not be determined. Providers used ACE* most commonly for chronic fatigue syndrome (n = 39), "hypoadrenalism" (n = 34), immune system enhancement, or infection (n = 11). One physician used ACE* extensively for weight loss. Dose per injection was 0.1 to 15 cc (median 2 cc), most commonly as a weekly injection (n = 31 [36%]) but ranged from a single injection to daily injections. Providers (n = 80) estimated treating a median of 7 patients each with ACE* (1 to several hundred) in the preceding 2 years. In some cases the provider's reported practice differed markedly from the quantity of ACE* ordered. One practitioner reported treating 12 patients with ACE* with an average of 3.5 ml per dose on a weekly to monthly basis, but invoices recorded that 410 (30 ml) vials of ACE* were shipped to this practitioner over the 20-month period.

Most providers [n = 91] administered ACE* in their offices (n = 77 [85%]), although others provided ACE* to their patients for home administration. Providers (n = 89) injected ACE* intramuscularly (n = 38 [43%]), intravenously (n = 35 [39%]), subcutaneously (n = 2 [2%]), or by more than one route (n = 14 [16%]). When given intravenously, ACE* was commonly (n = 42 [47%]) mixed with other injectable preparations including vitamins, minerals, and (in a few cases) crude liver extract.

Laboratory Results

From at least 38 purchasers, CDC and FDA obtained 248 vials labeled ACE. Of these, 213 vials were tested for mycobacterial contamination (177 unopened and 36 opened vials). *M. abscessus* was cultured from 7 vials (6 unopened), *M. mucogenicum* from 17 unopened vials, and both from 1 unopened vial. The 11 patient isolates of *M. abscessus* were identical to the 8 vial isolates of *M. abscessus* by MEE but differed from control isolates. Three outbreak isolates identical by MEE (data not shown) and PFGE (Figure 4) differed from 27 control isolates. These 3 isolates were resistant to mercury (as were the isolates of *M. mucogenicum*) but susceptible to clarithromycin, imipenem, amikacin, and kanamycin; moderately susceptible to cefoxitin; and resistant to ciprofloxacin, tobramycin, and minocycline.

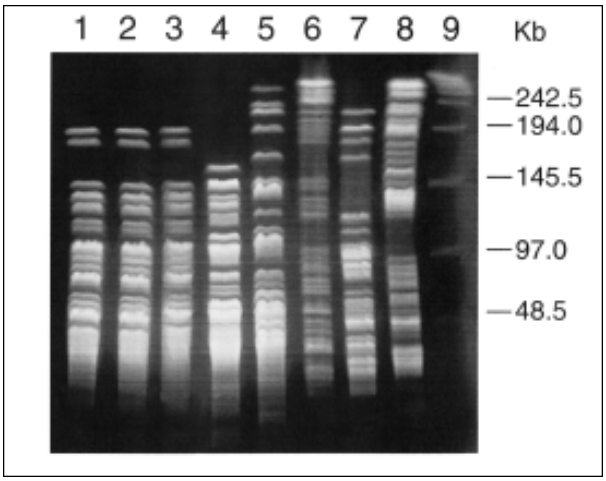


Figure 4. Pulsed-field gel electrophoresis patterns of three *Mycobacterium abscessus* isolates from the outbreak (lanes 1-3), five control isolates (lanes 4-8), and lambda DNA standards (lane 9). The chromosomal DNA was digested with *XbaI*.

Tracing ACE* Distribution

All implicated vials were from the same Arizona-based distributor. This distributor provided 337 invoices, representing shipment of 3,954 vials (each containing 30 ml of ACE*) to 148 purchasers between January 1, 1995, and August 18, 1996. ACE* was shipped to 30 states and two foreign countries. Of 146 U.S. purchasers, 103 (71%) were health-care providers, 8 (6%) were pharmacies or pharmaceutical companies, 11 (8%) were persons who purchased ACE* for self-administration; the remaining 24 (16%) could not be reached or declined to be interviewed. Purchasers received shipments of 1 to 200 vials (median 6). During the period of interest, one Dallas-based company received 13 shipments totaling 700 30-ml vials; the company declined to provide information about further distribution. We were able to trace 2,702 (68%) vials distributed to either health-care providers or persons who self-administered ACE*.

Manufacturing Process and Product Components

According to court documents, ACE* was manufactured in Florida under nonsterile conditions using a handwritten formula. The formula specified a low concentration of hydrocortisone mixed with the powdered preservative merthiolate (an organomercurial) dissolved in distilled water. Samples of the original distilled water were not available for testing. The product was placed in unlabeled vials and shipped to Montana, where labels bearing the names of nonexistent pharmaceutical companies were added. Vials contained 163 µg/ml to 234 µg/ml hydrocortisone and were found to contain mercury. There is no evidence that adrenal glands or other animal products were used to produce ACE* (13).

Discussion

Wide distribution of an unlicensed injectable preparation contaminated with *M. abscessus* led to a multistate outbreak of soft-tissue abscesses. The outbreak went undetected for longer than 1 year before cases were reported to public health authorities. Reporting led to a nationwide investigation and recall of the implicated product.

Nonsterile water has been suspected or implicated in most reported outbreaks of *M. abscessus*. Outbreaks have followed cardiac surgery (15), cosmetic surgery (16), podiatric procedures (17), invasive otologic procedures (18), and dialysis (19,20). In most outbreaks of postinjection abscesses due to rapidly growing nontuberculous mycobacteria, multidose vials appear to have become contaminated and served as a common source (21-23). Multidose vials of lidocaine were suspected in a recent large outbreak in which 350 patients given injections by an alternative medicine practitioner in Colombia developed abscesses or skin lesions (24,25). In this outbreak, distilled water used to dissolve the preservative merthiolate may have been the source of contamination. Although approximately 80% of *M. abscessus* isolates are susceptible to mercury (12), patient and vial isolates from this outbreak were uniformly resistant, which would explain their ability to grow in the presence of merthiolate. *M. mucogenicum* was cultured from unopened vials of ACE* but not from patient abscesses; however, in most cases abscesses were not cultured.

Despite a growing number of reports, *M. abscessus* and other mycobacteria remain an underappreciated cause of postinjection abscesses, particularly those of long incubation that are unresponsive to antibiotics active against more common bacterial species. If special culturing techniques and prolonged incubation of culture media are not performed, the diagnosis may not be established, and appropriate

treatment may be delayed or omitted. In this outbreak, mycobacteria were infrequently considered by the treating physician as a possible cause of the abscess. Only one fourth of case-patients were documented to have had mycobacterial cultures of an abscess; half of these patients were under the care of a single practitioner. Less than one third of patients with a postinjection abscess received an antibiotic active against mycobacteria, and most of those received an abbreviated course unlikely to have effected cure.

Randomized treatment trials of cutaneous disease, including localized abscesses, due to *M. abscessus* are lacking. Pretreatment isolates are susceptible to the oral antimicrobial agent clarithromycin (14), and most are susceptible to the parenterally administered antibiotics cefoxitin, imipenem, amikacin, and kanamycin (11); however, variations in resistance patterns make it imperative to determine the drug susceptibilities of each clinical isolate. In the largest reported outbreak of postinjection abscesses, the combination of surgical excision with clarithromycin appeared more efficacious (53% resolution at 3 months) than either treatment method alone (32% and 23%, respectively) (25). Optimal therapy would include two oral antimicrobial agents combined with incision and drainage. When only one oral antimicrobial agent is active against *M. abscessus*, combining incision and drainage with clarithromycin monotherapy may be practicable in cases of localized soft-tissue infection. This strategy was not associated with any adverse complications in this outbreak. Although monotherapy with clarithromycin has been shown to select for resistance in disseminated *M. chelonae* (26-29) and disseminated *M. abscessus* infections (29), the risk appears lower in localized disease. Clarithromycin should be continued for at least 3 to 6 months or longer depending on clinical response (25,28).

Tracing of contaminated vials of ACE* was hampered not only by the wide initial distribution and the secondary distribution by some recipients but also by the lack of lot numbers and expiration dates on the labels. We discovered 87 case-patients through distribution invoices and public alerts but this number probably underestimates the extent of the outbreak. The role of ACE* in the development of abscesses was underappreciated or overlooked by patients and their health-care providers: eight patients continued to receive injections of ACE* after abscesses developed; one of these received injections 8 months after the abscess formed.

Recent interest in assessing the efficacy of alternative medical practices in a scientifically rigorous manner (30,31) serves the public's health by differentiating between potentially beneficial medicines and ineffectual or dangerous compounds. This report underscores not only the dangers posed to public health by unlicensed products from manufacturers not following good manufacturing practices but also the delays that can result in investigating an outbreak due to such products. Unusual infectious agents and alternative health practices should be considered in the diagnosis of infections that do not respond to routine treatment.

Acknowledgments

We thank the many patients and health-care providers who provided information, William R. Mac Kenzie for his thoughtful review of the manuscript, Pam Lindholm-Levy for antimicrobial susceptibility testing, Barbara Brown for performing broth microdilution and mercury susceptibility testing, and Yansheng Zhang for performing PFGE. The investigation would not have been possible without the assistance of Lynn Weaver, the cooperation of FDA, and many state and local health departments. We also thank David Ashford, Ulana Bodnar, Kristy Bradley, Mary Anne Caine, Lisa Cairns, Debra Chew, Sara Cody, Todd Damrow, David Dassey, Annie Fine, Jesse Greenblatt, Richard Hoffman, Daniel Jernigan, Daniel Jorgensen, J.P. Lofgren, Laurene Mascola, Gayle Miller, Craig Nichols, Pekka Nuorti, Barbara Ray, Larry Shireley, Kathleen Toomey, Andre Weltman, and Kenneth Wilcox.

Dr. Galil is a medical epidemiologist with the National Immunization Program, CDC.

References

1. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, et al. Trends in alternative medicine use in the United States. JAMA 1988;280:1569-75.
2. Osler W. On six cases of Addison's disease with the report of a case greatly benefited by the use of suprarenal extract. International Medical Magazine 1895;4:3-11.
3. Eggleston C, Weiss S. Adrenal cortical hormone therapy. Am J Med Sci 1939;197:718-29.
4. Hypoglycemia Association. 1998. Bulletin #96. Available from: URL: http://fred.net/slowup/hai96.html.
5. Infection with *Mycobacterium abscessus* associated with intramuscular injection of adrenal cortex extract—Colorado and Wyoming, 1995-1996. MMWR Morb Mortal Wkly Rep 1996;45:713-5.
6. Smithwick RW, Bigbie MR Jr, Ferguson RB, Karlix MA, Wallis CK. Phenolic acridine orange fluorescent stain for mycobacteria. J Clin Microbiol 1995;33:2763-4.

7. Butler WR, Jost KC Jr, Kilburn JO. Identification of mycobacteria by high-performance liquid chromatography. J Clin Microbiol 1991;29:2468-72.
8. Yakrus MA, Reeves MW, Hunter SB. Characterization of isolates of *Mycobacterium avium* serotypes 4 and 8 from patients with AIDS by multilocus enzyme electrophoresis. J Clin Microbiol 1992;30:1474-8.
9. Wallace RJ Jr, Zhang Y, Brown BA, Fraser V, Mazurek GH, Maloney S. DNA large restriction fragment patterns of sporadic and epidemic nosocomial strains of *Mycobacterium chelonae* and *Mycobacterium abscessus*. J Clin Microbiol 1993;31:2697-701.
10. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233-9.
11. Swenson JM, Wallace RJ Jr, Silcox VA, Thornsberry C. Antimicrobial susceptibility of five subgroups of *Mycobacterium fortuitum* and *Mycobacterium chelonae* [published erratum appears in Antimicrob Agents Chemother 1986 Apr;29:720]. Antimicrob Agents Chemother 1985;28:807-11.
12. Steingrube VA, Wallace RJ Jr, Steele LC, Pang YJ. Mercuric reductase activity and evidence of broad-spectrum mercury resistance among clinical isolates of rapidly growing mycobacteria. Antimicrob Agents Chemother 1991;35:819-23.
13. U.S. District Court, Southern District of Florida. Complaint for preliminary and permanent injunction. Miami (FL): The Court; 1997. CIV-Case 97-1083.
14. Brown BA, Wallace RJ Jr, Onyi GO, De Rosas V, Wallace RJ III. Activities of four macrolides, including clarithromycin, against *Mycobacterium fortuitum, Mycobacterium chelonae*, and *M. chelonae*-like organisms. Antimicrob Agents Chemother 1992;36:180-4.
15. Kuritsky JN, Bullen MG, Broome CV, Silcox VA, Good RC, Wallace RJ Jr. Sternal wound infections and endocarditis due to organisms of the *Mycobacterium fortuitum* complex. Ann Intern Med 1983;98:938-9.
16. Safranek TJ, Jarvis WR, Carson LA, Cusick LB, Bland LA, Swenson JM, et al. *Mycobacterium chelonae* wound infections after plastic surgery employing contaminated gentian violet skin-marking solution. N Engl J Med 1987;317:197-201.
17. Wenger JD, Spika JS, Smithwick RW, Pryor V, Dodson DW, Carden GA, et al. Outbreak of *Mycobacterium chelonae* infection associated with use of jet injectors. JAMA 1990;264:373-6.
18. Lowry PW, Jarvis WR, Oberle AD, Bland LA, Silberman R, Bocchini JA Jr, et al. *Mycobacterium chelonae* causing otitis media in an ear-nose-and-throat practice. N Engl J Med 1988;319:978-82.
19. Bolan G, Reingold AL, Carson LA, Silcox VA, Woodley CL, Hayes PS, et al. Infections with *Mycobacterium chelonei* in patients receiving dialysis and using processed hemodialyzers. J Infect Dis 1985;152:1013-9.
20. Lowry PW, Beck-Sague CM, Bland LA, Aguero SM, Arduino MJ, Minuth AN, et al. *Mycobacterium chelonae* infection among patients receiving high-flux dialysis in a hemodialysis clinic in California. J Infect Dis 1990;161:85-90.
21. Inman PM, Beck A, Brown AE, Stanford JL. Outbreak of injection abscesses due to *Mycobacterium abscessus*. Arch Dermatol 1969;100:141-7.
22. Borghans JG, Stanford JL. *Mycobacterium chelonei* in abscesses after injection of diphtheria-pertussis-tetanus-polio vaccine. American Review of Respiratory Disease 1973;107:1-8.
23. Gremillion DH, Mursch SB, Lerner CJ. Injection site abscesses caused by *Mycobacterium chelonei*. Infection Control 1983;4:25-8.
24. Camargo D, Saad C, Ruiz F, Ramirez ME, Lineros M, Rodriguez G, et al. Iatrogenic outbreak of *M. chelonae* skin abscesses. Epidemiol Infect 1996;117:113-9.
25. Villanueva A, Calderon RV, Vargas BA, Zhang Y, Sander P, Onyi GO, et al. Report on an outbreak of postinjection abscesses due to *Mycobacterium abscessus*, including management with surgery and clarithromycin therapy and comparison of strains by random amplified polymorphic DNA polymerase chain reaction. Clin Infect Dis 1997;24:1147-53.
26. Tebas P, Sultan F, Wallace RJ Jr, Fraser V. Rapid development of resistance to clarithromycin following monotherapy for disseminated *Mycobacterium chelonae* infection in a heart transplant patient. Clin Infect Dis 1995;20:443-4.
27. Driscoll MS, Tyring SK. Development of resistance to clarithromycin after treatment of cutaneous *Mycobacterium chelonae* infection. Journal of the American Academy of Dermatology 1997;36:495-6.
28. Wallace RJ Jr, Tanner D, Brennan PJ, Brown BA. Clinical trial of clarithromycin for cutaneous (disseminated) infection due to *Mycobacterium chelonae*. Ann Intern Med 1993;119:482-6.
29. Wallace RJ Jr, Meier A, Brown BA, Zhang Y, Sander P, Onyi GO, et al. Genetic basis for clarithromycin resistance among isolates of *Mycobacterium chelonae* and *Mycobacterium abscessus*. Antimicrob Agents Chemother 1996;40:1676-81.
30. Alternative medicine: a report to the National Institutes of Health on alternative medical systems and practices in the United States, Workshop on Alternative Medicine; 1992 Sep 14-16; Chantilly, Virginia. Washington: U.S. Government Printing Office; 1993.
31. Villaire M. Centers provide OAM with 'deliverables'. Altern Ther Health Med 1997;3:20-4.

Tracking Drug-Resistant *Streptococcus pneumoniae* in Oregon: An Alternative Surveillance Method

Arthur E. Chin,*† Katrina Hedberg,* Paul R. Cieslak,* Maureen Cassidy,* Karen R. Stefonek,* and David W. Fleming*
*Oregon Health Division, Portland, Oregon, USA; and †Centers for Disease Control and Prevention, Atlanta, Georgia, USA

With the emergence of drug-resistant *Streptococcus pneumoniae,* community-specific antimicrobial susceptibility patterns have become valuable determinants of empiric therapy for *S. pneumoniae* infections. Traditionally, these patterns are tracked by active surveillance for invasive disease, collection of isolates, and centralized susceptibility testing. We investigated whether a simpler and less expensive method—aggregating existing hospital antibiograms—could provide community-specific antimicrobial susceptibility data. We compared 1996 active surveillance data with antibiogram data from hospital laboratories in Portland, Oregon. Of the 178 *S. pneumoniae* active surveillance isolates, 153 (86% [95% confidence interval (CI) = 80% to 91%]) were susceptible to penicillin. Of the 1,092 aggregated isolates used by hospitals to generate antibiograms, 921 (84% [95% CI = 82%-87%]) were susceptible to penicillin. With the exception of one hospital's erythromycin susceptibility results, hospital-specific *S. pneumoniae* susceptibilities to penicillin, cefotaxime, trimethoprim-sulfamethoxazole, and erythromycin from the two methods were statistically comparable. Although yielding fewer data than active surveillance, antibiograms provided accurate, community-specific drug-resistant *S. pneumoniae* data in Oregon.

Streptococcus pneumoniae infections are a major cause of illness and death worldwide. Penicillin-resistant *S. pneumoniae* were first described in 1967 (1). Since then, the proportions of isolates resistant to penicillin and other antimicrobial agents have increased worldwide (2-5). In the United States, the combined percentage of *S. pneumoniae* isolates with either intermediate (MIC = 0.1-1.0 μg/ml) or high (MIC 2.0 μg/ml) levels of penicillin resistance is higher than 60% in some areas (5). Strains with multidrug resistance to penicillins, macrolides, sulfonamides, and third-generation cephalosporins have been well documented (3,5-9). Despite the increasing proportion of drug-resistant *S. pneumoniae* and the importance of knowing the drug resistance status in determining empiric therapy, community-specific surveillance for drug-resistant *S. pneumoniae* is limited and its proportion is unknown in many areas (10,11).

Address for correspondence: Arthur E. Chin, 63 Indian Mountain Road, Lakeville, CT 06039, USA; fax: 860-364-4427; e-mail: gchin@javanet.com <mailto:gchin@javanet.com.

Active surveillance for invasive *S. pneumoniae* disease includes collection of isolates, centralized susceptibility testing, and collection of patient data (4).

Although such a resource-intensive system for providing community-specific and case-specific *S. pneumoniae* data is beyond the means of most local and many state health agencies, hospital-specific data already exist in many areas. Many hospital laboratories perform antimicrobial susceptibility testing on *S. pneumoniae* isolates from sterile and nonsterile sites, and results are often tabulated for local clinicians in a summary table called an antibiogram. Antibiogram data represent invasive and noninvasive *S. pneumoniae* disease isolates collected from normally sterile and nonsterile sites; may include multiple isolates from the same patient; and are based on hospital laboratory antimicrobial susceptibility testing, a process that may differ between laboratories. In contrast, *S. pneumoniae* active surveillance data are limited to invasive disease isolates collected

from normally sterile sites, specifically exclude duplicate isolates collected from the same patient, and are based on a centralized and standardized susceptibility testing protocol.

We examined preexisting antibiogram data to assess if they could provide local health agencies with an accurate, inexpensive means of estimating the community-specific proportion of drug-resistant *S. pneumoniae*. The Oregon Health Division performs active surveillance for drug-resistant *S. pneumoniae* through a cooperative agreement with the Centers for Disease Control and Prevention's Emerging Infections Program. We conducted a cross-sectional survey of the 12 hospital laboratories that serve the Portland Tri-County area (Multnomah, Washington, and Clackamas counties, population 1.2 million) and compared 1996 Portland *S. pneumoniae* susceptibility results and costs of the aggregated antibiogram surveillance system with the *S. pneumoniae* susceptibility results and costs of our active surveillance system. We determined the community-specific proportion of *S. pneumoniae* susceptible to penicillin and performed a limited analysis of hospital-specific susceptibilities to cefotaxime, trimethoprim-sulfamethoxazole, and erythromycin.

Methods

Active Surveillance

Case Definition

Our goal was to determine the proportion of drug-resistant isolates among all *S. pneumoniae* isolates collected by the active surveillance system in 1996. Therefore, an active surveillance case was defined as an *S. pneumoniae* isolate from a normally sterile site collected from a Portland Tri-County resident in 1996 and analyzed at a Portland Tri-County hospital microbiology laboratory.

Surveillance Protocol

All Portland-area hospital microbiology laboratories were asked to send all *S. pneumoniae* sterile-site isolates from both inpatients and outpatients to the Oregon State Public Health Laboratory. Health Department staff regularly contacted each laboratory to assess interim isolate recovery rates and to encourage ongoing participation in the surveillance system and (twice a year) performed on-site laboratory audits to compare the number of patients with invasive *S. pneumoniae* infections with the number of isolates submitted to the state laboratory.

To avoid duplication, only one isolate from each patient was sent to the reference laboratory, even if multiple isolates were obtained from the same person. Isolates were sent twice a year from the Oregon State Public Health Laboratory to a national reference laboratory for antimicrobial susceptibility testing by National Committee for Clinical Laboratory Standards broth microdilution protocols (12). *S. pneumoniae* antimicrobial-susceptibility percentages for Portland were calculated from the national reference laboratory results. Invasive cases did not have reference laboratory susceptibility testing if the hospital laboratory did not forward the isolate to the Oregon State Public Health Laboratory or if the isolates received by the Oregon or the reference laboratory were not viable.

Cost Calculations

Annual costs for this surveillance system included direct and indirect health department staff costs and the expense of isolate storage, processing, and transport incurred by the Oregon State Public Health Laboratory and the national reference laboratory. Hospital laboratory isolate testing, which would have been performed regardless of our request for surveillance data, were not included in these calculations. Time calculations included laboratory audits, patient chart reviews, data entry and analysis, coordination of isolate movement, and communication among hospital laboratories, the health department, the state public health laboratory and the reference laboratory.

Antibiogram Surveillance

Case Definition

An antibiogram case was defined as any *S. pneumoniae* isolate identified in 1996 by a Portland Tri-County hospital microbiology laboratory that was tabulated on the respective 1996 *S. pneumoniae* antibiogram. Specimens were submitted from inpatients and outpatients and from sterile and nonsterile sites.

Surveillance Protocol

We requested antibiograms from all 12 Portland Tri-County hospital laboratories.

Antibiogram data were aggregated to produce antimicrobial susceptibility percentages for the Portland area. All susceptibility testing was performed at individual hospital laboratories. We did not routinely survey laboratory techniques or reporting criteria, nor did the Oregon State Public Health Laboratory perform confirmation susceptibility testing of any hospital isolates.

Cost Calculations

The cost of the antibiogram method included direct and indirect health department staff expenses but excluded the cost of hospital laboratory isolate testing, a process performed regardless of our surveillance requests. Time calculations included staff time spent requesting antibiograms and performing data entry and analysis.

Statistical Methods

The Mantel-Haenzel chi-square and Fisher's exact tests were used to compare the proportions of susceptible *S. pneumoniae* isolates determined by the two surveillance methods. P values ≤0.05 were considered statistically significant. Statistical calculations were performed by using Epi-Info (Epi-Info version 6.04b; Centers for Disease Control and Prevention, Atlanta, GA).

Findings

Penicillin

Of the 12 Portland-area hospital laboratories participating in the active surveillance system, 10 (83%) submitted isolates to our active surveillance system in 1996. One hospital (A) had no sterile-site isolates in 1996. A second hospital (F) had two sterile-site isolates but did not submit them to the state laboratory. Of 266 invasive *S. pneumoniae* infections identified by health department staff through audits, 178 (67%) *S. pneumoniae* isolates were tested by the reference laboratory. Of the 88 identified cases that were not analyzed by the reference laboratory, in 81 cases the hospital did not submit an isolate to the state laboratory, and in 7 the isolate submitted was not viable. The number of isolates collected from each hospital was 2 to 59 (Table 1). The mean and median numbers of active surveillance isolates collected per hospital were 18 and 9.5, respectively. Of the 178 isolates tested, 153 (86% [95% CI = 80% to 91%]) were susceptible to penicillin (MIC 0.06 μg/ml).

Table 1. *Streptococcus pneumoniae* penicillin susceptibility as determined by two surveillance methods, Portland, Oregon, 1996

	Active surveillance			Antiobiograms		
	No.	Susceptible		No.	Susceptible	
Hospital	isolates	N	%	isolates	N	%
A	[a]			20	17	85
B	34	29	85	134	112	84
C	59	52	88	274	227	83
D	6	4	67	120	89	74
E	12	11	92	41	34	83
F	[a]			61	58	95
G	33	28	85	110	100	91
H	11	10	91	161	137	85
I	8	6	75	64	56	88
J	7	6	86	107	91	85
K	6	5	83	[b]		
L	2	2	100	[b]		
Total	178	153	86	1,092	921	84

[a]No isolates submitted to the Oregon Public Health Laboratory.
[b]Antibiogram data not available from hospital.

Penicillin antibiogram data were collected from 10 (83%) of 12 Portland-area hospitals (Table 1). Eight of the 10 hospitals listed only the proportion of susceptible *S. pneumoniae* isolates on their antibiogram and did not specify the number of intermediate- or high-resistance isolates. Of the aggregated 1,092 *S. pneumoniae* isolates used by Portland-area hospitals to generate penicillin antibiogram data, 921 (84% [95% CI = 82% to 86%]) were listed as susceptible to penicillin.

The proportion of penicillin-susceptible isolates at each hospital was 67% to 100% by the active surveillance method and 74% to 95% by antibiogram data (Figure). The median hospital-specific difference between the two methods

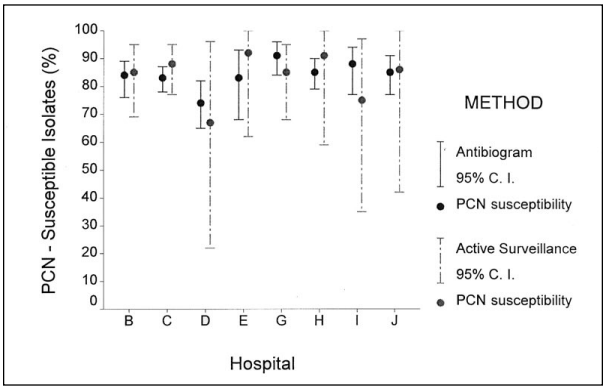


Figure. Hospital-specific *Streptococcus pneumoniae* penicillin-susceptibility determined by antibiogram and active surveillance, Portland, Oregon, 1996.

was 6%. In no instance did hospital-specific penicillin-susceptibility estimates from the two methods differ statistically (p >0.05). We found no statistical difference between the overall *S. pneumoniae* penicillin-susceptibility proportion determined by active surveillance and by the antibiogram method (p >0.05).

Other Antibiotics

We compared active surveillance and antibiogram *S. pneumoniae* susceptibilities to cefotaxime, trimethoprim-sulfamethoxazole, and erythromycin (Table 2). Of the 178 isolates collected and tested through the active surveillance system, 165 (93% [95% CI = 88% to 96%]) were susceptible to cefotaxime (MIC 0.50 µg/ml), 141 (79% [95% CI = 73% to 85%]) were susceptible to trimethoprim (MIC 0.50 µg/ml)-sulfamethoxazole (MIC 9.50 µg/ml), and 169 (95% [95% CI = 91% to 98%]) were susceptible to erythromycin (MIC 0.50 µg/ml).

In hospitals where antibiogram data were available for cefotaxime, trimethoprim-sulfamethoxazole, and erythromycin, 539 (94%) of 575 aggregated isolates (95% CI = 91% to 95%) were susceptible to cefotaxime, 251 (84%) of 300 isolates (95% CI = 79% to 88%) were susceptible to trimethoprim-sulfamethoxazole, and 649 (86%) of 751 isolates (95% CI = 84% to 89%) were susceptible to erythromycin. Hospital-specific antibiogram and active surveillance data from four institutions were available for direct comparison for cefotaxime and from three institutions for trimethoprim-sulfamethoxazole. In each instance, the hospital-specific proportion of *S. pneumoniae* isolates susceptible to cefotaxime or trimethoprim-sulfamethoxazole did not differ significantly by surveillance method. We were able to directly compare *S. pneumoniae* erythromycin susceptibility by antibiogram and active surveillance at six hospitals. The proportions of erythromycin-susceptible *S. pneumoniae* isolates determined by each surveillance method were statistically comparable in five of the six hospitals (p >0.05). One hospital (C) had a significantly higher proportion (p = 0.01) of erythromycin-susceptible isolates determined by active surveillance (97% [95% CI = 88% to 100%]) than reported by the corresponding antibiogram (84% [95% CI = 79% to 88%]).

Cost Comparison

The antibiogram survey required 20 hours of health department staff time, for a total cost of $700: $650 for personnel expenses and $50 for miscellaneous support expenses. The active surveillance method required 570 hours of staff time and cost $52,000: $40,000 for direct and indirect personnel expenses and $12,000 for laboratory costs.

Conclusions

Accurate, community-specific drug-resistant *S. pneumoniae* data are important for several reasons. First, most outpatient illnesses caused

Table 2. *Streptococcus pneumoniae* susceptibility to cefotaxime, trimethoprim-sulfamethoxazole, and erythromycin, by two surveillance methods, Portland, Oregon, 1996

	Cefotaxime						Trimethoprim-Sulfamethoxazole						Erythromycin					
	Active surveillance			Antibiograms			Active surveillance			Antiobiograms			Active surveillance			Antiobiograms		
Hospital	No. isolates	Susceptible N	Susceptible %	No. isolates	Susceptible N	Susceptible %	No. isolates	Susceptible N	Susceptible %	No. isolates	Susceptible N	Susceptible %	No. isolates	Susceptible N	Susceptible %	No. isolates	Susceptible N	Susceptible %
A	[a]			[b]			[a]			[b]			[a]			[b]		
B	34	32	94	10	8	80	34	26	76	[b]			34	33	97	134	126	94
C	59	54	92	274	255	93	59	49	83	274	230	84	59	57	97	274	230	84
D	6	4	67	120	112	93	6	3	50				6	5	83	120	88	73
E	12	12	100	[b]			12	9	75	16	13	81	12	12	100	42	39	93
F	[a]			61	59	97	[a]			[b]			[a]			61	56	92
G	33	31	94	110	105	95	33	27	82	[b]			33	30	91	110	100	91
H	11	11	100	-			11	10	91	[b]			11	11	100	[b]		
I	8	7	88	-			8	6	75	[b]			8	8	100	[b]		
J	7	6	86	-			7	5	71	10	8	80	7	6	86	10	10	100
K	6	6	100	-			6	4	67	[b]			6	5	83	[b]		
L	2	2	100	-			2	2	100	[b]			2	2	100	[b]		
Total	178	165	93	575	539	94	178	141	79	300	251	84	178	169	95	751	649	86

[a]Isolates submitted to the Oregon Public Health Laboratory.
[b]Antibiogram data not available from hospital.

by *S. pneumoniae* are treated empirically, without identification of the organism. Community-specific data may be a valuable determinant of empiric therapy for these infections and of initial empiric therapy for invasive disease. Second, communities with a high percentage of drug-resistant *S. pneumoniae* may benefit from efforts to reduce inappropriate antibiotic prescriptions. Increased drug-resistant *S. pneumoniae* carriage is directly related to antibiotic therapy, and reduced antimicrobial use in the community can decrease rates of antimicrobial resistance (13-16). Finally, clinicians in areas with a low percentage of drug-resistant *S. pneumoniae* and minimal penicillin resistance might gain confidence in treating presumptive outpatient infections with empiric penicillin therapy, thereby reducing the risk for multidrug resistance.

Despite the clinical and public health importance of drug-resistant *S. pneumoniae* surveillance, community-specific surveillance data are not uniformly available. A 1996 study determined that 54% of states either conducted or were planning to implement surveillance for drug-resistant *S. pneumoniae* by June 1997 (17). Our study supports the usefulness of *S. pneumoniae* antibiogram data, commonly available at many hospitals, in estimating the community-specific proportion of penicillin-susceptible *S. pneumoniae*. In no instance did hospital-specific penicillin susceptibility estimates from the two methods differ statistically. More importantly, the overall Portland penicillin susceptibility proportions determined by the active surveillance and antibiogram methods were statistically comparable.

Antibiogram data also hold promise for estimating *S. pneumoniae* susceptibilities to other antimicrobial drugs. The hospital-specific proportion of *S. pneumoniae* isolates susceptible to cefotaxime and trimethoprim-sulfamethoxazole did not differ for the two methods in hospitals where comparisons were possible. The erythromycin susceptibility proportions by antibiogram and active surveillance were statistically comparable at each of the hospitals for which erythromycin data were available, except for hospital C. The reason for this discordance is not clear but may be influenced by statistical chance.

Time and financial requirements for the antibiogram method were minimal and probably within reach of many local health departments. Laboratory effort was limited to mailing a current antibiogram to the health department. However, the antibiogram method can only estimate the proportion of drug-resistant *S. pneumoniae* in a community. The active surveillance system collects patient-specific (e.g., risk factors, demographics) and infection-specific information, permits serotyping and molecular analysis of isolates, provides data on the actual *S. pneumoniae* disease effect in the population, permits evaluation of targeted vaccination campaigns and antimicrobial guideline efforts, provides specific MIC data for a range of antimicrobial agents, and allows for validation of alternative surveillance methods.

Prior surveillance studies have documented equal or greater proportions of penicillin-resistant isolates collected from nonsterile sites than from sterile sites (18,19). Our active surveillance system captures only isolates from sterile sites collected from invasive *S. pneumoniae* disease. Most isolates in the antibiograms were from noninvasive diseases and nonsterile sites. Our study showed no statistical difference between the proportion of penicillin-susceptible *S. pneumoniae* determined by either method and therefore no difference between the penicillin-susceptible proportion of invasive and noninvasive isolates. Ninety-six percent of our active surveillance isolates were from outpatients or inpatients hospitalized less than 48 hours and are unlikely to represent nosocomial infections. These data support the traditional epidemiologic characterization of *S. pneumoniae* as a community rather than nosocomially acquired organism.

Several potential limitations deserve comment. The active surveillance system had a case-isolate recovery rate of 67%. The current performance indicator for the active surveillance system, instituted in 1998, is a case-isolate recovery rate of 85% (A. Schuchat, pers. comm.) We were unable to characterize the susceptibilities of the missing isolates, which may have biased our active surveillance results. Antibiograms were tabulated from all isolates submitted to a particular hospital laboratory. Multiple isolates from a single patient may have disproportionately influenced these results. Unlike the active surveillance system, in which chart reviews excluded nonresidents, antibiogram data may have included isolates from patients who were not Portland-area residents and should not have been included in Portland *S. pneumoniae* antimicrobial-susceptibility results. We were unable to estimate the number of duplicate

isolates or non-Portland-area residents in our antibiogram data.

This study suggests that antibiogram data already available in hospitals may be useful in estimating the community-specific proportion of drug-resistant *S. pneumoniae*. We recommend further validation of these results at sites where active surveillance and antibiogram data can be directedly compared. The most effective use of antibiogram drug-resistant *S. pneumoniae* surveillance may require that hospitals routinely and consistently perform *S. pneumoniae* susceptibility testing to multiple antimicrobial drugs. Communities considering this surveillance method may need to work with local hospitals to develop a cost-effective susceptibility testing regimen. Although yielding less information than active surveillance, antibiogram surveillance might be most useful in communities where hospital antibiogram data are available but more intensive surveillance is limited by a lack of financial or personnel resources.

Acknowledgments

We thank the laboratory directors, managers, and staff at the Oregon State Public Health Laboratory and the Portland Tri-County hospital microbiology laboratories for their work in this study and Drs. Anne Schuchat, Thomas Van Gilder, and Andrew Pelletier for manuscript review.

This study was funded, in part, by a cooperative agreement between the Oregon Health Division and the Centers for Disease Control and Prevention's Emerging Infections Program.

At the time this study was performed, Dr. Chin was an Epidemic Intelligence Service Officer with the Centers for Disease Control and Prevention, assigned to the Oregon Health Division. He currently practices emergency medicine in Sharon, Connecticut.

References

1. Hansman D, Bullen MM. A resistant pneumococcus. Lancet 1967;July 29, 1967:264-5.
2. Breiman RF, Butler JC, Tenover FC, Elliott JA, Facklam RR. Emergence of drug resistant-pneumococcal infections in the United States. JAMA 1994;271:1831-5.
3. Friedland IR, McCracken GH. Management of infections caused by antibiotic-resistant *Streptococcus pneumoniae*. N Engl J Med 1994;331:377-82.
4. Butler JC, Hofmann J, Cetron MS, Elliott JA, Facklam RR, Breiman RF. The continued emergence of drug-resistant *Streptococcus pneumoniae* in the United States: an update from the Centers for Disease Control and Prevention's pneumococcal sentinel surveillance system. J Infect Dis 1996;174:986-93.
5. Doern GV, Pfaller MA, Kugler K, Freeman J, Jones RN. Prevalence of antimicrobial resistance among respiratory tract isolates of *Streptococcus pneumoniae* in North America: 1997 results from the SENTRY antimicrobial surveillance program. Clin Infect Dis 1998;27:764-70.
6. Centers for Disease Control and Prevention. Drug-resistant *Streptococcus pneumoniae*—Kentucky and Tennessee, 1993. MMWR Morb Mortal Wkly Rep 1994;43:23-6.
7. Friedland IR, Shelton S, Paris M, Rinderknecht S, Ehrett S, Krisher K, et al. Dilemmas in diagnosis and management of cephalosporin-resistant *Streptococcus pneumoniae* meningitis. Pediatr Infect Dis J 1993;12:196-200.
8. Kleiman MB, Weinberg GA, Reynolds JK, Allen SD. Meningitis with beta-lactam-resistant *Streptococcus pneumoniae*: the need for early repeat lumbar puncture. Pediatr Infect Dis J 1993;12:782-4.
9. Frick PA, Black DJ, Duchin JS, Deliganis S, McKee WM, Fritsche TR. Prevalence of antimicrobial drug-resistant *Streptococcus pneumoniae* in Washington State. West J Med 1998;169:364-9.
10. Jernigan DB, Cetron MS, Breiman RF. Minimizing the impact of drug-resistant *Streptococcus pneumoniae* (DRSP): a strategy from the DRSP working group. JAMA 1996;275:206-9.
11. Centers for Disease Control and Prevention. Defining the public health impact of drug-resistant *Streptococcus pneumoniae*: report of a working group. MMWR Morb Mortal Wkly Rep 1996;45(RR-1):1-20.
12. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 4th ed. Approved standard. NCCLS [document M7-A4]. Vol 17. Wayne (PA): The Committee; 1997.
13. Reichler MR, Allphin AA, Breiman RF, Schreiber JR, Arnold JE, McDougal LK, et al. The spread of multiply resistant *Streptococcus pneumoniae* at a day care center in Ohio. J Infect Dis 1992;166:1346-53.
14. Pallares R, Gudiol F, Linares J, Ariza J, Rufi G, Murgui L, et al. Risk factors and response to antibiotic therapy in adults with bacteremic pneumonia caused by penicillin-resistant pneumococci. N Engl J Med 1987;317:18-22.
15. Leach AJ, Shelby-James TM, Mayo M, Gratten M, Laming AC, Currie BJ, et al. A prospective study of the impact of community-based azithromycin treatment of trachoma on carriage and resistance of *Streptococcus pneumoniae*. Clin Infect Dis 1997;24:356-62.
16. Seppala H, Klaukka T, Vuopio-Varkila J, Muotiala A, Helenius H, Lager K, et al. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. N Engl J Med 1997;337:441-6.
17. Centers for Disease Control and Prevention. Assessment of national reporting of drug-resistant *Streptococcus pneumoniae*—United States, 1995-1996. MMWR Morb Mortal Wkly Rep 1996;45:947-50.
18. Heffernan R, Henning K, Labowitz A, Hjelte A, Layton M. Laboratory survey of drug-resistant *Streptococcus pneumoniae* in New York City, 1993-1995. Emerg Infect Dis 1998;4:113-6.
19. Kellner J, McGeer A, Cetron M, Low D, Butler J, Matlow A, et al. The use of *Streptococcus pneumoniae* nasopharyngeal isolates from healthy children to predict features of invasive disease. Pediatr Infect Dis J 1998;17:279-86.

Diphtheria Antitoxin Levels in the Netherlands: a Population-Based Study

H.E. de Melker, G.A.M. Berbers, N.J.D. Nagelkerke, and M.A.E. Conyn-van Spaendonck
National Institute of Public Health and the Environment, Bilthoven, the Netherlands

In a population-based study in the Netherlands, diphtheria antitoxin antibodies were measured with a toxin-binding inhibition assay in 9,134 sera from the general population and religious communities refusing vaccination. The Dutch immunization program appears to induce long-term protection against diphtheria. However, a substantial number of adults born before the program was introduced had no protective diphtheria antibody levels. Although herd immunity seems adequate, long-term population protection cannot be assured. As more than 60% of orthodox reformed persons have antibody levels lower than 0.01 IU/ml, introduction of diphtheria into religious communities refusing vaccination may constitute a danger of spread of the bacterium.

The recent diphtheria epidemics in eastern Europe are a warning that diphtheria can make a comeback in susceptible populations (1). The World Health Organization (WHO) recommends the assessment of diphtheria immunity in nonepidemic countries, to prevent any indigenous cases in the European region by the year 2000 (2).

In the Netherlands, the last diphtheria epidemic occurred during World War II (220,000 cases in 1940 to 1946). Diphtheria vaccination was introduced in 1952 for persons born after 1945. Under the current schedule, children are vaccinated at ages 3, 4, 5, and 11 months with diphtheria, tetanus, pertussis, and inactivated polio vaccine (DTP-IPV) and at ages 4 and 9 years with DT-IPV. For the past 25 years, the vaccine coverage for at least three vaccinations at the age of 12 months has been 97%. Rare exposure to *Corynebacterium diphtheriae* may have led to lack of boosting opportunities (1). As in other industrialized countries, lack of immunity in older persons is a reason for concern (3,4). Furthermore, in the Netherlands, the immune status of sociogeographically clustered members of religious communities who refuse vaccination may be even more unfavorable. Inadequate herd immunity to diphtheria in these groups could lead to outbreaks similar to the poliomyelitis outbreaks in the Netherlands (5). A large population-based serum bank allowed us to assess the diphtheria immunity in the general Dutch population and in persons refusing vaccination (6).

Address for correspondence: H.E. de Melker, National Institute of Public Health and the Environment, Department of Infectious Diseases Epidemiology, P.O. Box 1, 3720 BA Bilthoven, The Netherlands; fax: 31-30-274-4409; e-mail: H.de.Melker@rivm.nl.

The Study

From October 1995 through December 1996, a population-based serum bank with specimens from 9,948 persons was established (6). Our objective was to select 40 municipalities with samples proportional to population size. In each of five regions, eight municipalities were included. For each of these 40 municipalities, an age-stratified sample of 380 persons was drawn from the population register (7). Participants were requested to have a blood sample drawn, complete a questionnaire, and provide immunization and military service records. Participants were also selected from eight additional municipalities with low vaccine coverage to assess the immunity of members of religious communities that refuse vaccination. The nationwide sample had 8,357 (55%) participants, and the low vaccine coverage sample had 1,589 (52.5%). Sufficient serum was available for testing 7,715 of the nationwide participants and 1,419 of the participants in the sample with low vaccine coverage.

Methods

Sera were stored at -86°C. The level of diphtheria antitoxin antibodies was measured with a toxin-binding inhibition assay (8). In brief, twofold serum dilution series were incubated with a fixed amount of toxin, and the nonneutralized toxin was measured in an enzyme-linked immunosorbent assay (ELISA) with equine antitoxin purified from hyperimmune serum as coat and peroxidase-labeled horse antidiphtheria IgG as conjugate. International units were calculated according to the WHO reference standard serum (10 IU/ml) by the four-parameter fit method in Kineticalc (KC4, Biolyse) with a Bio-Tek plate reader (EL312d). The minimum level of detection was 0.01 IU/ml, and samples below this level were set to 0.005 IU/ml for calculating geometric mean titers. The correlation of this method with the Vero neutralization assay has been confirmed recently ($r \geq 0.95$) (9).

Antitoxin antibody levels were classified according to international standards as < 0.01 IU/ml (no protection), 0.01 IU/ml to 0.1 IU/ml (basic protection) and > 0.1 IU/ml (full protection) (10).

Analysis

Frequencies and geometric mean titers in each municipality were weighted by the proportion of the age group in the population. To produce national estimates, the weighted frequencies and geometric mean titers were averaged over the 40 municipalities (7). For the low vaccine coverage sample, they were averaged by weighting the population of the municipality.

Data on age, sex, marital status, country of nationality, degree of urbanization, region, and contact information for all participants and nonparticipants were available. The effect of differential probabilities of response for these variables on both sample estimates was less than one standard error and was therefore disregarded.

Linear regression analysis was used to study the persistence of diphtheria antitoxin antibodies after full immunization in the national immunization program. The association between diphtheria antibody titer (^{2}log) and age in ^{2}log years was studied for persons who received the sixth documented vaccination at 8 to 9 years of age, without self-reported or documented revaccination or history of military service.

Age-Specific Immunity Levels to Diphtheria Antitoxin

In the nationwide sample, 58.1%, 30.0%, and 11.9% of persons ≤ 79 years of age had full, basic, or no diphtheria protection, respectively (Table 1). Women had lower levels of full protection and geometric mean titers. A greater percentage of persons from the municipalities with low vaccine coverage and of members of religious communities in the low vaccine coverage sample had no protection (Table 2). When members of the religious community were excluded from this low

Table 1. Diphtheria immunity in a nationwide sample of persons ≤ 79 years of age, the Netherlands

Sample	No.	<0.01 IU/ml	(95% CI[a])	0.01-0.1 IU/ml	(95% CI)	≥ 0.1 IU/ml	(95% CI)	Geometric mean titer	(95% CI)
Overall	7,715	11.9	(10.7-13.1)	30.0	(28.3-31.7)	58.1	(56.2-59.9)	0.12	(0.11-0.13)
Men	3,644	9.3	(8.0-10.6)	28.1	(25.9-30.2)	62.6	(60.1-65.2)	0.14	(0.13-0.16)
Women	4,071	14.4	(12.6-16.2)	31.6	(29.7-33.6)	54.0	(51.9-56.0)	0.10	(0.09-0.11)

[a]CI, confidence interval.

Table 2. Diphtheria immunity in a sample of persons ≤ 79 years of age with low vaccine coverage, the Netherlands

Nationwide sample[a]	No.	<0.01 IU/ml	(95% CI[b])	<0.01 IU/ml	(95% CI)	≥ 0.1 IU/ml	(95% CI)	Geometric mean titer	(95% CI)
OR									
Overall	233	60.9	(42.9-78.9)	12.4	(6.0-18.7)	26.7	(13.8-39.7)	0.02	(0.01-0.04)
Men	116	69.7	(54.2-85.2)	10.4	(4.2-16.6)	19.9	(8.2-31.5)	0.01	(0.01-0.03)
Women	117	52.3	(27.7-76.8)	15.0	(6.9-23.2)	32.7	(15.3-50.1)	0.03	(0.01-0.06)
NMCR									
Overall	1,259	17.5	(15.5-19.4)	25.2	(21.3-29.2)	57.3	(52.2-62.4)	0.10	(0.09-0.12)
Men	590	11.5	(8.2-14.8)	25.5	(20.5-30.6)	63.0	(55.4-70.5)	0.14	(0.13-0.16)
Women	669	21.7	(18.0-25.4)	25.0	(21.2-28.9)	53.3	(48.6-58.0)	0.08	(0.07-0.10)
TLVC									
Overall	1,492	24.3	(20.5-28.0)	23.6	(20.1-27.1)	52.1	(48.2-56.0)	0.08	(0.07-0.09)
Men	706	20.6	(16.6-24.7)	23.4	(18.9-27.8)	56.0	(50.3-61.7)	0.10	(0.08-0.12)
Women	786	26.7	(21.1-32.4)	23.3	(19.9-26.7)	50.0	(45.3-54.7)	0.07	(0.06-0.09)

[a]OR, orthodox reformed; NMCR, Not member of religious communities; TLVC, total low vaccine coverage.
[b]CI, confidence interval.

vaccine coverage sample, the percentages of full, basic, and no protection were 57.3%, 25.2%, and 17.5%, respectively (Table 2).

For the ages of 1, 4, and 8 to 9 years, the geometric mean titer and percentages of persons with full protection increased (Table 3). The

Table 3. Age-specific prevalence of diphtheria immunity and geometric mean titers for children ≤ 14 years of age and for men and women ≤ 79 years of age in a nationwide sample, the Netherlands

Age group (yrs)	No.	<0.01 IU/ml	(95% CI[a])	0.01-0.1 IU/ml	(95% CI)	≥ 0.1 IU/ml	(95% CI)	Geometric mean titer	(95% CI)
<1	187	3.7	(1.1-6.4)	25.5	(18.6-32.4)	70.7	(63.4-78.1)	0.19	(0.15-0.24)
1	185	1.2	(0.0-3.0)	9.9	(5.4-14.3)	88.9	(84.3-93.5)	0.57	(0.45-0.72)
2	156	1.9	(0.1-3.7)	34.9	(24.2-45.5)	63.3	(52.9-73.6)	0.15	(0.12-0.19)
3	215	1.7	(0.1-3.3)	35.5	(27.7-43.3)	62.8	(54.7-70.9)	0.16	(0.12-0.22)
4	153	0.6	(0.0-1.7)	8.7	(3.7-13.7)	90.7	(85.7-95.7)	0.81	(0.60-1.10)
5	102	1.4	(0.0-3.3)	6.3	(1.9-10.7)	92.3	(87.7-97.0)	0.43	(0.35-0.52)
6	121	0.9	(0.0-2.6)	20.0	(11.7-28.3)	79.1	(70.9-87.4)	0.26	(0.21-0.34)
7	101	2.3	(0.0-5.4)	31.8	(20.3-43.3)	65.9	(54.7-77.2)	0.16	(0.13-0.20)
8	127	0.7	(0.0-2.0)	23.6	(14.9-32.3)	75.8	(66.9-84.6)	0.28	(0.20-0.38)
9	97	0.0	(0.0-0.0)	11.8	(3.2-20.4)	88.2	(79.6-96.8)	0.71	(0.51-0.99)
10	113	0.7	(0.0-2.1)	6.7	(1.9-11.5)	92.6	(87.7-97.5)	0.54	(0.42-0.69)
11	111	0.7	(0.0-1.9)	8.3	(3.5-13.2)	91.0	(85.8-96.2)	0.36	(0.30-0.44)
12	122	0.6	(0.0-1.9)	17.0	(9.2-24.8)	82.4	(74.2-90.5)	0.28	(0.22-0.35)
13	126	0.0	(0.0-0.0)	27.3	(18.0-36.7)	72.7	(63.3-82.0)	0.23	(0.18-0.29)
14	102	0.4	(0.0-1.1)	26.7	(14.0-39.3)	72.9	(60.3-85.6)	0.22	(0.15-0.31)
Men									
<1	104	3.5	(0.04-7.0)	25.2	(15.8-34.6)	71.3	(61.4-81.2)	0.18	(0.13-0.24)
1-4	376	1.8	(0.5-3.1)	22.9	(17.0-28.8)	75.3	(69.1-81.5)	0.31	(0.25-0.38)
5-9	296	0.6	(0.0-1.4)	16.9	(12.4-21.4)	82.5	(77.9-87.1)	0.34	(0.28-0.40)
10-14	280	0.6	(0.0-1.7)	16.5	(11.7-21.3)	83.0	(78.0-88.0)	0.32	(0.27-0.38)
15-19	209	1.0	(0.0-2.4)	25.8	(19.7-31.9)	73.1	(67.1-79.1)	0.19	(0.16-0.23)
20-24	139	4.0	(0.4-7.6)	25.5	(17.8-33.2)	70.5	(62.0-79.0)	0.15	(0.12-0.19)
25-29	150	2.9	(0.0-6.2)	30.6	(21.7-39.5)	66.5	(56.9-76.1)	0.18	(0.14-0.24)
30-34	188	7.0	(1.6-12.4)	31.5	(23.0-40.0)	61.5	(52.1-70.9)	0.14	(0.10-0.19)
35-39	220	5.1	(1.3-8.9)	27.4	(20.7-34.1)	67.5	(60.8-74.2)	0.18	(0.14-0.23)
40-44	230	3.8	(1.1-6.5)	16.3	(11.0-21.6)	79.9	(73.9-85.9)	0.25	(0.20-0.31)
45-49	208	13.7	(7.8-19.6)	26.6	(21.4-31.8)	59.7	(51.8-67.6)	0.13	(0.09-0.18)
50-54	228	15.0	(10.4-19.6)	31.1	(24.7-37.5)	53.9	(46.5-61.3)	0.10	(0.08-0.13)
55-59	251	8.8	(4.0-13.6)	38.8	(32.1-45.5)	52.4	(46.0-58.8)	0.10	(0.08-0.12)
60-64	216	10.3	(6.2-14.4)	52.3	(43.8-60.8)	37.4	(29.9-44.9)	0.07	(0.06-0.09)
65-69	200	26.5	(19.6-33.4)	39.4	(30.4-48.4)	34.1	(26.2-42.0)	0.05	(0.04-0.06)
70-74	193	46.2	(36.9-55.5)	26.6	(19.2-34.0)	27.1	(19.2-35.2)	0.03	(0.02-0.04)
75-79	156	45.0	(35.8-54.2)	26.2	(17.8-34.6)	28.8	(20.8-36.8)	0.03	(0.02-0.04)
Women									
<1	83	4.2	(0.0-8.9)	26.6	(13.7-39.5)	69.3	(56.7-81.9)	0.19	(0.13-0.27)
1-4	333	2.2	(0.5-3.9)	22.2	(16.7-27.7)	75.6	(70.0-81.2)	0.30	(0.25-0.37)
5-9	252	1.8	(0.0-3.7)	23.8	(16.2-31.4)	74.5	(68.2-80.8)	0.29	(0.24-0.36)
10-14	294	0.3	(0.0-0.8)	18.6	(12.9-24.3)	81.1	(75.4-86.8)	0.29	(0.24-0.33)
15-19	243	0.7	(0.0-1.7)	28.2	(21.9-34.5)	71.1	(64.8-77.4)	0.17	(0.15-0.20)
20-24	199	2.8	(0.4-5.2)	31.3	(23.6-39.0)	65.9	(58.3-73.5)	0.14	(0.12-0.18)
25-29	226	5.0	(1.5-8.5)	27.1	(20.3-33.9)	67.9	(60.5-75.3)	0.14	(0.11-0.18)
30-34	244	10.0	(5.7-14.3)	36.9	(30.5-43.3)	53.0	(47.3-58.7)	0.11	(0.09-0.13)
35-39	282	5.6	(2.5-8.7)	37.0	(29.6-44.4)	57.4	(50.6-64.2)	0.13	(0.11-0.15)
40-44	247	8.7	(4.8-12.6)	24.4	(18.5-30.3)	66.9	(60.9-72.9)	0.16	(0.12-0.20)
45-49	261	22.0	(15.8-28.2)	37.6	(30.6-44.6)	40.3	(32.3-48.3)	0.06	(0.04-0.08)
50-54	264	28.2	(20.2-36.2)	41.7	(34.5-48.9)	30.1	(23.0-37.2)	0.04	(0.03-0.05)
55-59	250	22.3	(16.3-28.3)	49.7	(43.6-55.8)	28.0	(22.3-33.7)	0.04	(0.03-0.05)
60-64	237	29.2	(22.9-35.5)	45.9	(39.7-52.1)	24.8	(19.0-30.6)	0.03	(0.02-0.04)
65-69	263	38.0	(30.6-45.4)	34.3	(29.0-39.6)	27.7	(21.3-34.1)	0.03	(0.02-0.05)
70-74	218	58.2	(50.8-65.6)	25.0	(18.9-31.1)	16.8	(11.0-22.6)	0.02	(0.01-0.02)
75-79	175	52.7	(43.8-61.6)	26.6	(19.4-33.8)	20.7	(12.7-28.7)	0.02	(0.01-0.03)

[a]CI, confidence interval.

percentage with full protection decreased after the age of 10 to 14 years, but increased for the 35- to 44-year age group (Figure 1). After the age of 40 to 44 years, the percentage with full protection and the geometric mean titer decreased. Although the geometric mean titers differed statistically significantly by gender only after the age of 30 years, they were slightly lower for females 5 to 9 years of age and older (Table 3).

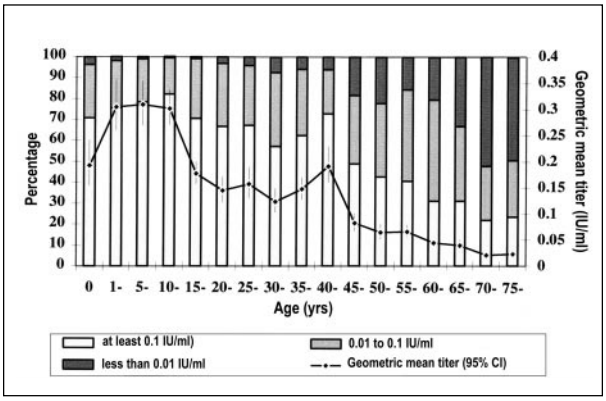


Figure 1. Age-specific diphtheria immunity in a nationwide sample (n = 7715), the Netherlands.

Both for orthodox reformed persons less than 50 years and for those at least 50 years of age, the proportion with no protection was higher than for persons in the nationwide sample (Table 4).

Men and women ages 20 to 49 years without a military service history had similar proportions of full, basic, and no protection, while the proportion with full protection was higher for men with a military service history (Table 5).

Persistence of Diphtheria Antitoxin Levels

The geometric mean titer decreased with age (or time since last vaccination) for persons who had received their sixth and last vaccination at 8 to 9 years of age (n = 961) from 0.30 IU/ml for 10 to 14 years to 0.09 IU/ml for 30 to 34 years (Table 6, Figure 2). According to linear regression analysis, the decrease corresponds to a decrease of -1.27 ^{2}log IU/ml with each ^{2}log increase in years. The percentage with full protection decreased from 82.5% to 41.7%, and the percentage with no protection increased from 0% to 4.3% for persons 10 to 14 years of age and 30 to 34 years of age, respectively (Table 6).

The geometric mean titer for persons 20 to 34 years of age with documented revaccination (n = 37) was 0.29 IU/ml. Percentages of full (81.0%), basic (19.0%), and no protection (0.0%) were similar to recently vaccinated 10- to 14-year-olds, without further documented or reported revaccination (Table 6).

Conclusions

Our population-based study showed that 58% of the Dutch population had full, 30% basic, and 12% no protection against diphtheria. These estimates and the geometric mean titer (0.12 IU/ml) are in between findings for other European countries (4,11-20). The Dutch immunization program appeared to induce long-term protection. However, approximately one third of adults age 50 to 79 years, who were born before the introduction of the immunization program, and approximately two-thirds of orthodox reformed persons had no protective diphtheria antibodies.

Table 4. Diphtheria immunity in a nationwide sample and in orthodox reformed persons in municipalities with low vaccine coverage, the Netherlands.

Sample[a] (yrs)	No.	<0.01 IU/ml	(95% CI[b])	0.01-0.1 IU/ml	(95% CI)	≥0.1 IU/ml	(95% CI)	Geometric mean titer	(95% CI)
NW									
0-49	5,064	5.3	(4.4-6.2)	27.3	(25.3-29.4)	67.4	(65.2-69.5)	0.17	(0.16-0.19)
50 -79	2,651	29.2	(26.4-31.9)	37.4	(35.3-39.5)	33.4	(31.3-35.6)	0.04	(0.04-0.05)
ORLVC									
0-49	170	60.8	(40.2-81.4)	7.4	(2.3-12.6)	31.8	(13.8-49.7)	0.02	(0.01-0.05)
50 -79	63	59.3	(30.1-88.6)	34.6	(9.5-59.6)	6.1	(0.0-18.2)	0.01	(0.005-0.025)

[a]NW, nationwide; ORLVC, Orthodox reformed from low vaccine coverage sample.
[b]CI, confidence interval.

Table 5. Diphtheria immunity in a nationwide sample among persons 20 to 49 years of age, according to sex and military service, the Netherlands

Sample[a]	No.	<0.01 IU/ml	(95% CI[b])	0.01-0.1 IU/ml	(95% CI)	≥0.1 IU/ml	(95% CI)	Geometric mean titer	(95% CI)
M, SH	425	2.9	(1.2-4.6)	14.9	(11.1-18.7)	82.2	(78.0-86.5)	0.30	(0.25-0.36)
M, NSH	710	7.8	(5.2-10.3)	33.1	(28.0-38.1)	59.2	(54.0-64.4)	0.12	(0.10-0.14)
W, NSH	1,456	9.1	(7.0-11.2)	32.5	(29.0-36.0)	58.4	(54.5-62.3)	0.12	(0.10-0.13)

[a]M, men; W, women; SH, service history; NSH, no service history.
[b]CI, confidence interval.

Table 6. Diphtheria immunity for persons in a nationwide sample who were completely vaccinated in the national immunization program and received the sixth diphtheria vaccination at 8 or 9 years of age, the Netherlands

Sample[a] (yrs)	No.	<0.01 IU/ml	(95% CI[b])	0.01-0.1 IU/ml	(95% CI)	≥0.1 IU/ml	(95% CI)	Geometric mean titer (IU/ml)	(95% CI)
NRE									
10-14	392	0.0	--	17.5	(12.8-22.1)	82.5	(77.9-87.1)	0.30	(0.26-0.34)
15-19	282	0.4	(0.0-1.3)	30.1	(23.7-36.5)	69.4	(63.2-75.7)	0.17	(0.14-0.21)
20-24	155	1.2	(0.0-3.0)	29.4	(20.7-38.2)	69.4	(60.7-78.1)	0.16	(0.12-0.19)
25-29	80	1.6	(0.0-3.7)	28.3	(17.2-39.4)	70.2	(58.7-81.6)	0.14	(0.11-0.18)
30-34	52	4.3	(0.0-9.2)	54.0	(39.3-68.8)	41.7	(27.3-56.1)	0.09	(0.07-0.13)
RE									
20-34	37	0.0	--	19.0	(3.2-34.9)	81.0	(65.1-96.8)	0.29	(0.18-0.46)

[a]NRE, no evidence of revaccination; RE, evidence of revaccination.
[b]CI, confidence interval.

The toxin inhibition test used to measure diphtheria antitoxin concentrations shows good correlation with the in vitro neutralization test in Vero cells, but is faster, simpler, and combines the measurement of diphtheria and tetanus antitoxin antibodies (8,9).

Although the participation rates in the nationwide sample and low vaccine coverage sample were 55% and 52.5%, respectively, our population-based estimates of diphtheria immunity were considered representative, because they do not seem to be affected by nonparticipation. Our participants included a large percentage of persons with diphtheria protection who were born after the vaccination was introduced in 1952 and after the virtual disappearance of diphtheria in 1960. High levels of immunity in this group reflect the success of the national vaccination program.

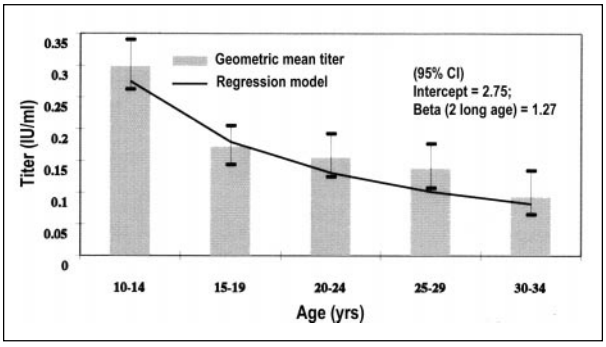


Figure 2. Diphtheria antitoxin titer (geometric mean titer ± 2 standard errors) by age group and linear regression of diphtheria antitoxin antibody titer (in ^{2}log) with age (in ^{2}log years) for persons who received the sixth diphtheria vaccination at the age of 8 or 9 years (n = 961) in the nationwide sample, the Netherlands.

For persons born before the introduction of vaccination, diphtheria immunity is largely derived from natural infection. However, immunity levels in persons older than 49 years in the general population are higher than those of orthodox reformed persons, suggesting that immunity was partly induced by vaccinations (e.g., for military service, travel).

The sharp increase in the percentage of persons older than 44 years with no protective diphtheria antitoxin levels is consistent with findings of other studies (4,11,12,14-19). The increase supports the phenomenon of waning immunity after natural infection without boosting.

In our study, higher immunity levels among men are associated with military service, as previously reported (15,19). However, some researchers have found similar immunity levels for men and women, while others have reported lower immunity for men (11,16,21). Furthermore, lower immunity for women that could not be ascribed to vaccinations during military service has also been reported (4,20). Women might maintain immunity after vaccination for a shorter time than men (14). The slightly lower geometric mean titers for girls age 5 to 19 years in our study are consistent with the latter possibility. As more than 60% of orthodox reformed persons have no protection against diphtheria, introduction of diphtheria into this group may constitute a danger of spreading the bacterium.

Since the Netherlands does not have a mandatory vaccination policy, protection of persons who refuse vaccination is problematic. For poliomyelitis the solution seems to be eradication of the causative agent (5). For diphtheria such a goal has not yet been formulated by WHO. However, even though systematic assessment has not been performed, no signs of persistent circulation of *C. diphtheriae* exist in the Netherlands.

When our data are interpreted longitudinally, the log linear decrease in diphtheria antibody level with age for completely vaccinated persons corresponds with a continuous decline in vaccine-induced antibodies (13,22). However, relatively few 30- to 34-year-old persons (4.3%) who received their last vaccination approximately 25 years ago had a diphtheria antitoxin level of less than 0.01 IU/ml. This compares favorably with observations in other countries (13,21-23). Our immunization program, in which children are vaccinated at 3, 4, 5, and 11 months with 15 Lf diphtheria toxoid and at the ages of 4 and 9 years with 2.5 Lf, appears to induce long-term protection against diphtheria.

In the Netherlands, booster vaccinations are only advised for persons at increased risk for exposure (e.g., travelers to endemic-disease countries and those who work with injection drug users and alcoholic patients). The need for routine boosters to guarantee population protection depends mainly on the proportion of vaccinated persons necessary to confer diphtheria herd immunity. This proportion is estimated at 70% to 80%, but no antitoxin level has been precisely defined for complete protection (10,13,24-26). The Dutch immunity level exceeds this threshold (a minimum level of 0.01 IU/ml [88%]), but is below a minimum level of 0.1 IU/ml (58%).

The absence of cases in the Netherlands associated with the diphtheria epidemic in Eastern Europe suggests that herd immunity is sufficient. This herd immunity might result from sufficient protective levels of antitoxin or immunologic memory. Our results, like those of others, indicate good immunologic memory after revaccination for persons who had been previously vaccinated (17, 27). However, the memory response of adults after initial vaccination is unknown. Furthermore, unknown protective mechanisms might be involved. Only sporadic cases and no outbreaks have occurred in other European countries where gaps have been found in the diphtheria antitoxin levels of adults. The only recent epidemic in western Europe, which occurred before the epidemic in eastern Europe, was among alcoholics (23). Perhaps unfavorable social conditions, like those that appear to have contributed to the epidemics in eastern Europe, play a role in the spread of diphtheria.

In conclusion, a substantial percentage of adults born before the introduction of the immunization program has low diphtheria antitoxin levels. Although herd immunity seems sufficient, long-term population protection cannot be assured. Possibly vaccination might fill the gaps of diphtheria antitoxin antibodies. Diphtheria vaccination could be efficiently combined with other vaccines (e.g., tetanus, influenza) as part of an adult immunization program.

Acknowledgments

We thank the Public Health Services, the Pienter Project Team, and C.J.P. van Limpt and H.A.T. Kuijken for their useful contributions. We also thank J. Huisman, H.C. Rümke, J.F.P. Schellekens, and J.K. van Wijngaarden for their valuable comments on our manuscript.

H.E. de Melker is an epidemiologist in the Department of Infectious Diseases Epidemiology, National Institute of Public Health and the Environment, the Netherlands. Her work involves epidemiologic research directed to vaccine-preventable diseases and evaluation of the national vaccination program.

References

1. Hardy IRB, Dittmann S, Sutter RW. Current situation and control strategies for resurgence of diphtheria in newly independent states of the former Soviet Union. Lancet 1996;347:1739-44.
2. World Health Organization. The expanded programme on immunisation in the European Region of WHO. Diphtheria. Plan of action for the prevention and control of diphtheria in the European Region (1994-1995). Copenhagen, 1994. ICP/EPI 038 (A).
3. Prospero E, Raffo M, Bagnoli M, Appignanesi R, D'Errico M. Diphtheria: epidemiological update and review of prevention and control strategies. Eur J Epidemiol 1997;13:527-34.
4. Maple PA, Efstratiou A, George RC, Andrews NJ, Sesardic D. Diphtheria immunity in UK blood donors. Lancet 1995;345:963-5.
5. Oostvogel PM, van Wijngaarden JK, van der Avoort HGAM, Mulders MN, Conyn-van Spaendonck MAE, Rümke HC, et al. Poliomyelitis outbreak in an unvaccinated community in the Netherlands, 1992-93. Lancet 1994;344:665-70.
6. de Melker HE, Conyn-van Spaendonck MAE. Immunosurveillance and the evaluation of national immunisation programmes: a population-based approach. Epidemiol Infect 1998;21:637-43.
7. Cochran WG. Sampling techniques. 3rd ed. New York: John Wiley & Sons; 1977.
8. Hendriksen CFM, van de Gun JW, Kreeftenberg JG. Combined estimation of tetanus and diphtheria antitoxin in human sera by the in vitro Toxin Binding Inhibition (ToBI) test. Journal of Biological Standardization 1989;17:191-200.
9. Knipping CT, Berbers GAM. Vergelijking van de Toxine Bindings Inhibitie test (ToBI) met de Dubbel Antigeen ELISA (DAE) voor het bepalen van antistoftiters gericht tegen difterie toxine in humaan serum. RIVM report no. 126000001, 1998, Bilthoven, the Netherlands.
10. Galazka AM. The immunological basis for immunisation. Diphtheria. Geneva: World Health Organization. Geneva; 1993. WHO/EPI/GEN/93.12.
11. Stark K, Barg J, Molz B, Vormwald A, Bienzle U. Immunity against diphtheria in blood donors in East Berlin and West Berlin. Lancet 1997;350:932.
12. Matheï C, van Damme P, Bruynseels P, Goossens H, Vranckx R, Metheus A. Diphtheria immunity in Flanders. Eur J Clin Microbiol Infect Dis 1997;16:631-6.
13. Simonsen O, Kjeldsen K, Bentzon MW, Heron I. Susceptibility to diphtheria in populations vaccinated before and after elimination of indigenous diphtheria in Denmark. A comparative study of antitoxic immunity. Acta Pathol Microbiol Immunol Scand Sect C 1987;95:225-31.
14. Kjeldsen K, Simonsen O, Heron I. Immunity against diphtheria and tetanus in the age group 30-70 years. Scand J Infect Dis 1988;20;177-85.
15. Jenum PA, Skogen V, Danilova E, Eskild A, Sjursen H. Immunity to diphtheria in northern Norway and northwestern Russia. Eur J Clin Microbiol Infect Dis 1995;14:794-8.
16. Klouche M, Lühmann D, Kirchner H. Low prevalence of diphtheria antitoxin in children and adults in northern Germany. Eur J Clin Microbiol Infect Dis 1995;14:682-5.
17. World Health Organization. Expanded programme on immunization. Immunization of adults against diphtheria. Wkly Epidemiol Rec 1995;70:56-9.
18. World Health Organization. Expanded programme on immunization. Diphtheria immunity in the adult French population. Wkly Epidemiol Rec 1995;70:252-5.
19. Gasparini R, Pozzi T, Fragapane E, Severini R, Cellesi C, Fabrizi P, et al. Immunity to diphtheria in Siena. Epidemiol Infect 1997;119:203-8.
20. Miller E, Rush M, Morgan-Capner P, Hutchinson D, Hindle L. Immunity to diphtheria in adults in England. BMJ 1994;308:598.
21. Cellesi C, Michelangeli C, Rossolini GM, Giovannoni F, Rossolini A. Immunity to diphtheria, six to 15 years after a basic three-dose immunization schedule. Journal of Biological Standardization 1989;17:29-34.
22. Kjeldsen K, Simonsen O, Heron I. Immunity against diphtheria 25-30 years after primary vaccination in childhood. Lancet 1985;1:900-2.
23. Böttiger M, Pettersson G. Vaccine immunity to diphtheria: a 20-year follow-up study. Scand J Infect Dis 1992;24:753-8.
24. Anderson RM, May RM. Infectious diseases of humans: dynamics and control. 2nd ed. New York: Oxford University Press; 1991.
25. Ipsen J. Circulating antitoxin at the onset of diphtheria in 425 patients. J Immunol 1946;54:325-47.
26. Schneerson R, Robbins JB, Taranger J, Lagergärd T, Trollfors B. A toxoid vaccine for pertussis as well as diphtheria? Lessons to be relearned. Lancet 1996;348:1289-92.
27. Simonsen O, Kjeldsen K, Vendborg HA, Heron I. Revaccination of adults against diphtheria I: response and reactions to different doses of diphtheria toxoid in 30-70-year-old persons with low serum antitoxin levels. Acta Pathol Microbiol Immunol Scand Sect C 1986;94:213-8.

Acute Sin Nombre Hantavirus Infection without Pulmonary Syndrome, United States

Paul T. Kitsutani,* Robert W. Denton,† Curtis L. Fritz,‡ Robert A. Murray,§ Randall L. Todd,¶# W. John Pape, J. Wyatt Frampton,†† Joni C. Young,* Ali S. Khan,* Clarence J. Peters,* and Thomas G. Ksiazek***

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; †Bishop, California, USA; ‡California Department of Health Services, Sacramento, California, USA; §California Department of Health Services, Berkeley, California, USA; ¶Bureau of Disease Control and Intervention Services, Carson City, Nevada, USA; #Nevada State Health Division, Carson City, Nevada, USA; **Colorado Department of Public Health & Environment, Denver, Colorado, USA; and ††Utah Department of Health, Salt Lake City, Utah, USA

Hantavirus pulmonary syndrome (HPS) occurs in most infections with Sin Nombre virus and other North American hantaviruses. We report five cases of acute hantavirus infection that did not fit the HPS case definition. The patients had characteristic prodromal symptoms without severe pulmonary involvement. These cases suggest that surveillance for HPS may need to be expanded.

Hantavirus pulmonary syndrome (HPS) is an emerging infectious disease, often characterized by rapid, dramatic clinical progression and high case-fatality rates. Most cases in the United States are caused by Sin Nombre virus (SNV); like other viruses causing HPS, SNV has a single rodent host belonging to the subfamily *Sigmodontinae* (1). The virus is transmitted to humans through inhalation of aerosolized feces, urine, or saliva from infected rodents.

Since the initial outbreak in the Four Corners region in 1993 (2), 217 cases of HPS were reported in the United States as of May 28, 1999; 32 of these occurred before May 1993. These cases have provided information on the clinical symptoms, disease progression, and laboratory characteristics of HPS. An incubation period of 2 to 3 weeks is typically followed by high fever, myalgia, headache, fatigue, and gastrointestinal symptoms (3,4). This phase is followed 4 to 6 days later by abrupt onset of dyspnea and hypoxia, typically associated with noncardiac pulmonary edema and respiratory failure, requiring hospitalization and intensive-care management (4,5). Hypotension or shock with myocardial depression is present in most patients; renal dysfunction of varying severity is sometimes observed. Common laboratory findings include elevated hematocrit, leukocytosis with left shift and immature myelocytes and immunoblasts, and thrombocytopenia (6,7). The diagnosis is confirmed by serologic testing for hantavirus SNV immunoglobulin (Ig)M and IgG, although reverse transcriptase polymerase chain reaction (RT-PCR) or immunohistochemical analysis (IHC) can also be done.

In a study of the prevalence of SNV antibody in patients who had mild febrile illness during the 1993 HPS outbreak, asymptomatic and mild infections were uncommon (8). This observation contrasts with reports of hantaviruses that cause hemorrhagic fever with renal syndrome; mild disease can occur after infection with Hantaan virus and is predominately associated with Puumala virus infections (9,10). Since May 1993, five persons with mild acute HPS illness have

Address for correspondence: Paul T. Kitsutani, Centers for Disease Control and Prevention, 1600 Clifton Road, Mail Stop A26, Atlanta, GA 30333, USA; fax: 404-639-1509; e-mail: pdk8@cdc.gov.

been identified; one was a 4-year-old boy whose case has been described (11). We describe the other four cases, two of which were detected in 1998 and one in 1999.

HPS is clinically defined as a febrile illness (temperature >38.3°C) with bilateral diffuse infiltrates that cause respiratory compromise requiring supplemental oxygen within 72 hours of hospitalization (12). A case may also be defined postmortem as an unexplained, fatal respiratory illness, with noncardiogenic pulmonary edema of unknown cause. Clinically suspected cases are confirmed by fulfilling one of three criteria at a reference laboratory: detection of hantavirus-specific IgM or rising titers of IgG antibodies, or hantavirus-specific RNA sequence by RT-PCR, or hantavirus antigens in tissues by IHC.

These four atypical cases were identified through the National HPS Surveillance System, although they did not meet the clinical criteria for HPS. In Patients 2 and 3, infection with SNV was suspected early in the illness, and sera were tested promptly for hantavirus antibodies. Serum from Patient 1 was tested for SNV antibody retrospectively, after a friend with a common exposure history was diagnosed with HPS. Acute- and convalescent-phase sera from each patient were also tested at CDC for hantavirus IgM and IgG antibodies by using a panel of prototypic hantavirus strains (13). Cases were confirmed as acute SNV infections if there were substantial titers of anti-SNV IgM and either substantial acute-phase titers of IgG or a fourfold rise in convalescent-phase IgG titers.

Case Report 1

A 38-year-old previously healthy man from Nevada visited a local emergency room in October 1993 with a 3-day history of fever, headache, fatigue, malaise, dizziness, progressive myalgia, dry cough, and shortness of breath (Table). His temperature was 38.3°C, pulse 103 per minute, and blood pressure 130/77 mm Hg. His oxygen saturation was 85% on room air, improving to 94% on 2 liters of oxygen by nasal cannula. Except for frontal sinus tenderness, his physical examination was otherwise unremarkable.

Approximately 2 weeks later, a friend of the patient's died of a respiratory illness diagnosed as HPS. A case investigation of all household and social contacts led retrospectively to the diagnosis of acute hantavirus infection in the patient, as demonstrated by positive SNV IgM and IgG titers. The patient and his friend had worked together at a ranch in rural Nevada, where they slept for 2 days in a rodent-infested guest house (14).

Case Report 2

A 36-year-old woman from California visited her physician in July 1998 with a 2-day history of fever, headache, and malaise. Her temperature was 37.5°C, pulse 130 per min, and blood pressure 90/60/mm Hg. Physical examination was unremarkable.

Two days later, she visited the emergency room for the same complaints, as well as myalgia, dry cough accompanied by substernal burning and pain, sore throat, vomiting, and photophobia (Table). She was not in acute respiratory distress. An infectious mononucleosis test was positive. She was diagnosed with a viral syndrome probably secondary to mononucleosis and dehydration, treated with intravenous fluids, and discharged. Her clinical course improved without hospitalization.

The patient worked as a registered nurse and lived on a ranch. She had no history of recent travel. She reported three exposures to rodent excreta in the month before becoming ill, twice while cleaning a barn and once while cleaning her mobile home. Because of this history, she was tested for SNV antibodies on day 2 of illness. This acute-phase serum, as well as a convalescent-phase serum, tested positive for both SNV IgM and IgG antibodies.

Case Report 3

A previously healthy 19-year-old man visited a local Colorado emergency room in June 1999 with a 2-day history of fever, chills, myalgia, nausea, and vomiting, but no shortness of breath. His vital signs included a temperature of 39.5°C, pulse of 93 per minute, blood pressure 114/71 mm Hg, and oxygen saturation of 89.9% on room air. A platelet count was 96,000/mm^3. A chest X-ray was unremarkable. A diagnosis of HPS was suspected because of a history of rodent exposure in the community, with two recent fatal cases, but the patient refused hospitalization.

The patient was admitted 2 days later after his initial serum specimen was noted to have SNV IgM and IgG antibodies. He felt better, although he remained febrile and had developed a slight cough. A repeat chest X-ray was initially reported as normal but was retrospectively read

Table. Characteristics of five acute cases of Sin Nombre Virus infection without pulmonary syndrome, 1993–1999

	Case 1, 1993	Case 2, 1998	Case 3, 1999	Case 4, 1998[a]	Case 5, 1993[b]
Age (yr)	38	36	19	32	4
Sex	M	F	M	M	M
Race	White	White	White	White	American Indian
State	Nevada	California	Colorado	Utah	New Mexico
Hospitalized	No	No	Yes	Yes	No
Symptoms					
Fever	+[c]	+	+	+	+
Headache	+	+	-	+	-
Malaise	+	+	-	-	-
Myalgia	+	+	+	+	-
Cough	+	+	+	-	+
Shortness of breath	+	-	-	-	-
Chest/substernal pain	-	+	-	-	-
Sore throat	-	+	-	-	NR[d]
Nausea/vomiting	+	+	+	-	NR
Dizziness	+	-	+	-	NR
Photophobia	-	+	-		
Abdominal pain	-	-	-	-	NR
Diarrhea	-	-	-	-	NR
Arthralgia	-	-	-	+	NR
Vital signs					
Max temp (°F)	102.5	104.0	103.1	102.8	100.6
Blood pressure	normal	normal	normal	normal	NR
Lowest O_2 sat. (RA[e])	85%	94%	89.9%	94%	NR
Laboratory results					
Highest Hct (%)	47.0	44.7	50.2	44.4	40.2
Highest WBC	9,100	8,000	10,200	7,300	NR[f]
% seg. neutrophils	68	40	39	88	
% bands	9	30	24		
% lymphocytes	19	14	20	7	
% atypical lymphocytes	NR	4	NR	NR	
Lowest platelet (/mm^3)	127,000	115,000	28,000	163,000	NR[g]
Highest SGOT[h] (U/L)	NR	81	NR	26	NR
Highest LDH[i] (U/L)	NR	337	488	240	NR
Lowest albumin (g/dL)	NR	3.1	NR	4.3	NR
Chest X-ray	2-cm granuloma	normal	Mild left lower lobe infiltrate	normal	NR
Anti-SNV[j] antibody					
IgM	positive	positive	positive	positive	positive
IgG	positive	positive	positive	positive	positive

[a]Obtained from Zavasky D-M, Hjelle B, Peterson M, et al. Acute infection with Sin Nombre hantavirus without pulmonary edema. Clin Infect Dis, in press.
[b]Obtained from Armstrong et al., 1995 (12).
[c]+, present; -, absent.
[d]NR = not recorded or obtained.
[e]RA = room air.
[f]Patient did not have leukocytosis.
[g]Patient did not have thrombocytopenia.
[h]SGOT = serum glutamic oxalacetic acid.
[i]LDH = lactic dehydrogenase.
[j]SNV = Sin Nombre virus.

as having a slight left lower lobe interstitial infiltrate. His symptoms gradually resolved and he was discharged 2 days after admission.

Conclusions

These patients are among the first adults in the United States to have had acute SNV infections resulting in illnesses less severe than HPS; a fourth is being described elsewhere (Table). These patients had the characteristic HPS-like prodromal symptoms of high fever, headache, and mylagia. Some of the other typical features of HPS (malaise, nausea, vomiting, dizziness, cough, chest pain) were also observed. Patient 1 initially had signs and symptoms of pulmonary involvement, documented by low oxygen saturation. In contrast, Patients 2 and 3 did not have respiratory distress, although Patient 3 had one oxygen saturation measurement of 89.5% on room air. All four patients had normal lung findings on physical examination and characteristic diffuse bilateral interstitial edema was not seen on chest X-rays.

Several of the typical laboratory findings of HPS were noted, including a left shift on the white blood count differential, atypical lymphocytes, mildly elevated serum glutamic oxalacetic acid or lactic dehydrogenase, and low albumin. All the reported patients had unequivocal thrombocytopenia, and Patient 4 had a decreasing platelet count. The hematocrit of Patient 2 rose from 42.3% to 44.7% during her acute illness, then decreased to 36.3%, suggesting a period of substantial hemoconcentration, as seen in HPS.

All three patients became ill in areas of the United States where reservoirs of other known pathogenic U.S. hantaviruses are not found and where all RT-PCR-typed HPS cases have been caused by SNV.

During the initial 1993 outbreak, an intensive search for SNV IgM and IgG antibodies was conducted among household contacts of patients (15), as well as among patients with acute fever and myalgia resembling HPS prodromal symptoms (8). IgM antibodies reacting with SNV were not found in the study population, which suggests that mild acute hantavirus infections were uncommon.

The first case of mild SNV illness with positive SNV IgM and IgG antibodies was described in a 4-year-old boy who had upper respiratory infection symptoms and otitis media but no other abnormal laboratory findings (11).

Mild cases of HPS have been observed in patients who did not have severe pulmonary disease or respiratory failure. In addition, a few patients with HPS with an initial normal chest X-ray have been described (16). However, chest X-rays 24 to 48 hours later demonstrated interstitial or alveolar edema in all these patients. These cases of mild HPS must be distinguished from the three cases reported in this article, which had no or minimal radiologic pulmonary involvement.

It is unclear why severe respiratory distress, pulmonary edema, and hypotension or shock, the hallmarks of HPS, did not develop in these patients. Histopathologic and immunologic studies of acute HPS patients have shown antibodies, significant CD8 and CD4 T-lymphocyte activation, and lymphokine involvement, suggesting the hypothesis that HPS is an immunopathologic response to hantavirus infection (6,7,13,17,18). Patients with mild SNV illness may have a weaker immune response to the virus than patients whose illness progresses to HPS. In addition, integrins expressed on platelets and endothelial cells have recently been implicated as a vehicle for HPS-associated SNV and NY-1 cellular entry and pathogenicity (19). Physiologic or genetic variations in these receptor molecules may provide another potential explanation for differing hantavirus pathogenesis.

Virologic factors may also play a role in the development of mild illness. Like the hantaviruses that cause a clinical spectrum of hemorrhagic fever with renal syndrome, less pathogenic strains of SNV or other American hantaviruses may not yet have been characterized. Further virologic, molecular, and immunologic analyses of these and other cases may provide better insights into the pathophysiologic mechanisms of mild SNV disease.

Domestic exposure to rodent excreta continues to be a major risk factor for contracting HPS. Public health education of risk-reducing measures against hantavirus infection should remain a high priority. Moreover, SNV infection should be considered in the differential diagnoses of patients with nonspecific febrile illness and a history of possible exposure to rodents.

Acknowledgments

The authors thank Daniel Safranek for the clinical details of Case 3 and Michael C. Peterson for a medical description of Case 4. We also thank Stuart T. Nichol and Pierre E. Rollin for their helpful review of this manuscript.

Dr. Kitsutani is an officer in CDC's Epidemic Intelligence Service.

References

1. Monroe MC, Morzunov SP, Johnson AM, Bowen MD, Artsob H, Yates T, et al. Genetic diversity and distribution of *Peromyscus*-borne hantaviruses in North America. Emerg Infect Dis 1999;5:75-86.
2. Centers for Disease Control and Prevention. Outbreak of acute illness-southwestern United States, 1993. MMWR Morb Mortal Wkly Rep 1993;42:421-4.
3. Khan AS, Khabbaz RF, Armstrong LR, Holman RC, Bauer SP, Graber J, et al. Hantavirus pulmonary syndrome: the first 100 U.S. cases. J Infect Dis 1996;173:1297-303.
4. Duchin JS, Koster FT, Peters CJ, Simpson GL, Tempest B, Zaki SR, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. N Engl J Med 1994;330:949-55.
5. Hallin GW, Simpson SQ, Crowell RE, James DS, Koster FT, Mertz GJ, et al. Cardiopulmonary manifestations of hantavirus pulmonary syndrome. Crit Care Med 1996;24:252-8.
6. Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foucar K, et al. Hantavirus pulmonary syndrome: pathogenesis of an emerging infectious disease. Am J Pathol 1995;146:552-79.
7. Nolte KB, Feddersen RM, Foucar K, Zaki SR, Koster FT, Madar D, et al. Hantavirus pulmonary syndrome in the United States: a pathological description of a disease caused by a new agent. Hum Pathol 1995;26:110-20.
8. Simonsen L, Dalton MJ, Breiman RF, Hennessy T, Umland ET, Sewell CM, et al. Evaluation of the magnitude of the 1993 hantavirus outbreak in the southwestern United States. J Infect Dis 1995;172:729-33.
9. Lee HW, van der Groen G. Hemorrhagic fever with renal syndrome. Prog Med Virol 1989;36:62-102.
10. Settergren B. Nephropathia epidemica (hemorrhagic fever with renal syndrome) in Scandinavia. Reviews of Infectious Diseases 1991;13:736-44.
11. Armstrong LR, Bryan RT, Sarisky J, Khan AS, Rowe T, Ettestad PJ, et al. Mild hantaviral disease caused by Sin Nombre virus in a four-year-old child. Pediatr Infect Dis J 1995;14:1108-10.
12. Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance. MMWR Morb Mortal Wkly Rep 1997;46(RR-10):16.
13. Ksiazek TG, Peters CJ, Rollin PE, Zaki S, Nichol S, Spiropoulou C, et al. Identification of a new North American hantavirus that causes acute pulmonary insufficiency. Am J Trop Med Hyg 1995;52:117-23.
14. Wells RM, Young J, Williams RJ, Armstrong LR, Busico K, Khan AS, et al. Hantavirus transmission in the United States. Emerg Infect Dis 1997;3:361-5.
15. Zeitz PS, Butler JC, Cheek JE, Samuel MC, Childs JE, Shands LA, et al. A case-control study of hantavirus pulmonary syndrome during an outbreak in the southwestern United States. J Infect Dis 1995;171:864-70.
16. Ketai LH, Williamson MR, Telepak RJ, Levy H, Koster FT, Nolte KB, et al. Hantavirus pulmonary syndrome: radiographic findings in 16 patients. Radiology 1994;191:665-8.
17. Ennis FA, Cruz J, Spiropoulou CF, Waite D, Peters CJ, Nichol ST, et al. Hantavirus pulmonary syndrome: CD8+ and CD4+ cytotoxic T lymphocytes to epitopes on Sin Nombre virus nucleocapsid protein isolated during acute illness. Virology 1997;238:380-90.
18. Simpson SQ, Mapel V, Koster FT, Montoya J, Bice DE, Williams AJ. Evidence for lymphocyte activation in the hantavirus pulmonary syndrome. Chest 1995;106:97S.
19. Gavrilovskaya IN, Shepley M, Shaw R, Ginsberg MH, Mackow ER. ß3 integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc Natl Acad Sci U S A 1998;95:7074-9.

Cryptosporidium parvum in Oysters from Commercial Harvesting Sites in the Chesapeake Bay

Ronald Fayer,* Earl J. Lewis,† James M. Trout,* Thaddeus K. Graczyk,‡ Mark C. Jenkins,* James Higgins,* Lihua Xiao,§ and Altaf A. Lal§

*U.S. Department of Agriculture, Beltsville, Maryland, USA; †National Oceanic and Atmospheric Administration, Oxford, Maryland, USA; ‡Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, USA; and §Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Oocysts of *Cryptosporidium parvum*, a zoonotic waterborne pathogen, can be removed by bivalve molluscs from contaminated water and retained on gills and in hemolymph. We identified oocysts of *C. parvum* in oysters from seven sites in the Chesapeake Bay area. These findings document the presence of *C. parvum* infectious for humans in oysters intended for human consumption.

Oocysts of *Cryptosporidium parvum* from human feces can enter surface waters through wastewater, leaky septic tanks, or recreational activities. Oocysts from other mammals, including wildlife, pets, and livestock (especially neonatal ruminants) can enter surface waters either directly or through runoff. Oysters can remove *C. parvum* oocysts from artificially contaminated water and retain them in hemocytes, on gills, and within the body for at least 1 month (1). Oocysts retained for 1 week by oysters were still infectious, as determined by testing in mice (1). Oocysts of *C. parvum* were found in oysters collected from tributaries of the Chesapeake Bay, at six sites selected for proximity to wastewater outfalls and cattle farms where high levels of fecal contamination might be expected (2). We examined oysters at sites where oysters are harvested for human consumption to determine if *C. parvum* oocysts were present. Oocysts recovered from these oysters were examined to determine the possible sources of contamination through oocyst genotyping and to determine if the oocysts were infectious.

Address for correspondence: Ronald Fayer, USDA, ARS, IDRL, 10300 Baltimore Avenue, Building 1040, Beltsville, MD 20705, USA; fax: 301-504-5306; e-mail: rfayer@lpsi.barc.usda.gov.

The Study

From 43 commercial oyster harvesting sites where the Maryland Department of Natural Resources makes routine annual collections, seven were selected to test for the presence of *C. parvum* oocysts (Table). Approximately 30 oysters were examined from each site on three occasions (Table). From each oyster, 3 to 5 ml of hemolymph was aspirated from the adductor muscle. All gill tissue from each oyster was excised and washed in 5 ml of PBS. For examination by immunofluorescence microscopy, 200 µl of hemolymph and gill washing from each oyster was air dried overnight. Slides were stained with Merifluor fluorescein-labeled anti-*Giardia* and anti-*Cryptosporidium* monoclonal antibodies (Merifluor; Meridian Diagnostics, Cincinnati, OH) and examined with an epifluorescence microscope equipped with a fluorescein isothiocyanate-Texas Red dual wavelength filter. Specimens were considered positive when round bodies 4.5 to 5.5 µm in diameter with distinct green fluorescing walls were identified.

Hemolymph and gill washings from six oysters were pooled, resulting in five aliquots from each collection site. Pooled aliquots were tested for infectivity in mice and examined by polymerase chain reaction (PCR) for the presence of *C. parvum*-specific DNA.

Table. Identification of *Cryptosporidium parvum* oocysts recovered from oysters in the Chesapeake Bay

Site	Location	Bay location or river system	Fall 1997			Winter 1998			Fall 1998			
			IFA	PCR[a]	Mice infectivity	IFA	PCR[a]	Mice infectivity	IFA	Cp11	Mice infectivity	Water[b]
A	Mt. Vernon Wharf	Wicomico	28[c]	ND	Neg[d]	15	BT	Pos	4	Pos	Neg	ND
B	Wetipquin	Nanticoke	29	BT	Neg	3	Neg	Neg	8	Pos	Neg	79
C	Halfway Mark	Fishing Bay	29	HT	Neg	0	BT & HT	Neg	1	Pos	Neg	ND
D	Beacon	Potomac	26	BT	ND	ND	ND	ND	2	Pos	Pos	10
E	Holland Point	Patuxent	28	BT	Pos	ND	ND	ND	1	Pos	Neg	31
F	Back Cove	Tangier Sound	ND	ND	ND	2	BT	Neg	6	ND	Neg	8
G	Old Woman's Leg	Tangier Sound	ND	ND	ND	0	BT	Neg	0	ND	Neg	ND

[a]Polymerase chain reaction and restriction fragment-length polymorphism (PCR-RFLP) on small subunit rRNA gene, 18s.
[b]Number of oocysts recovered per liter of filtered bay water.
[c]Number of oysters found positive for oocysts out of 30 oysters examined from each site.
[d]Neg indicates that PCR using Cp11 primers failed to detect *Cryptosporidium* DNA in the DNA extracted from the ilea of mice that were intubated with pooled hemolymph and gill washings from oysters.
HT, human genotype; BT, bovine genotype; ND, not done; IFA, immunofluorescent assay; PCR, polymerase chain reaction.

Three hundred to 400 µl of each of the five aliquots of pooled hemolymph and gill washings from each site was administered to each of four 7- to 10-day-old BALB/c mice by gastric intubation. Mice were necropsied 96 hours postinoculation, and 1 cm of terminal ileum was placed in DNA extraction buffer to obtain total DNA as described (3). Mouse ileum DNA (100-1,000 ng/reaction) was analyzed for *Cryptosporidium* DNA by PCR, using CP11-P5 and CP11-P6 primers (3). The PCR products were analyzed by polyacrylamide gel electrophoresis and ethidium bromide staining, followed by image capture on a charge coupled device camera.

Pooled hemolymph and gill washings shipped to the Centers for Disease Control and Prevention within 1 week of collection were rinsed three times by repeatedly suspending in 10 ml sterile distilled water and centrifuging at 1,500 X g for 10 min. Supernate was decanted, and pelleted specimens were stored at 4°C until subjected to five freeze-thaw cycles, followed by phenol-chloroform extraction to extract DNA. Purified DNA was dissolved in 50 µl distilled water and stored at -20°C until PCR analysis.

A small subunit rRNA gene-based, nested PCR and restriction fragment-length polymorphism (RFLP) technique developed for species- and genotype-specific diagnosis of *Cryptosporidium* (4,5) was used to characterize oocysts from oysters.

To confirm PCR-RFLP results, all positive secondary PCR products were sequenced. Samples collected in the fall of 1998 were also assayed by nested CP11 PCR (Figure).

At least 50 L of water from each site was filtered by the membrane disk (393-mm diameter, 3-µm pore size, white SSWP [Millipore Corp., Bedford, MA]) method (7). After filtration, an elution protocol (Method 1622) was followed (7). To test the recovery efficiency of this method, 10-L samples of bay water were processed as above, except that four samples were spiked with 10^5 and four others with 10^6 purified *C. parvum* oocysts. The concentration of oocysts in Chesapeake Bay water (Table) was adjusted for the recovery efficiency of the membrane disk method.

Findings

During three collection periods, oocysts corresponding in size and shape to those of *C. parvum* and labeled with fluoresceinated anti-*Cryptosporidium* antibody were detected in oysters collected at six of seven sites (Table). These findings were confirmed in all but one case by positive PCR results for the 18s rRNA gene. Specimens from the one site at which oocysts were not detected by microscopy were found positive by PCR. The presence of oocysts in oysters obtained at the last collection period was confirmed by PCR for the CP11 gene sequence (GenBank accession no. AF124243).

PCR-RFLP testing for the 18s rRNA gene identified two genotypes of *C. parvum* in hemolymph and gill washings from oysters. All

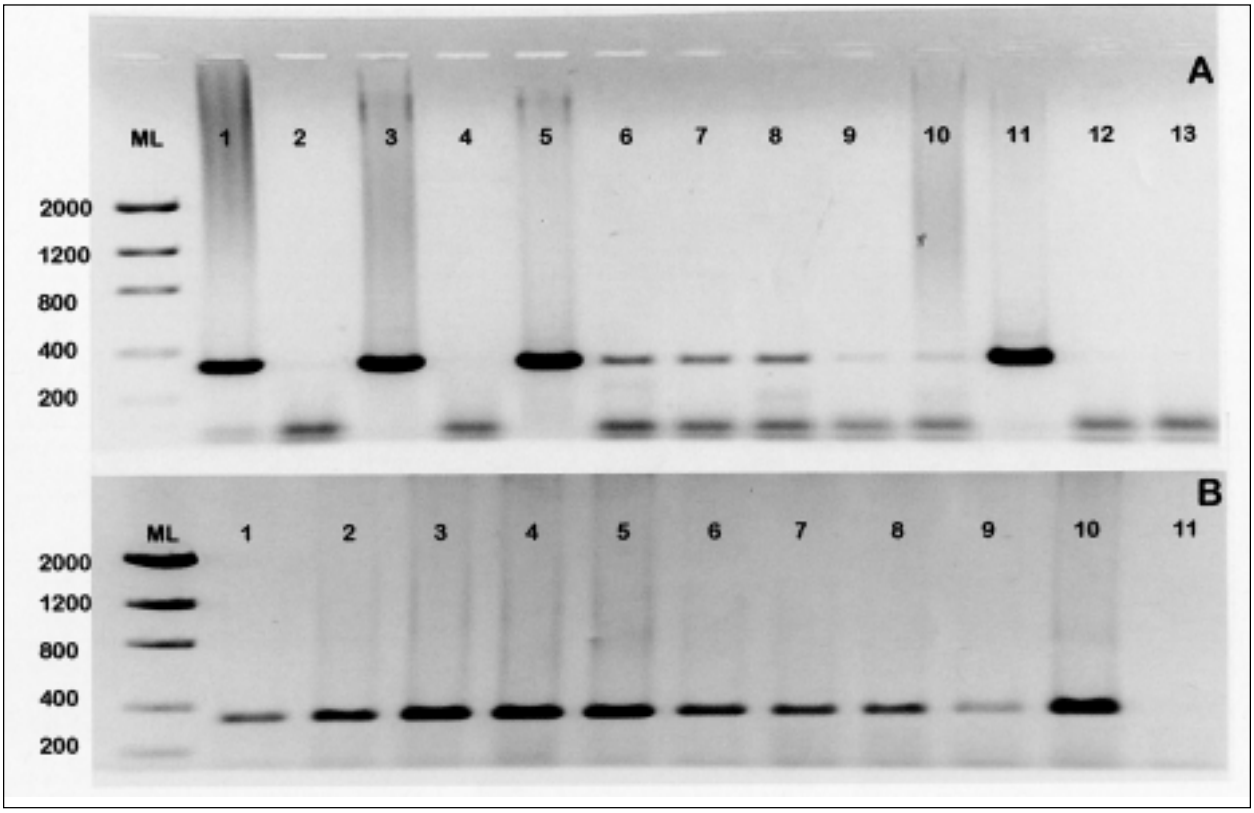


Figure.[1,2] Panel A. Results of nested *Cryptosporidium parvum* CP11 gene PCR performed on pooled oyster hemolymph and gill tissues. Expected PCR product size is 344 bp. Samples analyzed were collected from Maryland Department of Natural Resources oyster harvesting sites at Mt Vernon Wharf (lanes 1-5), Wetipquin (lanes 6-8), Beacon (lane 9), and Holland Point (lane 10). Lane 11: *C. parvum* positive control. Lanes 12 and 13 are 1° and 2° no template controls, respectively. Panel B: Results of oyster (*Crassostrea virginica*) small subunit ribosomal RNA PCR performed on the same oyster tissues analyzed in Panel A, lanes 1-10. Lane 11: no template control. Expected PCR product size is 340 bp.

[1]A PCR product of approximately 1,325 bp was amplified first in primary PCR, by using primers 5'-TTCTAGAGCTAATACATGCG-3' and 5'-CCCTAATCCTTCGAAACAGGA-3'. The PCR reaction contained 10 µl of Perkin-Elmer 10X PCR buffer, 6 mM of $MgCl_2$, 200 µM of each dNTP, 100 nM of each primer, 2.5 U Taq polymerase, and 0.25 µl of DNA template, for a total of 100 µl reaction. Thirty-five cycles each consisted of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1 minute, with an initial hot start at 94°C for 3 minutes and a final extension at 72°C for 7 minutes. A secondary PCR product of 826 or 864 bp (depending on species) was then amplified from 2 µl of the primary PCR reaction, using primers 5'-GGAAGGGTTGTATTTATTAGATAAAG-3' and 5'-AAGGAGTAAGGAACAACCTCCA-3'. The PCR and cycling conditions were identical to primary PCR, except that 3 mm of $MgCl_2$ was used in the PCR reaction. For restriction fragment analysis, 20 µl of the secondary PCR products were digested in a total of 50 µl reaction mix consisting of 20 units of *Ssp* I (New England BioLabs, Beverly, MA; for species diagnosis), or *Vsp* I (Gibco BRL, Grand Island, NY; for genotyping of *C. parvum*) and 5 µl of restriction buffer at 37°C for 1 hour. The digested products were fractionated on 2.0% agarose gel and stained with ethidium bromide.

[2]PCR products were purified first by the Wizard PCR Preps DNA purification system (Promega, Madison, WI), then sequenced by fluorescent cycle sequencing by using dye terminator chemistry on an ABI 377 Automated Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA). Sequences obtained from oyster samples were then aligned with sequences obtained from known *Cryptosporidium* spp. (*C. parvum, C. baileyi, C. muris, C. serpentis, C. meleagridis, C. wrairi*, and *C. felis*) and various *C. parvum* genotypes (human, bovine, monkey, dog, pig, mouse, ferret, marsupial, and desert monitor). Isolates corresponding to those transmissible to humans are reported as human genotype or genotype 1, and isolates corresponding to those transmissible among cattle, mice, and humans are reported as bovine genotype or genotype 2.

As an adjunct to the 18S rRNA assay, a nested PCR was also performed by using primers derived from an 11 kDa protein, extracted from *C. parvum* oocysts. The sequence of outer forward primer P5 is: 5' AAC ATC CAT CGA GTT TAG TA 3' and of outer reverse primer P6 is: 5'GCA AGA GCG CAT TGG TGA AT 3'; the expected PCR product size is 541 bp. The sequence of inner forward primer Cp 11/F is: 5' GTC TAG AAC CGT TAC TGT TAC TGG 3', and of outer reverse primer CP11/R is: 5' CAA CTC CTG GAA GCA TCT TAA CAG 3'; the expected PCR product size is 334 bp.

As a control for the quality of DNA extracted from the oyster tissues, PCR was performed by using primers derived from the small subunit ribosomal RNA sequence of the oyster, *Crassostrea virginica* (6). The OyF forward primer sequence is: 5' GTC TCA AAG ATT AAG CCA TGC ATG 3' (corresponding to nucleotides 34-57), and the OyR reverse sequence is: 5' TGA TTC CCC GTT ACC CGT TAC AA 3' (nucleotides 354-376). The predicted size of the PCR product is 340 bp.

For PCR, pooled oyster gill washings and hemolymph were subjected to nucleic acid extraction by using the Ambion Totally RNA® kit, followed by isopropanol precipitation of nucleic acids and a 70% ethanol wash. The pellet was resuspended in 30-50 µl of molecular biology grade water, and 5-10 µl used as template for PCR. These reactions were performed in 50-µl volumes containing 1.5 mM $MgCl_2$, 1U taq polymerase, 1X PCR buffer, 50 pmol each primer, and 10 mM dNTP mix (Life Technologies, Gaithersburg, MD). Cycling parameters for all reactions were 2 minutes at 95°C, followed by 40 cycles of 95°C for 15 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. Three microliters of primary PCR product was used as template for secondary PCR. PCR products (13-15 µl) were electrophoresed on 1% agarose gels and visualized with ethidium bromide and UV illumination.

For cycle sequencing, the amplicon was cut from a 1% agarose gel, extracted by using the QIAquick Gel Extraction kit (Qiagen, Valencia, CA) and sequenced by using dye-terminator chemistry on the ABI 373 and 377 models fluorescence automated sequencers (Perkin-Elmer Applied Biosystems, Foster City, CA). Sequences were analyzed with AutoAssembler® (Perkin-Elmer Applied Biosystems, Foster City, CA, and Genetics Computer Group, Madison, WI) software.

18s PCR-positive specimens with the exception of those collected from Fishing Bay were bovine genotype. Of two specimens from that location, one contained human genotype alone and the other contained both genotypes.

Eighty aliquots of pooled hemolymph and gill washings were tested for infectivity in mice by PCR of mouse ileum (Table). Oocysts from sites E (2 of 5 aliquots), A (2 of 5 aliquots), and D (5 of 5 aliquots) were found to be infectious at all three collection periods. No other aliquots had positive PCR findings.

The CP11 gene nested PCR was performed on pooled samples, collected in October 1998 from five oyster beds; although no amplicons were observed for the outer primer set P5/P6, all beds were positive by nested PCR (Figure). The nested amplicons from specimens from sites A and C were sequenced and found to have a 99% homology with the *C. parvum* CP11 sequence (data not shown). As a control for the quality of DNA extracted from the oyster tissues, we used a PCR assay for the small subunit of the ribosomal RNA gene of *Crossostrea virginica* (Figure, Panel B). The amplification of this gene fragment from the oysters negative for *Cryptosporidium* by CP11 gene PCR (e.g., Figure, Panel A, sample 2) indicates that PCR-inhibitory substances were successfully removed by using our nucleic acid purification protocol. Accordingly, sample 2 can be considered a true negative for the presence of *Cryptosporidium* oocysts.

The mean recovery efficiency of the membrane disk filtration method was 71.1%, CV=13.3%. Oocysts were detected in water samples collected in fall 1998 from sites B, D, E, and F. The concentration of oocysts at each site ranged from 8 to 79 oocysts/L (Table), with a mean of 32 oocysts/L.

Conclusions

C. parvum oocysts were found in oysters collected from all seven commercial oyster harvesting sites sampled in the Chesapeake Bay. These findings confirm those of previous studies, in which oysters (1) and clams (8) acquired *Cryptosporidium* oocysts from artificially contaminated aquarium water, and oysters (2) and mussels (9) acquired oocysts in nature. Collectively, these findings establish that bivalve molluscs can effectively remove and retain oocysts of *Cryptosporidium* from feces-contaminated estuarine waters.

PCR-RFLP testing for the 18s rRNA gene identified two genotypes of *C. parvum* in hemolymph and gill washings from oysters. Although many species of migratory and residential waterfowl, as well as amphibians, reptiles, and numerous mammals, inhabit the drainage area of sites from which oysters were collected, only the human and bovine genotypes of *C. parvum* were recovered from the oysters.

Results from infectivity studies indicate that only three sites of 16 tested over three collection periods yielded oocysts that produced detectable infections in mice. Based on positive IFA and PCR findings, 16 collections contained *C. parvum* bovine genotype oocysts. The low rate of infectivity for mice may reflect the small number of oocysts that were administered to each mouse of a lack of infectivity due to age or unknown environmental effects.

Neither the age of the oocysts nor how long they may have been on land, in the water, or retained by the oysters could be determined. In a previous study, oysters retained oocysts for at least 1 month after exposure and the oocysts infected mice when tested 1 week after exposure (1). In this study, salinity values and water temperatures during the three successive collection periods (based on data recorded at site A) were 9.0, 6.0, and 15.0 ppt and 12.0, 9.0, and 16.0°C, respectively. Oocysts suspended in 10 and 20 ppt artificial seawater at 20°C retained infectivity for mice when held for 12 and 8 weeks, respectively (2). Therefore, freshly deposited oocysts at these sites could have retained infectivity for 2 to 3 months.

At all sites sampled, examination of gill washings and hemolymph by both IFA microscopy and PCR revealed the presence of *C. parvum* oocysts. This finding indicates that water at these sites contained human or animal feces when oysters were filtering and that oocysts excreted in those feces were acquired by the oysters. Because oocysts of this species are infectious for humans but can be rendered noninfectious by heating to temperatures above 72°C (10), we recommend that oysters be cooked before being eaten, especially by persons with any type of immunodeficiency. Oocysts can also be rendered noninfectious by freezing at -20°C for 24 hours (11), but because viral or bacterial pathogens might also be acquired by oysters from water contaminated with feces and can survive freezing, we recommend cooking rather than freezing.

Acknowledgments

We thank John Collier for assisting in the collection of oysters and Colleen Carpenter and Anjeli Sonstegard for technical assistance.

This study was funded in part by Maryland SeaGrant, R/F-88 and funding from CDC's Food Safety Initiative.

Dr. Fayer is a senior scientist at the U.S. Department of Agriculture's Agricultural Research Service, Beltsville, Maryland. His primary area of research is zoonotic diseases with an emphasis on foodborne and waterborne parasitic protozoa.

References

1. Fayer R, Farley CA, Lewis EJ, Trout JM, Graczyk TK. Potential role of the Eastern oyster, *Crassostrea virginica*, in the epidemiology of *Cryptosporidium parvum*. Appl Environ Microbiol 1997;63:2086-8.
2. Fayer R, Graczyk TK, Lewis EJ, Trout JM, Farley CA. Survival of infectious *Cryptosporidium parvum* oocysts in seawater and Eastern oysters (*Crassostrea virginica*) in the Chesapeake Bay. Appl Environ Microbiol 1998;64:1070-4.
3. Jenkins MC, Trout J, Fayer R. Development and application of an improved semiquantitative technique for detecting low-level *Cryptosporidium parvum* infections in mouse tissue using polymerase chain reaction. J Parasitol 1998;84:182-6.
4. Xiao L, Escalante L, Yang C, Sulaiman I, Escalante AA, Montali J, et al. Phylogenetic analysis of *Cryptosporidium* parasites based on the small subunit ribosomal RNA gene locus. Appl Environ Microbiol 1999;65:111578-83.
5. Xiao L, Morgan U, Limor J, Escalante AA, Arrowood M, Shulaw W, et al. Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl Environ Microbiol 1999;65:3386-96.
6. Littlewood DT, Ford SE, Fong D. Small subunit rRNA gene sequence of *Crassostrea virginica* (Gmelin) and a comparison with similar sequences from other bivalve molluscs. Nucleic Acids Res 1991;19:6048.
7. U.S. Environmental Protection Agency. Method 1622: *Cryptosporidium* in water by filtration/IM/FA. Washington: The Agency; 1997. EPA 821-R-97-023:51.
8. Graczyk TK, Fayer R, Cranfield MR, Conn DB. Recovery of waterborne *Cryptosporidium parvum* oocysts by freshwater benthic clams (*Corbicula fluminea*). Appl Environ Microbiol 1998;64:427-30.
9. Graczyk TK, Fayer R, Lewis EJ, Trout JM, Farley CA. *Cryptosporidium* oocysts in Bent mussels (*Ischadium recurvum*) in the Chesapeake Bay. Parasitol Res 1999;85:30-4.
10. Harp JA, Fayer R, Pesch BA, Jackkson GJ. Effect of pasteurization on infectivity of *Cryptosporidium parvum* oocysts in water and milk. Appl Environ Microbiol 1996;62:2866-8.
11. Fayer R, Nerad T. Effects of low temperatures on viability of *Cryptosporidium parvum* oocysts. Appl Environ Microbiol 1996;62:1431-3.

Seroepidemiology of *Bartonella vinsonii* subsp. *berkhoffii* Infection in California Coyotes, 1994-1998[1]

Chao-chin Chang,* Kazuhiro Yamamoto,* Bruno B. Chomel,* Rickie W. Kasten,* Darren C. Simpson,† Charles R. Smith,‡ and Vicki L. Kramer‡

*University of California, Davis, California, USA; †Santa Clara County Department of Health Services, San Jose, California, USA; ‡California Department of Health Services, Sacramento, California, USA

The prevalence of antibodies to *Bartonella vinsonii* subsp. *berkhoffii* in coyotes (*Canis latrans*) in California ranged from 51% in central to 34% in southern and 7% in northern California. Seropositive coyotes were more likely to be from coastal than inland counties ($p < 0.05$). The clustered distribution of *Bartonella* seropositivity in coyotes suggests that *B. vinsonii* subsp. *berkhoffii* infection is vectorborne. Further investigation is warranted to evaluate which arthropods are vectors and what the mode of transmission is from wildlife to domestic dogs and possibly humans.

Bartonella species are small gram-negative bacteria, which have been identified in a wide range of mammalian species, including felines and rodents (1-3). Several *Bartonella* species are human pathogens (4). Bacillary angiomatosis, initially recognized in AIDS patients, was related in the early 1990s to a new bacterium, *Bartonella henselae* (5,6), which was later associated with cat scratch disease (4). Clinical entities have been associated with several *Bartonella* species in humans; for example, *B. henselae*, *B. quintana*, and *B. elizabethae* have been associated with human cases of endocarditis (7-11). *B. vinsonii*, isolated only from small rodents, has not yet been confirmed as a cause of human disease (12). *B. vinsonii* subsp. *berkhoffii*, was recently isolated from a dog with endocarditis (13). It was found that 3.6% of 1,920 dogs in North Carolina and Virginia were seropositive to *B. vinsonii* subsp. *berkhoffii* antigen (14). *Bartonella*-seropositive dogs were 5.6 times more likely to be flea infested than were seronegative dogs, but 14 times more likely to have a history of heavy tick exposure. For the dogs with known tick exposure, a high correlation was found between seroreactivity to *B. vinsonii* subsp. *berkhoffii* and seroreactivity to *Ehrlichia canis* and *Babesia canis*, both well-known tickborne infections. Furthermore, Breitschwerdt et al. (15) reported that dogs infected with *Ehrlichia* species were frequently coinfected with *B. vinsonii* subsp. *berkhoffii*, suggesting that this infection in dogs could also be tickborne.

In July 1996, a 3 1/2-year-old boy was bitten by a coyote (*Canis latrans*) in Santa Clara County, CA, and became ill with fever and lymphadenopathy. The incident prompted an investigation of possible *Bartonella* infection in the boy and coyotes. Because of the clinical signs in the child and the absence of *Francisella tularensis* antibodies in two coyotes, the coyotes were tested for *Bartonella* antibodies by an immunofluorescence test with a *B. henselae* antigen, and both tested positive (titers of 1:128 and 1:512). *B. vinsonii* subsp. *berkhoffii*, identified by both polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) of the citrate synthase and 16S rRNA genes and by partial sequencing of the 16S rRNA gene, was isolated from several coyotes from Santa Clara County (3). The PCR/RFLP profiles of the coyote isolates and the domestic dog isolate were the same (ATCC 51672 strain).

Address for correspondence: Bruno B. Chomel, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, 95616, USA; fax: 530-752-2377; e-mail: bbchomel@ucdavis.edu.

[1]An earlier version of this paper was presented at the Second International Conference on Emerging Zoonoses, Strasbourg, France, November 5–9, 1998.

To determine the geographic distribution of *B. vinsonii* subsp. *berkhoffii* infection in California coyotes, we analyzed data for age, sex, and origin of the animals to establish possible risk factors associated with seropositivity and to identify high-risk coyote populations. Since coyotes (*C. latrans*) belong to the same genus as domestic dogs (*C. familiaris*), wild canids may serve as a reservoir of *B. vinsonii* subsp. *berkhoffii*, and transmission to domestic dogs could occur through common ectoparasites.

The Study

Coyote blood samples (nobuto filter strips or serum) collected from 34 of the 58 counties in California from 1994 to 1998 were tested serologically for *B. vinsonii* subsp. *berkhoffii* antibodies. Age, sex, county of sample collection, and collection date were recorded.

For 869 specimens, specific antibodies against a *B. vinsonii* subsp. *berkhoffii* purified antigen (outer membrane proteins) were detected by enzyme-linked immunosorbent assay (16). Cut-off values were established as described (17,18). The cut-off value (Optical Density [OD] > 0.2) for seropositivity was determined by the average OD plus three standard deviations (SD) of 76 nobuto strips from counties where all OD values were < 0.190. Statistically, animals with OD values > 0.2 can be considered to be seropositive with 99% confidence.

Univariate analysis by chi-square and Fisher exact tests was applied first to screen for factors associated with seropositivity to *B. vinsonii* subsp. *berkhoffii*. Potential confounders were evaluated by 10% change of estimates, i.e., odds ratio. Multiple logistic regression analysis was applied to evaluate the adjusted effects of the associated factors.

The overall prevalence of *B. vinsonii* subsp. *berkhoffii*-seropositive coyotes was 35% (95% confidence interval [CI]: 25% to 48%). *Bartonella* antibody prevalence varied from 51% in central California to 34% in southern and 7% in northern California (Table 1). The prevalence of seropositive coyotes from coastal counties was significantly higher than that of seropositive coyotes from inland counties (Figure) in all three geographic areas ($p < 0.05$). No gender difference in seropositivity was detected. Antibody prevalence was higher in adult coyotes (37%) than in young coyotes (< 1 year of age) (29%) (Table 2). The prevalence of high antibody titers (OD > 0.5) was

Table 1. Seroprevalence of *Bartonella vinsonii* subsp. *berkhoffii* infection in 869 coyotes, California, 1994–1998

Geographic region	County	No. seropositive	(%)
Northern California		13/174	(7)
Coastal counties	Humboldt	2/12	(17)
	Mendocino	7/30	(23)
Inland counties	Siskiyou	0/26	(0)
	Modoc	0/47	(0)
	Lassen	1/7	(14)
	Plumas	1/40	(3)
	Butte	0/2	(0)
	Trinity	0/1	(0)
	Shasta	2/9	(22)
Central California		180/355	(51)
Coastal counties	Solano	0/4	(0)
	Sonoma	1/3	(33)
	Alameda	14/32	(44)
	Santa Clara	116/167	(69)
	Santa Cruz	1/1	(100)
Inland counties	Sierra	6/32	(19)
	Nevada	2/10	(20)
	Placer	0/1	(0)
	El Dorado	26/63	(41)
	Alpine	0/2	(0)
	Calaveras	0/2	(0)
	Tuolumne	4/8	(50)
	Mono	0/7	(0)
	Mariposa	4/7	(57)
	Merced	2/2	(100)
	Sacramento	3/3	(100)
	Madera	1/11	(9)
Southern California		115/340	(34)
Coastal counties	Monterey	4/10	(40)
	San Luis Obispo	18/56	(32)
	Santa Barbara	27/45	(60)
	Ventura	0/1	(0)
	Los Angeles	15/26	(58)
	San Diego	6/6	(100)
Inland counties	Kern	41/190	(22)
	San Bernardino	4/6	(67)

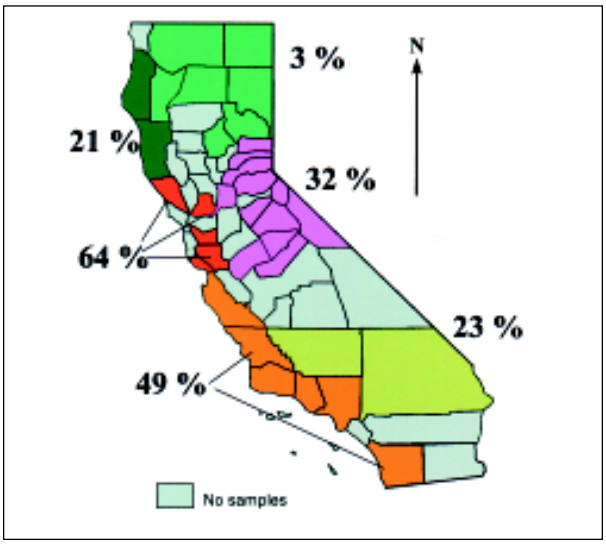


Figure. *Bartonella vinsonii* subsp. *berkhoffii* seroprevalence in 869 California coyotes (1994–1998).

Table 2. Seroprevalence of *Bartonella vinsonii* subsp. *berkhoffii* infection, by age, sex, and season, for 869 coyotes, California, 1994–1998

Characteristic	No. seropositive (%)
Age[a]	
<1 year old	35/122 (29)
≥1 year old	273/745 (37)
Sex[b]	
Female	155/407 (38)
Male	152/455 (33)
Season[c]	
Spring (Mar to May)	86/296 (29)
Summer (Jun to Aug)	121/286 (42)
Fall (Sep to Nov)	67/182 (37)
Winter (Dec to Feb)	27/85 (32)

[a]Not available for two coyotes.
[b]Not available for seven coyotes.
[c]Not available for 20 coyotes.

Table 3. Annual *Bartonella vinsonii* subsp. *berkhoffii* antibody prevalence in 869 coyotes, by age group, California, 1994–1998

Year	All ages no. (%)	≥1 year old no. (%)	<1 year old no. (%)
1994	22/62 (35)	21/61 (34)	1/1 (100)
1995	55/159 (35)	53/146 (36)	2/13 (15)
1996[a]	47/124 (37)	37/100 (37)	9/23 (39)
1997[a]	115/275 (42)	107/238 (45)	8/36 (19)
1998	70/249 (28)	55/200 (28)	15/49 (31)

[a]Age not available for one coyote.

similar in adults (20 [7.3%] of 273) and young coyotes (3 [8.6 %] of 35). There were no major differences in prevalence by age groups for each of the three following OD groups (OD > 0.2 and ≤ 0.3 [young coyotes, 37%; adults, 48%]; OD > 0.3 and ≤ 0.4 [young coyotes, 40%; adults, 27%]; and OD > 0.4 and ≤ 0.5 [young coyotes, 14%; adults; 19%]). Summer (June to August) had the highest prevalence (42%) of *Bartonella*-seropositive coyotes and spring had the lowest (29%) (Table 2). However, in young coyotes, antibody prevalence increased from 23% (3/13) in winter and 24% (6/25) in spring to 28% (12/43) in summer and 33% (13/39) in fall. Of the 25 coyotes < 6 months old, neither of the two captured in spring were seropositive, whereas 20% of the 10 captured in summer and 46% of the 13 captured in fall were seropositive.

Antibody prevalence was relatively constant during the 5-year period, from a low of 28% in 1998 to a high of 42% in 1997 (Table 3). In 1997, the prevalence was significantly higher in adults than in young coyotes (odds ratio [OR] = 2.86, 95% CI = 1.2, 7.5). This difference was associated with the high prevalence of positive adults from coastal central (50 [82%] of 61) and southern (20 [77%] of 26) California. That year, none of the young coyotes from inland areas were seropositive, but 5 (62%) of 8 and 4 (80%) of 5 from coastal central and southern California were positive. Overall, infection occurred more frequently in young coyotes from coastal central and southern California (29 [57%] of 51) than from inland areas (5 [15%] of 34) (OR = 7.65, 95% CI = 2.3, 26.9).

By multiple logistic regression analysis, after adjustment for age, odds ratios for central California and southern California were 9.6 (95% CI: 5.2, 17.7) and 5.2 (95% CI: 2.8, 9.7) times higher, respectively, than for northern California. The adjusted odds ratio for the coastal counties compared with inland counties was 3.7 (95% CI: 2.7, 5.0). Similarly, coyotes ≥ 1 year old were 60% more likely to be seropositive (OR = 1.6, 95% CI = 1.0, 2.6) than young coyotes.

Conclusions

Because the discovery of *B. vinsonii* subsp. *berkhoffii* is so recent, little is known about the epidemiology and the mode of transmission of this organism in domestic dogs and wild canids. Of 54 coyotes from Santa Clara County, 16 (30%) were *B. vinsonii* subsp. *berkhoffii* bacteremic (19). Therefore, coyotes could serve as a potential reservoir for *B. vinsonii* subsp. *berkhoffii*, which could also be transmitted to domestic dogs, either by mechanical means (biting and scratching) or through arthropod vectors. *Bartonella* spp. are usually transmitted by arthropod vectors. Cats are the main reservoir for *B. henselae* and cat fleas are the main vector for transmission between cats (20,21). No direct transmission of *B. henselae* from cat to cat has been documented experimentally (22,23). Therefore, *B. vinsonii* subsp. *berkhoffii* transmission among coyotes and between coyotes and dogs is more likely to be vectorborne. Furthermore, the seasonal trends of *Bartonella* antibody prevalence in coyotes, especially young coyotes, indicate a moderate seasonal peak in summer and fall compared with winter and spring, when ectoparasites are the most abundant (14).

The high *Bartonella* antibody prevalence, especially in young coyotes, in coastal areas of central and southern California, indicates active transmission in these counties compared with the low prevalence of infection in young coyotes in inland areas. The clustered distribution of infection in young coyotes in California may be

associated with the mode of transmission of the infection, possibly through arthropods.

Limited data on the actual distribution of various arthropods throughout California are available. As suggested by Pappalardo et al. (14) and Breitschwerdt et al. (15), ticks could be potential vectors for *Bartonella* transmission in domestic dogs. The clustered distribution of *Bartonella* infection in California coyotes shown in this study seems to coincide with the known geographic distribution of tick species (*Ixodes pacificus* and *Dermacentor variabilis*) that can feed on carnivores in California (24). According to the studies of Chaniotis et al. (25) and Ayala et al. (26), the distribution of sandflies in California is mainly in the Upper Sonoran zone of central California. This distribution could also match the coastal range of *Bartonella* infection in coyotes. Since sandflies are well-known vectors for *B. bacilliformis* transmission, they could also be considered for *Bartonella* spp. transmission in coyotes. Nevertheless, further studies need to be conducted to verify this hypothesis. In domestic carnivores, flea infestation is common and widespread in most of California. Cat fleas (*Ctenocephalides felis*) have been shown to be responsible for *B. henselae* infection in cats, and they also can infest domestic dogs and possibly coyotes. Although flea transmission cannot be completely ruled out, the dog study conducted in the Eastern United States showed that ten control dogs with heavy flea infestation but no known tick exposure had no seropositivity to *B. vinsonii* subsp. *berkhoffii* (14).

Only a few infectious agents can be transmitted by multiple vectors, e.g., tularemia and relapsing fever. Therefore, further investigation will be necessary to determine which of all the potential vectors mentioned above can harbor *B. vinsonii* subsp. *berkhoffii* under natural conditions and which one plays a major role for *B. vinsonii* subsp. *berkhoffii* transmission in canids. Furthermore, it is necessary to evaluate the risk of domestic dog infection with *Bartonella* spp. from wild canids and the potential risk of transmission to humans.

Acknowledgments

The authors thank the California County Health Offices and their trappers for providing blood samples from coyotes as part of the California Plague Surveillance Program and Pamela K. Swift for initiating the coyote testing.

Dr. Chang is a doctoral student in Epidemiology at the University of California, Davis, under the direction of Dr. Bruno B. Chomel. His research interests include molecular epidemiology of *Bartonella* infections and potential vectors for *Bartonella* spp. transmission.

References

1. Birtles RJ, Fichet-Calvet E, Raoult D, Ashford RW. Detection and genotypic differentiation of *Bartonella* species infecting a Tunisian *Psammomys obesus* population. Proceedings from 13th Sesqui-Annual Meeting of the American Society for Rickettsiology; 1997 Sep 21-24; Champion, Pennsylvania. Abstract 34.
2. Heller R, Kubina M, Delacour G, Mahoudeau I, Lamarque F, Artois M, et al. Prevalence of *Bartonella* spp. in blood of wild small rodents. Abstracts of the 97th General Meeting of the American Society for Microbiology; 1997 May 4-7;Miami Beach, Florida. Washington: The Society, 1997. p. 115.
3. Chomel BB, Kasten RW, Chang CC, Yamamoto K, Heller R, Maruyama S, et al. Isolation of *Bartonella* spp. from California wildlife. Abstracts of the International Conference on Emerging Infectious Diseases; 1998 Mar 8-11; Atlanta, Georgia. p. 21.10.
4. Anderson BE, Neuman MA. *Bartonella* spp. as emerging human pathogens. Clin Microbiol Rev 1997;10:203-19.
5. Slater LN, Welch DF, Min KW. *Rochalimaea henselae* causes bacillary angiomatosis and peliosis hepatitis. Arch Intern Med 1992;152:602-6.
6. Welch DF, Pickett DA, Slater LN, Steigerwalt AG, Brenner DJ. *Rochalimaea henselae* sp. nov., a cause of septicemia, bacillary angiomatosis, and parenchymal bacillary peliosis. J Clin Microbiol 1992;30:275-80.
7. Daly JS, Worthington MG, Brenner DJ, Moss CW, Hollis DG, Weyant RS, et al. *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. J Clin Microbiol 1993;31:872-81.
8. Spach DH, Callis KP, Paauw DS, Houze YB, Schoenknecht FD, Welch DF, et al. Endocarditis caused by *Rochalimaea quintana* in a patient infected with human immunodeficiency virus. J Clin Microbiol 1993;31:692-4.
9. Holmes AH, Greenough TC, Balady GJ, Regnery RL, Anderson BE, O'Keane JC, et al. *Bartonella henselae* endocarditis in an immunocompetent adult. Clin Infect Dis 1995;21:1004-7.
10. Drancourt M, Mainardi JL, Brouqui P, Vandenesch F, Carta A, Lehnert F, et al. *Bartonella* (*Rochalimaea*) *quintana* endocarditis in three homeless men. N Engl J Med 1995;332:419-23.
11. Drancourt M, Birtles R, Chaumentin G, Vandenesch F, Etienne J, Raoult D. New serotype of *Bartonella henselae* in endocarditis and cat-scratch disease. Lancet 1996;347:441-3.
12. Baker JA. A rickettsial infection in Canadian voles. J Exp Med 1946;84:37-50.
13. Breitschwerdt EB, Kordick DL, Malarkey DE, Keene B, Hadfield TL, Wilson K. Endocarditis in a dog due to infection with a novel *Bartonella* subspecies. J Clin Microbiol 1995;33:154-60.

14. Pappalardo BL, Correa MT, York CC, Peat CY, Breitschwerdt EB. Epidemiologic evaluation of the risk factors associated with exposure and seroreactivity to *Bartonella vinsonii* in dogs. Am J Vet Res 1997;58:467-71.
15. Breitschwerdt EB, Hegarty BC, Hancock SI. Sequential evaluation of dogs naturally infected with *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia equi*, *Ehrlichia ewingii*, or *Bartonella vinsonii*. J Clin Microbiol 1998;36:2645-51.
16. Chomel BB, Carlos ET, Kasten RW, Yamamoto K, Chang CC, Carlos RS, et al. *Bartonella henselae* and *Bartonella clarridgeiae* infection in domestic cats in the Philippines. Am J Trop Med Hyg 1999;60:593-7.
17. Richardson MD, Turner A, Warnock DW, Llewellyn PA. Computer assisted rapid enzyme linked immunosorbent assay (ELISA) in the serological diagnosis of Aspergillosis. J Immunol Methods 1983;56:201-7.
18. Magnarelli LA, Anderson CS, Apperson CS, Fish D, Johnson RC, Chappell A. Spirochetes in ticks and antibodies to *Borrelia burgdorferi* in white-tailed deer from Connecticut, New York State, and North Carolina. J Wildl Dis 1986;22:178-88.
19. Chang CC, Yamamoto K, Chomel BB, Kasten RW, Simpson D, Wilson B. Sero-epidemiology of *Bartonella* infection in wild canids in California. Proceedings of the International Conference on Emerging Zoonoses; 1998 Nov 5-9; Strasbourg, France. p. 58.
20. Koehler JE, Glaser CA, Tappero JW. *Rochalimaea henselae* infection—a new zoonosis with the domestic cat as reservoir. JAMA 1994;271:531-5.
21. Chomel BB, Kasten RW, Floyd-Hawkins K, Chi B, Yamamoto K, Roberts-Wilson J, et al. Experimental transmission of *Bartonella henselae* by the cat flea. J Clin Microbiol 1996;34:1952-6.
22. Abbott RC, Chomel BB, Kasten RW, Floyd-Hawkins KA, Kikuchi Y, Koehler JE, et al. Experimental and natural infection with *Bartonella henselae* in domestic cats. Comp Immunol Microbiol Infect Dis 1997;20:41-51.
23. Guptill L, Slater L, Wu CC, Lin TL, Glickman LT, Welch DF, et al. Experimental infection of young specific pathogen-free cats with *Bartonella henselae*. J Infect Dis 1997;176:206-16.
24. Furman DP, Loomis EC. The ticks of California (Acari: Ixodida). Berkeley: University of California press, 1984.
25. Chaniotis BN, Anderson JR. Age structure, population dynamics and vector potential of *Phlebotomus* in northern California. II. Field population dynamics and natural flagellate infections in parous females. J Med Entomol 1968;5:273-92.
26. Ayala SC, Lee D. Saurian malaria: development of sporozoites in two species of phlebotomine sandflies. Science 1970;167:891-2.

Epidemic Typhus Imported from Algeria

M. Niang,* P. Brouqui,*† and D. Raoult†
*Hopital Felix Houphoüet Boigny, Marseilles, France; and †Universitè de la Mèditerranèe, Facultè De Mèdecine, Marseilles, France

We report epidemic typhus in a French patient returning from Algeria. The diagnosis was confirmed by serologic testing and the isolation of *Rickettsia prowazekii* in blood. Initially the patient was thought to have typhoid fever. Because body lice are prevalent in industrialized regions, the introduction of typhus to pediculosis-endemic areas poses a serious public health risk.

Epidemic typhus, caused by *Rickettsia prowazekii*, is transmitted in the feces of the infected body louse. Body lice live in clothing and are easily controlled by good personal hygiene. Louse-infested populations are primarily those who live in extreme poverty. Typhus has been associated with war, famine, refugee camps, cold weather, and conditions that lead to domestic crowding and reduced personal hygiene, like those found in Algeria because of the ongoing civil war (1,2).

In October 1998, a 65-year-old man came to the Felix Houphoüet Boigny Hospital of Tropical Medicine in Marseilles, France, for evaluation of fever, nausea, vomiting, myalgias, and diarrhea. He was a native Algerian, who lived in France but had visited Msila, a small town in east central Algeria, for 3 months. The patient recalled pruritis and scratching during his stay in Algeria. However, no lice were found on his clothing. He had a temperature of 40°C, relative bradycardia with a heart rate of 70 beats per minute, blood pressure of 110/60 mm Hg, mild confusion, a discrete rash (a few rose spots on the trunk) (Figure 1), and splenomegaly. Laboratory findings showed a normal white blood cell count and increased serum lactate dehydrogenase (LDH 1032 IU/L) and aspartate aminotransferase (AST 85 IU/L) concentrations. The chest radiograph was normal. A presumptive diagnosis of typhoid fever was made, and treatment with intravenous ceftriaxone, 3 g/day (3), was started immediately after samples of blood, urine, and stool were obtained for bacterial cultures. All cultures remained negative on hospital day 3, but the patient's clinical condition continued to worsen. He had now become semicomatose, remained febrile, and had severe dyspnea and purpuric rash (Figure 2).

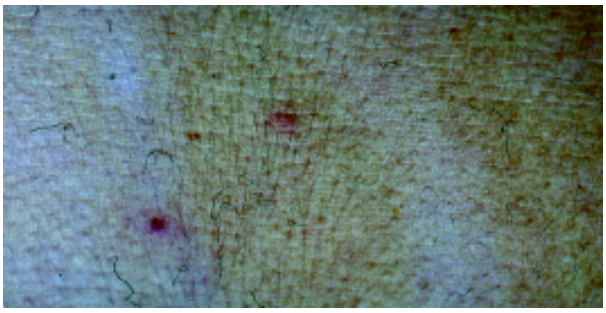


Figure 1. Rose spots-like skin lesion on the trunk (day 4).

Laboratory follow-up showed that the patient had severe thrombocytopenia (4,900

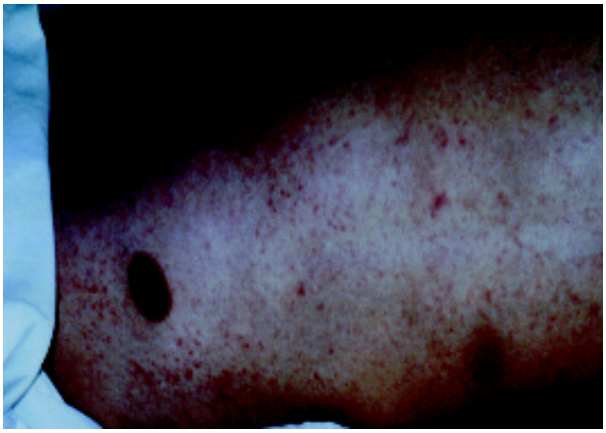


Figure 2. Diffuse petechial rash of epidemic typhus (day 7).

Address for correspondence: D. Raoult, Unitè des Rickettsies, CNRS UPRES A 6020, Universitè de la Mèditerranèe, Facultè de Mèdecine, 27 Boulevard Jean Moulin, 13385 Marseilles Cedex 05, France; fax: 334-91-83-0390; e-mail: Didier.Raoult@medecine.univ-mrs.fr.

thrombocytes/mm^3), and elevated serum creatinine phosphokinase (CK 1,4813 IU/L), AST (422 IU/L), LDH (2,373 IU/L), and creatinine (143 micromoles/L) concentrations. Coagulation studies suggested disseminated intravascular coagulation and acute renal failure (serum creatinine 143 micromoles/L). The patient was severely hypoxic, and a repeated chest radiograph showed bilateral interstitial pneumonia (Figure 3). The diagnosis was now suspected to be typhus (murine or epidemic) rather than typhoid fever, and the patient was transferred to the intensive care unit, where he was started on intravenous doxycycline, 200 mg/d. His clinical condition improved rapidly, and he became afebrile within 3 days. Doxycycline was stopped after 10 days, when the patient had recovered.

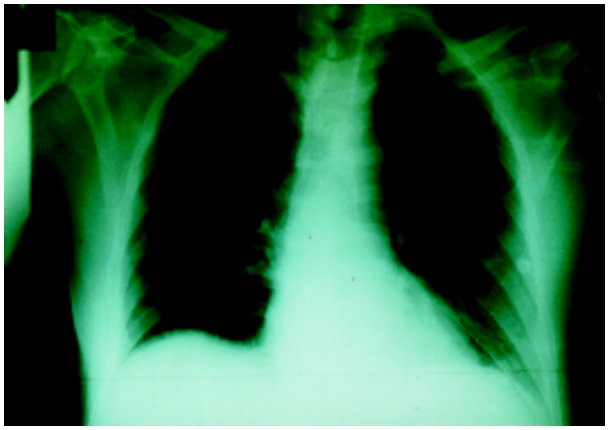


Figure 3. Bilateral interstitial pneumonia (day 7).

The diagnosis of epidemic typhus was established by demonstrating increasing antibody titers from the acute to the convalescent-phase of illness, with the presence of immunoglobulin (Ig) M to *R. prowazekii* (micro-immunofluorescence titer IgG <1:80 and IgM <80 to IgG 1:4,096 and IgM titer 1:256) in serum samples collected 6 days apart (4). The diagnosis was confirmed by the isolation of *R. prowazekii* in blood (shell vial technique), followed by polymerase chain reaction identification of specific DNA in blood by using primers reacting with the citrate synthetase gene and verified by gene product sequencing (4).

Because the patient had returned from Algeria, a country where enteric fever is prevalent, the illness was misdiagnosed as typhoid fever, and the patient was treated with ceftriaxone (3). On average, the rash of epidemic typhus appears on day 5 of illness and consists initially of pink macules in the axillary areas, which subsequently spread over the trunk and limbs and eventually become petechial. While petechiae are reported in 33% of patients with typhus, the initial pinkish macules are reported in only 5% of typhus cases (5). Thus, epidemic typhus may be very difficult to distinguish from typhoid fever in the early phase of illness.

Chloramphenicol has been effective against both typhoid fever and typhus. Co-trimoxazole, which may be used to treat typhoid fever, is not effective against rickettsial agents (6), and a patient who was treated with ciprofloxacin for suspected typhoid fever died of typhus (7). The persistently negative blood culture results, combined with the development of petechiae and failure to improve on ceftriaxone, led us to consider the diagnosis of typhus rather than typhoid fever, since rickettsia are resistant to beta-lactam antibiotics (6). The death rate of untreated epidemic typhus is approximately 15%; this rate is reduced to 0.5% with a single 200-mg dose of doxycycline (1). Therefore, epidemic typhus should be considered in the differential diagnosis of typhoid fever, in particular when epidemiologic conditions may have led to contact with body lice.

Typhus has not been reported in Algeria for several decades (8). Because infection is lifelong, humans are regarded as the main reservoir of the bacteria. Recrudescence in the form of Brill-Zinsser disease may act as the source of an outbreak, but wide spread cannot occur without louse infestation, as recent outbreaks in Burundi (1) and Russia (2) have shown. Body lice are prevalent among the homeless in industrialized regions such as Marseilles, France. The importation of typhus by an infected patient could start an outbreak within this exposed population, as has occurred with trench fever (9). Disease surveillance, delousing of exposed persons, and improvement of the living conditions of populations at risk should prevent the reemergence of louse-borne diseases in industrialized countries.

Acknowledgment

The authors thank J. Bakken for review of the manuscript for English.

Dr. Niang is a fellow in the infectious disease and tropical medicine unit at the Houphouet Boigny University Hospital, Marseilles, France. His fields of interest are rickettsial diseases and bacterial infections.

References

1. Raoult D, Ndihokubwayo JB, Tissot-Dupont H, Roux V, Faugere B, Abegbinni R, et al. Outbreak of epidemic typhus associated with trench fever in Burundi. Lancet 1998;352:353-8.
2. Tarasevich I, Rydkina E, Raoult D. Outbreak of epidemic typhus in Russia. Lancet 1998;352:1151.
3. Islam A, Butler T, North S. Randomized treatment of patients with typhoid fever by using ceftriaxone or chloramphenicol. J Infect Dis 1988;158:742-7.
4. La Scola B, Raoult D. Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. J Clin Microbiol 1997;35:2715-27.
5. Perine PL, Chandler BP, Krause DK, McCardle P, Awoke S, Habte-Gabr E, et al. A clinico-epidemiological study of epidemic typhus in Africa. Clin Infect Dis 1992;14:1149-58.
6. Raoult D, Roux V. Rickettsioses as paradigms of new or emerging infectious diseases. Clin Microbiol Rev 1997;10:694-719.
7. Zanetti G, Francioli P, Tagan D, Paddock CD, Zaki SR. Imported epidemic typhus. Lancet 1998;352:1709.
8. Le Corroller Y, Neel R, Lecubarri R. Le typhus exanthematique au Sahara. Arch Inst Pasteur Alger 1970;48:125-30.
9. Brouqui P, Lascola B, Roux V, Raoult D. Chronic *Bartonella quintana* bacteremia in homeless patients. N Engl J Med 1999;340:184-9.

Human and Animal Epidemic of *Yersinia enterocolitica* O:9, 1989–1997, Auvergne, France

Florence Gourdon,* Jean Beytout,* Alain Reynaud,† Jean-Pierre Romaszko,* Didier Perre,‡ Philippe Theodore,‡ Hélène Soubelet,‡ and Jacques Sirot*

*University Hospital of Clermont-Ferrand, Clermont-Ferrand, France; †Laboratoire Départemental d'Analyses Vétérinaires, Lempdes, France; ‡Direction Départementale de l'Agriculture et de la Forêt, Préfecture du Puy-de-Dôme, Lempdes, France

Yersinia enterocolitica O:9 infections were reported in Auvergne in 1988 to 1989, while brucellosis due to *Brucella abortus* was almost eliminated. The serologic cross-reactions between the two bacteria complicated the diagnosis of brucellosis cases. In 1996, human cases of *Yersinia enterocolitica* O:9 infection were detected, with a peak incidence of 12 cases. Veterinary surveillance could have predicted the emergence of this disease in humans.

In Auvergne, a cattle-raising area in central France, brucellosis control measures have been strictly observed since 1965, and systematic vaccination was stopped in 1983. Active surveillance is conducted on the basis of clinical findings (abortions or orchitis) and an annual serologic test performed for every animal (rose bengal plate agglutination test or complement fixation test); abortions and orchitis have to be bacteriologically confirmed. When infected animals are detected, a second test on a new sample drawn 2 weeks later is required for confirmation. When an animal on a farm is infected, the herd is slaughtered. This policy has resulted in a dramatic decrease in the prevalence of brucellosis, and very few cases were reported in 1988 (1). In 1988, however, several animals had positive tests for brucellosis. These positive reactions apparently were associated with an epizootic due to *Yersinia enterocolitica* O:9. The bacterium was isolated from the stools of cattle and goats in infected herds (2).

Yersinia enterocolitica O:9 shares antigens with *Brucella abortus,* and misdiagnosis can occur because both bacteria produce positive reactions with the Wright agglutination test and immunofluorescent assay (brucellosis) and the agglutination test (yersiniosis) (3). The clinical, biological, and epidemiologic features of the two diseases, however, are quite different. In the 1988 epizootic, *Yersinia* infection, commonly called "atypical brucellosis," affected a few young cattle (<2 years of age) and did not spread to the whole herd. No increase in abortion was noted, the titer of antibodies declined rapidly, and no reaction to the Brucellallergene (Rhone Merieux OCB) dermal test was observed. Epidemiologic and serologic surveillance of infected herds found no evidence of brucellosis infection and allowed restoring them to noninfected status after several months. Epidemiologic surveillance demonstrated that brucellosis decreased, whereas yersiniosis continued to spread throughout the region in the 1990s (Figure 1).

Before the 1988 epizootic, *Y. enterocolitica* human infections were rare in Auvergne. In a 1980-81 survey of infections due to *Yersinia* species, five patients had antibodies against *Y. enterocolitica* O:3, the serogroup commonly found in Europe in this period (4,5). No more than three cases of *Y. enterocolitica* infection were recorded each year at the University Hospital laboratory during 1982 to 1990: none had the serotype O:9. Two cases of human

Corresponding author: Florence Gourdon, University Hospital of Clermont-Ferrand, Hôtel Dieu, Boulevard Leon Malfreyt, 63000 Clermont-Ferrand, France; fax: 33-473-316-264; e-mail: fgourdon@chu-clermontferrand.fr.

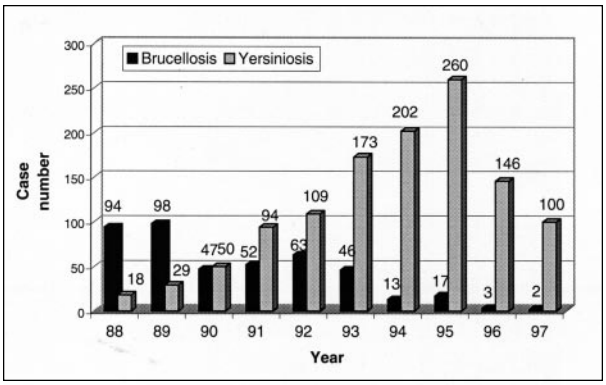


Figure 1. Annual incidence of cattle brucellosis and yersiniosis, Auvergne, France, 1989–1997.

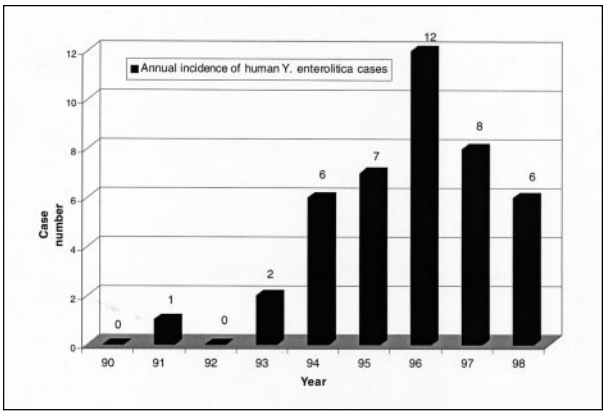


Figure 2. *Yersinia enterocolitica* O:9 infections in humans, Auvergne, France, 1990–1998.

autochthonous brucellosis were detected in 1988 to 1990; in both cases, brucellosis had been detected in the patients' cattle a few months before.

Awareness of *Yersinia* infection was heightened in the regional teaching hospital, but systematic surveillance for patients with diarrhea or abdominal symptoms could not be established. The first human case was detected in 1991; this patient also had positive serologic results for brucellosis but no history of contact with *Brucella*-infected animals; gastrointestinal symptoms suggested yersiniosis (5). Since then, the number of human cases diagnosed in Auvergne has increased, despite the lack of systematic screening for *Yersinia* infection. Human yersiniosis cases were defined by clinical symptoms (fever, gastrointestinal symptoms, arthritis, *erythema nodosum*) associated with a positive serologic test for brucellosis and lack of contact with *Brucella*-infected animals.

In 1996, a retrospective study was done among regional medical laboratories to identify positive brucellosis serologic tests from April 1995 to March 1996. Of eight cases detected, six met criteria for yersiniosis and two had evidence of past brucellosis. Through the end of 1998, 42 cases were recorded, with a peak incidence of 12 cases in 1996 (Figure 2). Gastrointestinal symptoms were found in 35 (83%) patients: diarrhea alone in eight, abdominal pain in six (four patients had surgery [6]), and both in 21. Twelve patients had fever with no other symptoms when they sought medical attention (7), six had arthritis in one or several joints (two with sacroilieitis), and five had *erythema nodosum*. The diagnosis of the last 18 cases was confirmed with an enzyme-linked immunosorbent assay (ELISA) developed and performed by the Laboratoire de Reference des Yersinia of Institut Pasteur. This new ELISA, which uses microtitration plates coated with plasmid-encoded *Yersinia* outer proteins (YOP), is more specific than the agglutination test (8). The results matched the clinical diagnosis of yersiniosis and were consistently negative in patients with brucellosis (six recent or past cases with a positive Wright agglutination test) tested in the same period. Stool samples were negative except for one, but in most cases, gastrointestinal symptoms disappeared before the patients were admitted to the hospital. None of these patients had had contact with *Brucella*-infected animals. Only six were cattle breeders, and seven had recent contact with animals through work or travel. We suspect that most of the patients acquired *Yersinia* through foodborne transmission. Two patients may have eaten the same cheese, although bacteriologic analysis of the cheese could not be performed. Serologic tests for yersiniosis and brucellosis were done for both patients; one was positive for *Brucella*, the other for *Yersinia*. Second specimens were both positive for *Yersinia* by the agglutination test and the new YOP ELISA test.

Yersinia enterocolitica infection is a protean disease (5). Gastrointestinal symptoms are the most frequent. In our series, many patients sought medical attention for persistent fever, night sweats, or secondary features of the disease (7); digestive symptoms were prominent in their history. At this stage of the disease, *Yersinia* could not be isolated from stools. As the common serologic tests (positive either with *Y. enterocolitica* or with *Brucella* antigens) were

not useful, the absence of contact with animals infected with brucellosis was an indication of yersiniosis. Diagnosis could be confirmed by positive YOP ELISA.

The 1996 *Yersinia* epizootic in Auvergne preceded an increase in human cases in central France, where no cases of *Y. enterocolitica* O:9 had previously been detected. The epizootic demonstrates that such emerging disease can be predicted by veterinary surveillance data (9).

Acknowledgments

The authors gratefully acknowledge the help of E. Carniel (Centre de Référence des Yersinia, Institut Pasteur, Paris).

References

1. Douzal Y. Policy of prevention against bovine brucellosis. Actual results. Medecine et Maladies Infectieuses 1993;23(S):507-12.
2. Reynaud A, Delmas C, Vidon JM, Marty-Garrec M, Dubourdieu V. Presence of *Yersinia enterolitica* O:9 in fecal samples of *Brucella abortus* ruminants revealing atypical serological reactions against antigens. Medecine et Maladies Infectieuses 1993;23(S):516-9.
3. Kittelberger R, Reichel MP, Joyce MA, Skaak C. Serological crossreactivity between *Brucella abortus* and *Yersinia enterocolitica* O:9. III. Specificity of the in vitro antigen-specific gamma interferon test for bovine brucellosis diagnosis in experimentally *Yersinia enterocolitica* O:9-infected cattle. Vet Microbiol 1997;57(4):361-71.
4. Verhaegen J, Charlier J, Lemmens P, Delmee M, Van Noyen R, Verbist L, et al. Surveillance of human *Yersinia enterocolitica* infections in Belgium: 1967-1996. Clin Infect Dis 1998;27(1):59-64.
5. Bottone EJ. *Yersinia enterocolitica*: the charisma continues. Clin Microbiol Rev 1997;10(2):257-276.
6. Shorter NA, Thompson MD, Mooney DP, Modlin JF. Surgical aspects of an outbreak of *Yersinia* enterocolitis. Pediatr Surg Int 1998;13(1):2-5.
7. de Kleijn EM, van Lier HJ, van der Meer JW. Fever of unknown origin (FUO). II. Diagnostic procedures in a prospective multicenter study of 167 patients. The Netherlands FUO Study Group. Medicine 1997;76(6):401-14.
8. Benoit C, Guiyoule A, Carniel E. Serodiagnosis of human pathogenic *Yersinia*. Presse Med 1996;25:1627-30.
9. Tauxe RV. Emerging foodborne diseases: an evolving public health challenge. Emerg Infect Dis 1997;3(4):425-34.

A Case of *Corynebacterium pseudodiphtheriticum* Nosocomial Pneumonia

To the Editor: *Corynebacterium pseudodiphtheriticum* has seldom been isolated from patients with upper respiratory tract infections and pneumonia. Most reported infections are community acquired and occur in patients with underlying disease and immunosuppression (1). We report a case characterized by hospital-acquired pneumonia in a debilitated patient. Review of the literature indicates that *C. pseudodiphtheriticum* should be regarded an emerging pathogen.

On April 1, 1998, a 68-year-old woman was admitted to the intensive care unit for acute respiratory distress. She had a 14-month history of amyotrophic lateral sclerosis. Three weeks before admission, she had been hospitalized for *Staphylococcus aureus* pneumonia and had recovered after treatment with amoxicillin and clavulanic acid plus ciprofloxacin. At the time of admission, the patient had a temperature of 38°C. Systolic blood pressure was 120 mm Hg. Ventilation was spontaneous, with respirations 24 per minute; pulmonary sibilants were noted. Respiratory acidosis was also identified, with a pH of 7.35, SaO2 92%, PaO2 60 mm Hg, and PaCO2 60 mm Hg. Laboratory data included 18,000 leukocytes per ml (90% polymorphonuclear cells) and a serum fibrin level of 7 g/L. A chest X-ray showed pneumopathy of the lower segment of the right lung, which was compatible with the diagnosis of inhalation pneumopathy. On day 2 of admission, the patient's temperature was 39°C, and she had paresis of the vocal cords. After *C. pseudodiphtheriticum* infection was diagnosed, treatment with intravenous cloxacillin (l g 3 times/day) and amoxicillin plus clavulanic acid (l g 3 times/day) was started. On day 3 after admission, the patient's breathing worsened, a radiograph showed bilateral pneumopathy, and she was intubated for mechanical ventilation. Two days later, her breathing improved, and a second bronchic aspiration was sterile. The patient eventually died of unrelated complications.

Direct microscopy examination of a Gram-stained bronchial aspiration sample showed numerous polymorphonuclear cells and gram-positive bacilli in parallel rows, which did not show pleomorphism. After 48 hours of incubation at 37°C, 10^6 colony-forming units/ml of a coryneform bacillus further identified as *C. pseudodiphtheriticum* grew in pure culture on blood agar gelose (BioMérieux, La Balme les Grottes, France) under a 5% CO_2 atmosphere and did not produce hemolysis. The test for catalase was positive, and the following biochemical characteristics were obtained by using a commercial identification strip (ApiCoryne, BioMérieux): absence of carbohydrate fermentation, urea hydrolysis, and nitrate reduction compatible with C. *pseudodiphtheriticum*. Minimum inhibitory concentrations (disk diffusion method) were 2 mg/L for amoxicillin, 2 mg/L for cefalotin, 0.09 mg/L for doxycycline, 0.03 mg/L for gentamicin, <4 mg/L for vancomycin, 16 mg/L for erythromycin, and 20 mg/L for trimethoprim-sulfamethoxazole. Identification was confirmed by analysis of the cell-wall fatty acid profile by the Sherlock system, by the trypticase soy broth agar database 3.9 (MIDI Inc., Newark, DE), and by 16S rRNA sequence analysis under previously described conditions (2). The 16S rRNA gene sequence was compared with all eubacterial 16S rRNA sequences available in the GenBank database by using the multisequence comparison Advanced Blast NCBI. The sequence had a 99% similarity to that of *C. pseudodiphtheriticum* (1039/1047 base pairs).

Eighty-nine cases of infection possibly caused by *C. pseudodiphtheriticum* have been identified in the last 57 years. Of these, 57 (62.9%) were upper respiratory infections, which included rhinosinusitis, tracheitis, tracheobronchitis, and bronchitis; 19 (21.3%) were pneumonia (3-7). Ten (11.2%) cases of endocarditis were reported (8); there was also one case each of urinary tract malakoplakia after renal transplantation (9), lung abcess (10), diskitis (11), and lymphadenitis (12).

Unlike *C. diphtheriae, C. pseudodiphtheriticum* is a commensal bacterium that does not produce toxins and needs predisposing factors to become a pathogen causing pneumonia. Of patients with hospital-acquired *C. pseudodiphtheriticum* upper respiratory tract infections and pneumonia (7 of 26 upper respiratory tract infections and 2 of 14 cases of pneumonia reported in the early 1990s), all had underlying pathologic features. Predisposing factors were as follows: 33.7% had lung and tracheobronchial diseases, including chronic obstructive pulmonary disease, angina

(5), chronic emphysema, asthma, and bronchitis (6); 32.5% had congestive heart failure (5). Of those with immunodepression, 5% had AIDS, 7.2% had undergone chemotherapy or prolonged steroid use; and 18.2% had other pathologic features, disseminated intravascular coagulation (6), chronic renal failure, diabetes mellitus (5), and connective tissue disease (5,6).

The first source of pneumonia is usually inhalation, as was the case for our patient, who had paresis of the vocal cords. She was not immunosuppressed but was debilitated by amyotrophic lateral sclerosis. The second factor is often an endotracheal intubation, as reported in a previously healthy 29-year-old trauma victim who contracted pneumonia due to *C. pseudodiphtheriticum* after 7 days of intubation (7). An increase in cases reported from 1932 to 1998 indicates the emergence of infections due to *C. pseudodiphtheriticum*. Thirty-four cases were reported from 1932 to 1989 (57 years), and 55 cases were reported from 1990 to 1998 (8 years). Reasons for the emergence of *C. pseudodiphtheriticum* infections may include confusion between *C. diphtheriae* and *C. pseudodiphtheriticum* infections. For example, two cases of *C. pseudodiphtheriticum* exudative upper respiratory tract infections with a pseudomembrane were first diagnosed as respiratory diphtheria. In the first case, *C. pseudodiphtheriticum* was isolated from a 32-year-old Uzbek man who had a severe sore throat and dysphagia lasting 2 days (3). In a second case, a 4-year-old girl had exudative pharyngitis with a pseudomembrane, which was possibly caused by *C. pseudodiphtheriticum* (4). The availability of commercial strips for the identification of *C. pseudodiphtheriticum* and 16S rRNA sequencing eliminates such confusion.

Cécile Martaresche, Pierre-Edouard Fournier, Véronique Jacomo, Marc Gainnier, Alain Boussuge, and Michel Drancourt
Hôpital Salvator, Assistance Publique-Hôpitaux de Marseille, Marseille, France

References

1. Brown AE. Other *Corynebacteria* and *Rhodococcus*. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 4th ed. New York: Churchill Livingstone; 1995. p. 1874.
2. Weisburg WC, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 1991;173:697-703.
3. Santos MR, Ghandi S, Vogler M, Hanna BA, Holzman RS. Suspected diphtheria in a Uzbek national; isolation of *C. pseudodiphtheriticum* resulted in a false positive presumptive diagnosis. Clin Infect Dis 1996;22:735.
4. Izurieta HS, Strebel PM, Youngblood T, Hollis DG, Popovic T. Exudative pharyngitidis possibly due to C. *pseudodiphtheriticum,* a new challenge in the differential diagnosis of diphtheria. Emerg Infect Dis 1997;3:65-8.
5. Manzella JP, Kellog JA, Parsey KS. *Corynebacterium pseudodiphtheriticum*: a respiratory tract pathogen in adults. Clin Infect Dis 1995;20:37-40.
6. Ahmed K, Kawakami K. *Corynebacterium pseudodiphtheriticum:* a respiratory tract pathogen. Clin Infect Dis 1995;20:41-6.
7. Miller RA, Rompalo A, Coyle MB. *Corynebacterium pseudodiphtheriticum* pneumonia in an immunologically intact host. Diagn Microbiol Infect Dis 1986;4:165-71.
8. Wilson ME, Shapiro DS. Native valve endocarditis due to *Corynebacterium pseudodiphtheriticum*. Clin Infect Dis 1992;15:1059-60.
9. Nathan AW, Turner DR, Aubrey C, Cameron JS, Williams DJ, Ogg CS, et al. *Corynebacterium hofmannii* infection after renal transplantation. Clin Nephrol 1982;17:315-8.
10. Andavolu RH, Venkita J, Lue Y, McLean T. Lung abscess involving *Corynebacterium pseudodiphtheriticum* in a patient with AIDS-related complex. New York State Journal of Medicine 1986;86:594-6.
11. Wright ED, Richards AJ, Edje AJ. Discitis caused by *Corynebacterium pseudodiphtheriticum* following ear, nose and throat surgery. Br J Rheumatol 1995;34:585-6.
12. LaRocco M, Robinson C, Robinson A. *Corynebacterium pseudodiphtheriticum* associated with suppurative lymphadenitis. European Journal of Clinical Microbiology 1987;6:79.

Family Outbreak of *Rickettsia conorii* Infection

To the Editor: Over a 15-day period, three young siblings were separately taken to an emergency room in Israel, with symptoms suggesting a contagious viral illness (fever, maculopapular rash, hepatosplenomegaly, lymphadenopathy, neutropenia, and thrombocytopenia). None of the children had been in direct contact with animals. Specific immunoglobulin (IgM) immunofluorescence assay (IFA) 7 to 8 days after admission of each child confirmed the diagnosis of *Rickettsia conorii* infection.

Spotted fever is the generic name given to a variety of tickborne rickettsial diseases distributed worldwide. In Mediterranean countries, including Israel, spotted fever is caused by members of the *R. conorii* complex. Spotted fever has been endemic in Israel for more than 40 years, with several hundred cases reported annually. In 1997, two fatal cases were reported

(1). Spotted fever is caused by a variant member of *R. conorii,* which is transmitted by the dog tick *Rhipicephalus sanguineous* (2,3). The disease has a broad spectrum of clinical signs, from asymptomatic to fatal (4,5). Symptoms and signs include fever, headache, vomiting, myalgia, conjunctivitis, and a typical maculopapular or purpuric rash. The tache noir at the site of the tick bite, which is found in patients in Europe, is seldom, if ever, seen in Israel.

The first patient, a 6-year-old boy, was taken to the pediatric emergency room with high fever and a diffuse rash, approximately 1 week after visiting a cousin who had similar complaints. Physical examination showed temperature of 40°C, chills, diffuse maculopapular rash all over the body, including the hands and feet, hepatosplenomegaly, and lymphadenopathy. Blood tests showed neutropenia, thrombocytopenia, and hyponatremia. Because *Rickettsia* was included in the differential diagnosis, immunofluorescent assay (IFA) for *Rickettsia* was performed and intravenous doxycycline (2 mg/kg/day) was initiated. One week later, the boy's 8-month-old sister was brought to the emergency room with similar complaints, and 2 days afterwards his 2-year-old sister began to have the same symptoms. A detailed history revealed that all children had played on a lawn frequented by dogs.

All three siblings had fever, chills, and diffuse maculopapular rash all over the body, including the hands and feet. An IgM IFA test for *R. conorii* from the first child was negative on the day of admission and became positive 8 days later. On the day of the boy's hospital discharge, his 8-month-old sister was taken to the emergency room. Her serology test was negative on admission but became positive 7 days later. The third (2-year-old) sibling's first blood test was negative, and the family did not agree to a second blood test. All three children responded well to doxycycline (2 mg/kg/day, with a double dose the first day) for 5 to 7 days. Most symptoms subsided within 48 hours.

Spotted fever is usually a sporadic illness and is not spread from person to person. Clusters of cases have been reported. Yagupsky reported spotted fever in Israel in a few children living near each other in an agricultural settlement (6). A report from the Delaware Division of Public Health described a group of children who had been camping together where contact with ticks was likely (7). This case illustrates that spotted fever may be acquired even without direct contact with animals, through exposure to ticks in places frequented by infected animals. Our report suggests that Rickettsial illness should be considered in the differential diagnosis of fever with rash in disease-endemic areas, even if the timing of similar complaints in several family members suggests a contagious viral illness.

G. Shazberg, J. Moise, N. Terespolsky, and H. Hurvitz
Bikur Cholim General Hospital, Jerusalem, Israel

References

1. Spotted fever. Jerusalem, Israel: Ministry of Health, Department of Epidemiology; 1997.
2. Goldwasser RA, Steiman Y, Klinberg W, Swartz TA, YJingberg MA. The isolation of strains of *Rickettsiae* of the spotted fever group in Israel and their differentiation from other members of the group by immunofluorescence methods. Scand J Infect Dis 1974;6:53-62.
3. Manor E, Ighbarieh J, Sarov B, Kassis 1, Regnery R. Human and tick spotted fever group rickettsia isolated from Israel: a genotypic analysis. J Clin Microbiol 1992;30:2653-6.
4. Wolach B, Franci S, Bogger-Goren S, Drucker M, Goldwasser RA, Sadan N, et al. Clinical and laboratory findings of spotted fever in Israeli children. Pediatr Infect Dis J 1989;8:152-5.
5. Yagupsky P, Wolach B. Fatal Israeli spotted fever in children. Clin Infect Dis 1993;17:850-3.
6. Agupsky P, Sarov B, Sarov I. A cluster of cases of spotted fever in a kibbutz in southern Israel. Scand J Infect Dis 1989;21:155-60.
7. Rotz L, Callejas L, McKechnie D, Wolfe D, Gaw E, Hathcock L, et al. An epidemiologic and entomologic investigation of a cluster of Rocky Mountain spotted fever cases in Delaware. Del Med J 1998;70:285-91.

Iron and the Role of *Chlamydia pneumoniae* in Heart Disease

To the Editor: Chronic infection of the coronary arteries by *Chlamydia pneumoniae* has been proposed as a heart disease risk factor (1). One reason for this proposal is the organism's association with one or more other risk factors for heart disease (2). However, an independent pathogenic role for *C. pneumoniae* in heart disease is unlikely if its presence is only a marker for another risk factor. In the Helsinki Heart Study (3), markers of chronic *C. pneumoniae* infection were a significant risk factor for a cardiac event, independent of most traditional

risk factors; however, some association with known risk factors was seen, including a positive association with smoking and an unexpected negative association with spare-time physical activity.

We postulate a key role for iron, a proposed risk factor for heart disease (4-6), in promoting the growth of *C. pneumoniae* in coronary arteries. Iron is an essential growth factor for nearly all pathogenic microorganisms (7). In particular, the growth of *C. pneumoniae* in a human lung cell line and in Hep-2 cells is strongly inhibited by iron restriction or by use of the iron chelator deferoxamine (8, P. Saikku, pers. comm.). Excess iron is present in atherosclerotic lesions. Seven times more iron is present in atherosclerotic than in healthy arteries (9).

Among proposed risk factors for heart disease, iron provides the most conceptually straightforward explanation for the presence of *C. pneumoniae* in coronary vessels. We propose that chronic infection of coronary arteries by *C. pneumoniae* occurs only if excess iron is present in vivo. Excess iron is defined as stored iron or iron in excess of the amount needed to maximize hematocrit. This implies that *C. pneumoniae* can establish infection in the coronary arteries only if a threshold level of available iron is present. Confirmation of the hypothesis could explain an association of *C. pneumoniae* with coronary atherosclerosis and, more generally, with ischemic heart disease and would be consistent with the greater susceptibility of men than women to *C. pneumoniae* infection (2) and myocardial infarction. Moreover, confirmation of the hypothesis would leave open the question of whether *C. pneumoniae* is directly atherogenic or merely finds fertile ground for growth in arteries because of the presence of iron above some threshold level.

Until age 20, men and women show few differences in prevalence of antibody titers against *C. pneumoniae*. After age 20, the prevalence of markers diverges sharply, with men showing a much steeper rise than women. This is similar to the patterns observed for both stored iron levels and rate of myocardial infarction in men and women, especially between the ages of 20 and 50 years (4,5). In later years, prevalence rates for *C. pneumoniae* markers do not rise as steeply for women as the curves for stored iron level or myocardial infarction rates (2). These patterns are compatible with associations between stored iron, myocardial infarction rate, and markers for infection with *C. pneumoniae*. Another relevant observation is the negative association of markers with spare-time physical activity (2). Such activity is associated with lower stored iron levels (10), which may decrease vulnerability to *C. pneumoniae*.

The presence of excess iron in regulating susceptibility to *C. pneumoniae* does not readily explain the geographic gradient in the frequency of antibodies (2). *C. pneumoniae* infection seems to be more prevalent near the equator. In general, acquisition of stored iron is more problematic among impoverished persons, many of whom live near the equator. Parasitic infections that cause chronic iron loss from bleeding in the gut and bladder, along with limited availability of easily absorbed heme iron in meat, tend to minimize iron acquisition in these areas. *C. pneumoniae* may be endemic in populations near the equator, especially among children in tropical urban slums, because of other factors that eliminate any differential effects on the basis of iron levels. In these areas chlamydial antibodies may be a good marker for invasion but not necessarily for disease.

We suggest that, above a modest threshold level of stored iron in vivo, *C. pneumoniae* acquires the ability to colonize coronary arteries. Invasion and colonization by the organism in vivo probably require a concentration of available iron similar to that needed for growth in cell culture. Even in a state of total iron depletion, iron is still present in the body in abundance. However, in iron depletion virtually all iron in the body is functional iron. Functional iron, i.e., iron in hemoglobin, may not be readily accessible to the organism. Our hypothesis implies that stored iron can be mobilized by *C. pneumoniae* for growth. An approach to testing the hypothesis would involve comparing the ability of *C. pneumoniae* to colonize macrophages from stored iron-replete persons with those from persons without stored iron. If the hypothesis is confirmed, maintenance of an iron-depleted state under medical supervision could be recommended as a preventive strategy against recolonization after a course of antibiotic therapy.

Acknowledgment

We thank Jane E. Raulston for review of the manuscript and for useful suggestions.

Jerome L. Sullivan* and Eugene D. Weinberg†
*University of Florida College of Medicine, Gainesville, Florida, USA; and †Indiana University, Bloomington, Indiana, USA

References

1. Saikku P, Mattila K, Nieminen MS, Huttunen JL, Leinonen M, Ekman M-R, et al. Serological evidence of an association of a novel *Chlamydia*, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet 1988;ii:983-5.
2. Saikku P. The epidemiology and significance of *Chlamydia pneumoniae*. J Infect 1992;25 Suppl I:27-34.
3. Saikku P, Leinonen M, Tenkanen L, Linnanmaki E, Ekman MR, Manninen V, et al. Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki Heart Study. Ann Intern Med 1992;15;116:273-8.
4. Sullivan JL. Iron and the sex difference in heart disease risk. Lancet 1981;1:1293-4.
5. Sullivan JL. Iron versus cholesterol—perspectives on the iron and heart disease debate. J Clin Epidemiol 1996;49:1345-52.
6. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation 1992;86:803-11.
7. Weinberg ED. Patho-ecologic implications of microbial acquisition of host iron. Reviews in Medical Microbiology 1998;9:171-8.
8. Freidank HM, Billing H. Influence of iron restriction on the growth of *Chlamydia pneumoniae* TWAR and *Chlamydia trachomatis*. Clinical Microbiology and Infection 1997;3 Suppl 2:193.
9. Thong PSP, Selley M, Watt F. Elemental changes in atherosclerotic lesions using nuclear microscopy. Cell Mol Biol 1996;42:103-10.
10. Lakka TA, Nyyssönen K, Salonen JT. Higher levels of conditioning leisure time physical activity are associated with reduced levels of stored iron in Finnish men. Am J Epidemiol 1994;140:148-60.

Filth Flies Are Transport Hosts of *Cryptosporidium parvum*

To the Editor: Infection with *Cryptosporidium parvum,* a zoonotic and anthroponotic coccidian parasite (1), may be fatal for persons with impaired immune systems (2), for whom a low number of oocysts can initiate life-threatening diarrhea (1). Insects such as promiscuous-landing synanthropic flies (i.e., coprophilic filth flies) are recognized transport hosts for a variety of parasites (3-5), but not for *C. parvum*. We assessed the role of synanthropic flies in the mechanical transmission of *C. parvum* oocysts.

Bovine diarrheic feces (20-ml specimens) containing 2.0 x 10^5 oocysts/ml were placed in petri dishes in each of five 4-liter paper cages with approximately 250 pupae of laboratory-reared house flies (*Musca domestica* F58WTZ strain). Three days after the flies emerged, fecal specimens were collected on glass microscope slides placed in each cage. Thirty flies aspirated from each cage on days 3, 5, 7, 9, and 11 after emergence were eluted, and the eluants were processed by the cellulose acetate membrane (CAM)-filter dissolution method (6). Digestive tracts dissected from randomly selected flies and the glass slides with fly excreta were examined by immunofluorescent antibody (IFA) (7), and *C. parvum* oocysts were counted (8). Maggots of *M. domestica* were reared in fly larvae medium (PMI FEEDS, Inc., St. Louis, MO) contaminated with calf diarrheic feces (50 ml) containing 2.0 x 10^5 *C. parvum* oocysts/ml. Resulting pupae were eluted, the eluants were processed by the CAM-filter dissolution method (6), and *C. parvum* oocysts were identified by IFA (7) and counted (8). Diarrheic fecal specimens from a *C. parvum*-uninfected calf were used as negative controls in similar experiments. Randomly selected samples containing fly-derived *C. parvum* oocysts were processed with acid-fast stain (AFS) (8) to check for normal cellular morphologic features.

Ten Victor-type flying-insect traps (Woodstream, Lititz, PA) were baited with rotten fish and placed inside a barn (approximately 880 m^2) in which a male Holstein calf infected with *C. parvum* (AUCP-1 strain) was housed. The traps were emptied weekly, the flies were counted and identified (5,9), and the inside surfaces of the traps (containing fly excreta), along with the flies, were eluted with 200 ml of eluting fluid (6). The eluting fluid was filtered through a CAM (Millipore, Bedford, MA) (6,8), which was then processed (6), and *C. parvum* oocysts were identified by IFA (7) and counted (8).

The mean number of *C. parvum* oocysts per droplet of *M. domestica* was 4 to 20 (mean 7.0 $\pm$ 3.2), and the number of droplets increased over time. All flies harbored *C. parvum* oocysts on their external surfaces. On average, 14.0 + 6.8 fly excreta were counted per 1.0 cm^2 of glass slide. From 1 to 8 *C. parvum* oocysts were

detected in digestive tracts of flies exposed to feces with oocysts. *C. parvum* oocysts were also numerous on maggot and pupa surfaces; approximately 150 and 320 oocysts were recovered per maggot and pupa, respectively.

Wild-caught flies belonged to the families *Calliphoridae* (96% of total flies), *Sarcophagidae* (2%), and *Muscidae* (2%). An average of eight flies was caught per trap, and more than 90% of flies harbored *C. parvum* oocysts. The number of trap-recovered *C. parvum* oocysts per fly was 2 to 246 (mean 73 oocysts per fly).

Synanthropic flies that breed in or come in contact with a fecal substrate contaminated with *C. parvum* oocysts can harbor these oocysts both externally and internally and will mechanically deposit them on other surfaces. Therefore, synanthropic flies can serve as mechanical vectors for *C. parvum* oocysts and under poor sanitary conditions could be involved in the transmission of human and animal cryptosporidiosis. The biology and ecology of synanthropic flies indicate that their potential for mechanical transmission of *C. parvum* oocysts can be high. The morphologic and AFS and IFA staining characteristics of *C. parvum* oocysts recovered from the exoskeletons of flies and identified in their fecal spots suggest that oocysts are still viable.

Thaddeus K. Graczyk,*† Ronald Fayer,‡ Michael R. Cranfield,*† Barbara Mhangami-Ruwende,* Ronald Knight,* James M. Trout,§ and Heather Bixler§

*Johns Hopkins University, Baltimore, Maryland, USA; †The Baltimore Zoo, Druid Hill Park, Baltimore, Maryland, USA; ‡U.S. Department of Agriculture, Beltsville, Maryland, USA; and §University of Pennsylvania, School of Veterinary Medicine, Philadelphia, Pennsylvania, USA

References

1. Fayer R, Speer CA, Dubey JP. The general biology of *Cryptosporidium*. *Cryptosporidium* and cryptosporidiosis. In: Fayer R, editor. Boca Raton (FL): CRC Press; 1997. 1-42.
2. Graczyk TK, Fayer R, Cranfield MR. Zoonotic potential of cross-transmission of *Cryptosporidium parvum*: implications for waterborne cryptosporidiosis. Parasitol Today 1996;13:348-51.
3. Wallace GD. Experimental transmission of *Toxoplasma gondii* by filth-flies. Am J Trop Med Hyg 1971;20:411-3.
4. Hedges SA. Flies, gnats and midges. In: Malis A, editor. Handbook of pest control. Cleveland (OH): Franzak & Foster Co.; 1990. p. 621-84.
5. Greenberg B. Flies and diseases, biology and disease transmission. Princeton (NJ): Princeton University Press; 1973. p. 221.
6. Graczyk TK, Cranfield MR, Fayer R. Recovery of waterborne oocysts of *Cryptosporidium parvum* from water samples by the membrane-filter dissolution method. Parasitol Res 1997;83:121-5.
7. Graczyk TK, Cranfield MR, Fayer R. Evaluation of commercial enzyme immunoassay (EIA) and immunofluorescent antibody (IFA) test kits for detection of *Cryptosporidium* oocysts of species other than *Cryptosporidium parvum*. Am J Trop Med Hyg 1996;54:274-9.
8. Graczyk TK, Fayer R, Cranfield MR, Owens R. *Cryptosporidium parvum* oocysts recovered from water by the membrane filter dissolution method retain their infectivity. J Parasitol 1997;83:111-4.
9. Borror DJ, DeLong DM, Triplehorn CA. An introduction to the study of insects. Philadelphia (PA): Saunders College Publishing; 1981. p. 827.

The Cost-Effectiveness of Vaccinating against Lyme Disease

To the Editor: The recent article by Meltzer and colleagues (1) is an important contribution to a pertinent public health issue: who should receive the newly licensed Lyme disease vaccine. Answering this question is a daunting task, given the scarcity of valid data. Estimates of the spectrum and prevalence of the long-term sequelae of Lyme disease remain controversial (2-4). In generating their cost-effectiveness model, Meltzer et al. examined the cost savings involved in preventing three categories of classic organ-specific Lyme disease sequelae (cardiovascular, neurologic, and arthritic); however, they did not take into account the potential cost savings from preventing cases of a generalized symptom complex known as post-Lyme syndrome, which includes persisting myalgia, arthralgia, headache, fatigue, and neurocognitive deficits. These generalized sequelae, which are recognized by the National Institutes of Health as late sequelae of Lyme disease, have been found to persist for years after antibiotic therapy (5,6). Two population-based retrospective cohort studies (7,8) among Lyme disease patients whose illness was diagnosed in the mid-1980s determined that one third to half had clinically corroborated post-Lyme syndrome symptoms years after the initial onset of disease. Although these studies were conducted 15 years ago, when optimal antibiotic regimen guidelines were still evolving, the estimated cost of averting these often-disabling nonorgan-specific symptoms should also be taken into account in estimated

sensitivity analyses of vaccine cost-effectiveness. The cost of treating sequelae is weighted heavily in the cost-effectiveness models presented by Meltzer and colleagues, which adds importance to considering post-Lyme syndrome. Nevertheless, we recognize the difficulty of this modeling, especially in the absence of validated cost-of-treatment data for these generalized symptoms.

A point of correction is that Meltzer et al. erroneously cite one of these studies (7) to infer that the long-term clinical sequelae of Lyme disease lasted a mean of 6.2 years from the onset of disease. In this retrospective study, Shadick et al. evaluated 38 persons with a clinical history of Lyme disease a mean of 6.2 years from the onset of disease regardless of the presence of persisting symptoms; 25 of these patients had no residual symptoms at follow-up. To accurately estimate the duration of clinical sequelae, longitudinal evaluations of representative populations of Lyme disease patients will be required because late manifestations have been demonstrated months to years after diagnosis (9,10).

Dimitri Prybylski
University of Maryland School of Medicine,
Baltimore, Maryland, USA

References

1. Meltzer MI, Dennis DT, Orloski KA. The cost-effectiveness of vaccinating against Lyme disease. Emerg Infect Dis 1999;5:321-8.
2. Ellenbogen C. Lyme disease. Shift in the paradigm? Arch Fam Med 1997;6:191-5.
3. Liegner KB. Lyme disease: the sensible pursuit of answers. J Clin Microbiol 1993;31:1961-3.
4. Sigal LH. Persisting symptoms of Lyme disease—possible explanations and implications for treatment [editorial]. J Rheumatol 1994;21:593-5.
5. National Institute of Allergy and Infectious Diseases. Research on chronic Lyme disease. National Institutes of Health Office of Communications Fact Sheet; May 1997.
6. National Institute of Allergy and Infectious Diseases. Emerging infectious diseases—NIAID research. National Institutes of Health Fact Sheet; Mar 1998.
7. Shadick NA, Phillips CB, Logigian EL, Steere AC, Kaplan RF, Berardi VP, et al. The long-term clinical outcomes of the disease. A population-based retrospective cohort study. Ann Intern Med 1994;121:560-7.
8. Asch ES, Bujak DI, Weiss M, Peterson MG, Weinstein A. Lyme disease: an infectious and post-infectious syndrome. J Rheumatol 1994;21:454-61.
9. Logigian EL, Kaplan RF, Steere AC. Chronic neurologic manifestations of Lyme disease. N Engl J Med 1990;323:1438-44.
10. Szer IS, Taylor E, Steere AC. The long-term course of Lyme arthritis in children. N Engl J Med 1991;325:159-63.

The Elusive Magic Bullet: The Search for the Perfect Drug. John Mann. Oxford University Press, New York, 1999.

Humans have been medicating themselves with one substance or another for thousands of years, first tapping into a vast natural arsenal of animal, vegetable, and mineral-based remedies, then creating still more in the chemistry laboratory. Millions of pills and potions later, we have not even a single perfect drug—a drug that can, in Paul Ehrlich's phrase, function as a "magic bullet" against disease, completely safe and completely effective, painlessly speeding through our bodies to vaporize invading organisms or errant cells without disturbing our equilibrium. A cynic might conclude at this point that our search for the magic bullet is as doomed as any alchemist's effort to spin straw into gold. However, the search continues at an ever more frantic pace as sophisticated technology launches increasingly devious and subtle efforts to undermine disease while leaving the patient whole.

John Mann, a professor of organic chemistry at Reading University in Great Britain, has written a readable overview of the last century's achievements in two interrelated categories of drug development: antimicrobial agents and antineoplastic agents. He starts with Paul Ehrlich's antitreponemal arsenicals and ends with the antisense agents now in early clinical trials for the treatment of autoimmune diseases and cancer, covering in the process the development of antibacterial and antiviral agents, vaccines, antimetabolites, and hormone-based antineoplastic agents.

The book is short and well written, linking together the old stories of Jenner, Pasteur, Fleming, and Waksman and placing them into a contemporary context at a nice, brisk pace. Among its strengths are a well-crafted beginning that clarifies the intellectual continuum between the German dye industry of the late 19th century and the beginning of the antibiotic era and a truly gripping overview of the first heady years of penicillin use. The last chapters on the evolution of chemotherapy lead into the genetic basis of tumor biology without missing a beat.

Thus, while the author is clearly an accomplished scientist, an enthusiastic historian, and a gifted raconteur, he could have used an editor or collaborator with some clinical background. When the discussion ventures from the development of drugs into their use, the text is peppered with factual errors, misstatements, and occasional off-base conclusions. For instance, Mann illustrates a short discussion of serious staphylococcal infections with the case of the puppeteer Jim Henson (who actually died of a fulminant group A streptococcal pneumonia); he misstates the name of the first neuraminidase inhibitor to be marketed for influenza infections (the drug is zanamivir, not anamivir, and warrants nowhere near the degree of enthusiasm lavished on it); he warns that the major toxicity of the tetracycline family of drugs is copious gastrointestinal blood loss (upset, yes; bleeding, no). He states that antibiotics can be purchased over the counter not only in the Third World, but also in the United States—any reader curious to know the source of this last piece of misinformation will be thwarted by the absence of both footnotes and endnotes in this book. The only reference materials cited are "suggestions for further reading" in the back. A good-sized section on the treatment of AIDS is oddly distorted, minimizing the revolution that the protease inhibitors and combination drug regimens have brought to the treatment of the disease and concluding that the future of AIDS treatment "looks increasingly rosy" because of the discovery of the chemokine receptors CCR5 and CXCR4. This view from the chemist's bench, is not one that most clinicians would share.

None of these (or other) misstatements is all that important in itself, but their cumulative effect is to erode some of the reader's confidence in the accuracy of this smooth and entertaining narrative. Perhaps the lesson to be drawn is that, just as the development of the antimicrobial and antineoplastic drugs has been the work of thousands of diverse collaborators, from the much-feted to the entirely unknown, so the telling of their full story requires more than a single person's talents, as multifaceted as they may be.

Abigail Zuger
Albert Einstein College of Medicine,
Bronx, New York

Meeting Summary

The 2nd International Symposium of the European Study Group on Enterohemorrhagic *Escherichia coli* (EHEC)

A total of 193 participants from 15 European countries, Canada, and China gathered in Brussels, Belgium, on April 16-17, 1999, to discuss progress in EHEC epidemiology and surveillance, origin of infection, virulence factors, and pathomechanisms, as well as diagnostics, molecular characterization, and typing methods. Alberto Tozzi (Italy); Helge Karch, Herbert Schmidt, Lothar Zimmerhackl, Matthias Pulz, and Jochen Bockemühl (Germany); Gad Frankel, Henry R. Smith, and Frederick J. Bolton (United Kingdom); Vincent Leclerc (France); and David Karpman (United States) provided overviews and poster session summaries.

Overview of EHEC Infections and Hemolytic Uremic Syndrome (HUS) in Europe (1)

In continental Europe, the incidence of Shiga-toxin producing *Escherichia coli* (STEC) is low ($<1/10^5$) and the incidence of HUS is $1.9/10^5$, while in the United Kingdom the incidence of STEC infections has been as high as $2.7/10^5$ (in 1997). The vehicles of transmission are often unknown, although EHEC infections in the United Kingdom were foodborne and (in contrast to those in continental Europe) associated with the prototype of EHEC O157:H7. In Germany (1992 and 1993) and in France (1992), person-to-person transmission seems to be predominant, while in Spain (1995) waterborne infections were reported. In the Netherlands (1993), Finland (1997), and Spanish Islands (1986, 1994, and 1997), infections due to swimming in lakes were reported. Continental Europe was faced with emerging non-O157-infections (e.g., O111, O103, and O26), although non-O157 STEC were isolated from every European country. Prevalence of O157 EHEC-induced HUS was 69% (Belgium, 1996), 61% (Germany, 1996-98), 58% (France, 1997-98), and 38% (Italy, 1996-98). STEC infections are more frequent in Northern Europe.

The introduction and establishment of Enter-Net (International Network for Surveillance of Enteric Infections, Salmonella, and STEC) should overcome the problem of "not speaking the same language" in Europe and contribute to better understanding and successful communication between countries regarding enteric and STEC outbreaks, with the benefit of a coordinated approach to international STEC standardized surveillance. Enter-Net started with 15 European countries, and the participants are now negotiating communicative exchange with the United States, South Africa, Japan, and Asia. The objectives include monitoring of *Salmonella* sp. antimicrobial resistance and STEC O157 typing-method harmonization. This concerted action will lead to an international *Salmonella* database. The STEC file specifications are reference, microbiologic, and epidemiologic data and data transfer. Long-term surveillance will identify ongoing changes in the epidemiologic situation in participating countries. As an example, a 20% increase in *Salmonella enteritidis* infections in Western Europe in 1995 to 1998 reflects successful Enter-Net communication. In addition, international outbreaks are being recognized. Thus, the coordinated approach will provide an international STEC surveillance.

Model of the Evolution and Origin of EHEC (2)

Grouping of at least four EHEC clone complexes, all related to an ancestral enteropathogenic *E. coli* (EPEC)-like strain O55:H7, harboring the locus of enterocyte effacement (LEE) pathogenicity island, was suggested on the basis of multilocus enzyme electrophoresis, DNA fingerprinting, polymerase chain reaction (PCR)-based techniques, and sequence analysis data. Additional characteristics, such as the ability to ferment sorbitol (SOR+), express ß-D-glucuronidase (GUD+), and express either Shiga toxin 1 (Stx 1+), Shiga toxin 2 (Stx 2+), or both were also considered. These four clone complexes are 1) O157:H7/H- and O55:H7; 2) O26:H11 and O111:H8; 3) O113:H21 and 4O91:H21; and 4) O103:H2 and O45:H2. While O157:H7 is found worldwide, the sorbitol fermenting group (SOR+) of O157:H- seems to be geographically restricted to Germany and the Czech Republic. O157:H- *E. coli* are important pathogens in Germany, with most infections occurring during winter. Compared with the prototype-like *E. coli* O157:H7, where infections occurred during summer, the SOR+ O157:H- strains are often negative for hemolysin (Hly-), negative for the

catalase-peroxidase (Kat P-), negative for the serin protease (Esp P-), and sensitive to tellurite. Microevolutional events (exchange of amino acids of individual genes, e.g., housekeeping genes) and macroevolutional events (horizontal gene transfer of plasmids, phages, and LEE) may have contributed to the evolution of STEC.

Pathogenic Mechanism of *E. coli* Leading to Diarrhea (3)

The attaching and effacing (A/E) lesions on epithelial cells caused by either EHEC or EPEC are mediated by binding of intimin (gene: *eae*) to its receptor (Tir) on the host cell. Both genes are located in LEE. Frankel and colleagues introduced four different classes of intimin (intimin α, ß, γ, and δ) on PCR-based data, which seem to exhibit different Tir/cell-binding properties, as shown by gel overlay and by the yeast two-hybrid system. As a hypothesis and a conclusion from the experiments presented, Frankel et al. suggested two binding activities involving a host cell receptor and Tir and binding of EHEC O157 to human intestinal mucosa. These interactions can produce A/E lesions in the Peyers Patches but not in all parts of the mucosa.

Virulence Factors of EHEC (4)

The most important characteristic elements of EHEC are located on mobile genetic elements—Shiga toxin (phage-encoded); the gene for hemolysin (*ehly*); and serin protease (*esp P*, plasmid-encoded), which is flanked by IS (Insertion) elements. LEE, which consists of a cluster of genes involved in A/E lesions, is located on a 35.5-kb fragment and inserted at 82 min in the *E. coli* chromosome (5). Besides the capability of expressing prophage-encoded Shiga toxins, the expressions of large plasmid (pO157)-encoded genes, such as hemolysin (*ehly*), *etp* (transporter of type II secretion pathway), *kat P* (bifunctional catalse-peroxidase), and *esp P* (serin protease) are considered additional or potentially important virulence factors. While *E. coli* O157 is associated to 100% with hemolysin and Etp expression and to 66% with Kat P and Esp P expression, 95% of the non-O157 strains express hemolysin, 52% Etp, 38% Kat P, and 36% Esp P. These virulence factors are not found in EPEC, diffuse enteroaggregative *E. coli,* and enteroaggregative *E. coli*. The hemolysin is a 107-kd pore-forming protein. It lyses eucaryotic cells, vero cells, and sheep red blood cells and is closely related to the α-hemolysin. It is cell-associated, exported in low amounts, and reacts with reconvalescent-phase sera from HUS patients. The ability of expressing hemolysin is proven after 20 hours of incubation at 37°C on indicator agar. The bifunctional catalase-peroxidase is located in the periplasm and interacts with the host cell defense mechanism on the reactive oxygen intermediate level. The serin protease (Esp P) is temperature regulated, expressed during infection, and cleaves the human coagulation factor V. The Shiga toxins consist of 1 A subunit and 5 B subunits (A1B5), inserted in the same region in the phage, and are most probably not randomly integrated in the *E. coli* chromosome.

A European and International Standard for the Detection of *E. coli* O157 in Food (6)

This is an approach to harmonize O157 STEC diagnostics. Precise recommendations are given on how to set up such a diagnostic procedure. The inquiry on the launch began in early 1999 among members of the European Committee for Standardization and the International Organization for Standardization.

HUS Surveillance in Europe (7)

HUS is a complex of symptoms (including anemia, acute renal failure, and thrombocytopenia) with multiple etiologies. Data from a prospective study indicate that 85% of the HUS cases in Germany in 1997 were associated with EHEC infections and thus involved postinfectious HUS. Most cases were due to eating contaminated food, but person-to-person transmission seems to be a prominent vehicle of transmission in Germany. Also, only a minority of European countries have a mandatory EHEC reporting system (Austria, since 1996; Finland, since 1994; Sweden, since 1996; and Germany, since 1998). In addition to a standardized definition, EHEC also requires standardized diagnostic procedures. Early diagnosis is a prerequisite for fewer deaths and fewer patients on dialysis.

Prevalence and Molecular Characterization of STEC in Asymptomatic Children (8)

In 1997, an increase in EHEC infections was observed after raw milk consumption in an EHEC-endemic area in Germany (Weser-Emsland). Four children contracted HUS after drinking raw milk. A total of 1,697 asymptomatic

children from 27 kindergartens were screened for STEC in stool specimens with enzyme immunoassay, and positive results were confirmed by PCR. The prevalence was 0.8% (15 cases), but the infectivity of these asymptomatic carriers was low, since during a 4-week monitoring phase of five children who were still shedding virus no new infection occurred. Nine of these 15 cases were positive for *eae* and *hly*, *as* well as *stx*. The predominant serotype found was *E. coli* O111:H-.

New Aspects on the Antibody Response in Children with Diarrhea and HUS (9)

The anti-LPS antibodies that can be screened for comprise a group of approximately 25 *E. coli* serotypes. Other *E. coli* proteins, such as Esp A and Esp B (secretory proteins) and intimin, can mount an antibody response. Although Western blot analysis did not show significant changes in pattern of acute- and convalescent-phase sera, further investigation is warranted for this *E. coli*-serotype-independent method of detecting EHEC infections.

Since diagnostics of EHEC from clinical specimens (stool samples) of patients with EHEC-induced diarrhea, hemorrhagic colitis, or HUS may fail (10), a further focus on serologic diagnostics could be one approach to meet the need for additional reliable diagnostics of the biochemical heterogeneous *E. coli* strains belonging to the STEC/EHEC group.

Overview of EHEC Infections and Detection in the Laboratory

Although progress has been made in harmonizing STEC/EHEC diagnostic procedures, in-vitro diagnostic manufacturers still concentrate on *E. coli* O157 detection systems. Outer membrane serotyping (OMS) has problems along with its advantages; for example, there are 1-173 O-groups (three times as many as for *Salmonella*), polysaccharides covering the O-specific side chain, heating is required for agglutination (boil strains for 1-2 hours), and cross-reactions between O- and H-antigens (1-53) serogroups might occur.

Typing and Characterization of EHEC

While a number of new molecular techniques are useful for analysis of clonal diversity and characterization of virulence factors, the optimal method should always be a combination of at least two methods (Tschäpe [Germany], Grimont [France]). These methods include such phenotyping procedures as phage-, colicin-, and serotyping and antibiotic resistance pattern, but also outer membrane protein pattern, LPS pattern, and multilocus enzyme electrophoresis. Genotyping procedures are plasmid profile analysis, restriction fragment length polymorphism, methods, such as ribotyping, virulence-associated gene probes, macrorestriction by pulse-field gel electrophoresis, PCR-based methods, multilocus sequence typing, and random amplified polymorphism DNA PCR fingerprinting (11).

Bacteriologic and Immunologic Techniques for Detecting EHEC in Food and Water (12)

Since *E. coli* O157 is usually present in low numbers (<10 cfu/g), methods of detection and selective enrichment procedures are required. Many factors affect the efficacy of protocols: the enrichment broth or agar used, the temperature of incubation, the choice of method used (e.g., immunomagnetic separation [IMS] for *E. coli* O157 only), and the subculturing methods. The detection level (number of bacteria) for IMS after enrichment is 1.7×10^1, for direct IMS 3.0×10^2, for direct culture 1.7×10^4, and for culture after enrichment 3.4×10^1. As a standard protocol, starting with 25 g food/ml broth for enrichment is recommended; water should be filtered first and may need to be concentrated. Bile salts as supplements often do not allow recovery and may inhibit the PCR reaction (A. Lehmacher, Germany). Also, the following incubation times should be used for different types of foods: raw meat, 6 hours; milk, 20 to 24 hours; cheese, 20 to 24 hours; and potable water, 20 to 24 hours. There are now suitable commercially available enzyme immunoassays that allow sensitive and specific detection of *E. coli* O157.

Conclusions

The European study group on EHEC has made enormous progress in the last 2 years in the surveillance of STEC/EHEC and efforts in harmonizing detection methods, above all the *E. coli* O157-specific techniques. Europe must confront the emerging group of non-O157 *E. coli*, although some European countries do not seem to have this problem, perhaps because of lack of recommended procedures and techniques for detecting non-O157 *E. coli*. For example, the Federal Republic of Germany is successfully

facing the problem of independently diagnosing STEC/VTEC serotype (13). There is still much to do, but we are now gaining a better understanding of this complex field.

Abstracts of other presentations from the Symposium of the European Study Group on EHEC were published in Acta Clinica Belgica, 1999, 54-1, pages 33-52.

Ralf D. Hess,* R. Lieske,* and B. Weber†
*HISS Diagnostics GmbH, Freiburg, Germany; and
†Laboratories Reunis Kutter-Lieners-Hastert, Luxemburg and Institut für Med. Virologie der Universitätskliniken Frankfurt, Germany

References

1. Tozzi E, Fisher IST. Epidemiology of VTEC infections in Europe: Enter-Net and other data. Acta Clin Belg 1999;54-1:34.
2. Karch H. Origins of EHEC. Acta Clin Belg 1999;54-1:33.
3. Frankel G, Phillips A, Connerton I, Matthews S, Knutton S, Dougan G. Specific aspects of attachment-effacement in EHEC. Acta Clin Belg 1999;54-1:33.
4. Schmidt H. New insight in virulence of EHEC. Acta Clin Belg 1999;54-1:34.
5. Paton JC, Paton AW. Pathogenesis and diagnosis of shiga toxin-producing *Escherichia coli*. Clin Microbiol Rev 1998;11:450-79.
6. Leclerc L, Lombard B. Development of a European international standard on the detection of *E. coli* O157 in foods. Acta Clin Belg 1999;54-1:43.
7. Zimmerhackl LB, Verweyen H, Karch H. HUS (hemolytic uremic syndrome) surveillance in Europe. Acta Clin Belg 1999;54-1:34.
8. Pulz M, Vogelsang E, Fuchs T, Hinz S, Frisch W, Windorfer A, et al. Prevalence and molecular characterization of shiga toxin-producing *Escherichia coli* (STEC) in asymptomatic children. Acta Clin Belg 1999;54-1:37.
9. Karpman D, Sorongon-Dubois ML, Karmali MA, Jarvis KG, Gansheroff LJ, O'Brien AD, et al. Antibody responses to enterohemorrhagic *Escherichia coli* antigens in children with diarrhea and hemolytic uremic syndrome. Acta Clin Belg 1999;54-1:43.
10. EHEC in Deutschland. Bundesgesundheitsblatt, Oktober 1998, Sonderheft.
11. Schmidt H, Geitz C, Tarr PI, Frosch M, Karch H. Non-O157 pathogenic shiga toxin-producing *Escherichia coli*: phenotypic and genetic profiling of virulence traits and evidence for clonality. J Infect Dis 1999;179:115-23.
12. Bolton FJ. Detection of EHEC in foods and water: bacteriological and immunological techniques. Acta Clin Belg 1999;54-1:35.
13. Hess RD, Lieske R, Klopfer G, Acheson DWK. Conclusions from a questionnaire on STEC/VTEC diagnostics in the Federal Republic of Germany. Journal of Laboratory Medicine 1999;23:427-36.

10th International Rabies in the Americas Meeting
November 14-19, 1999

The 10th International Rabies in the Americas Conference is scheduled for November 14-19, 1999, at the Town and Country Resort Hotel, San Diego, CA. Sponsors include the California Association of Public Health Laboratory Directors and the Viral and Rickettsial Disease Laboratory Branch and the Veterinary Public Health Section of the California Department of Health Services. Rabies in the Americas, an annual meeting that has been held since 1990 in the United States, Canada, Mexico, and South America, highlights current issues and research advances in rabies control in the Americas.

A call for papers is proposed in the following areas: animal populations susceptible to rabies; vaccination of animals; rabies transmission by bats, wildlife, and domestic animals; rabies diagnosis and epidemiologic surveillance; human rabies prophylaxis; clinical aspects of rabies and rabies pathology; molecular epidemiology; health education and rabies prevention; and the legal and economic aspects of rabies control. A special all-day session arranged by Dr. Hilary Koprowski, entitled "Rabies 2000," will be held November 19, 1999.

The proposed deadline for paper submission and registration is September 1, 1999. A late fee may apply to registrations after September 1. A registration discount will be given to presenters. To obtain registration materials or submit papers, please contact Donna Taclindo, California Department of Health Services, Viral & Rickettsial Disease Laboratory, 2151 Berkeley Way, Room 454, Berkeley, CA, 94704; phone (510) 540-2830, or e-mail dtaclind@dhs.ca.gov or bsun@dhs.ca.gov. Program and registration information is also available at the following website: http://www.caphld.org.

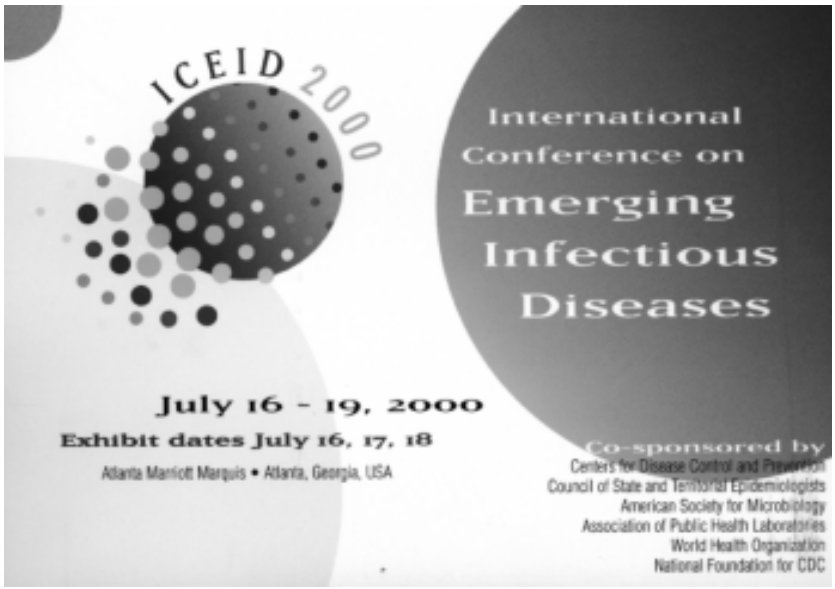
The 10th International Symposium on Viral Hepatitis and Liver Disease
Atlanta, Georgia, USA, April 9–13, 2000

Topics will include virology, epidemiology, diagnosis, treatment, and prevention. For more information contact: Organizing Secretariat, MediTech Media Ltd., Tower Place 100, 3340 Peachtree Road, Suite 550, Atlanta, GA 30326, USA; telephone: 404-233-4490; fax: 404-233-7464; e-mail: info@Hep2000.com.

Erratum Vol. 5, No. 3

In the article "Bacterial Vaccines and Serotype Replacement: Lessons from *Haemophilus influenzae* and Prospects for *Streptococcus pneumoniae*," by Marc Lipsitch, there is an error in the figure legend on page 339. Controls are incorrectly identified as black bars; however, in the text and the figure itself, controls are correctly represented by white bars and vaccine recipients by black bars. We regret any confusion this error may have caused.

ICEID 2000



Hold the dates of July 16–19, 2000, for the International Conference on Emerging Infectious Diseases, a meeting of 2,500 specialists in infectious diseases. The program will include plenary sessions and symposia with invited speakers, presentations on emerging infections activities, and oral and poster presentations. Major topics will include current work on surveillance, epidemiology, research, communication and training, as well as prevention and control of emerging infectious diseases, both in the United States and abroad. Abstracts are invited and will be accepted beginning in October 1999.

The Call for Abstracts and Preliminary Program will be mailed in October 1999.

For more information, call ICEID management at 202-942-9248, e-mail meetinginfo@asmusa.org, or www.cdc.gov/ncidod/iced2k.htm.

Editorial Policy and Call for Articles

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 has an international scope and 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, demography, sociology, and other disciplines. Inquiries about the suitability of proposed articles may be directed to the Editor at 404-639-4856 (tel), 404-639-3075 (fax), or eideditor@cdc.gov (e-mail).

Emerging Infectious Diseases is published in English and features the following types of articles: Perspectives, Synopses, Research Studies, Policy Reviews, and Dispatches. The purpose and requirements of each type of article are described in detail below. To expedite publication of information, we post journal articles on the Internet as soon as they are cleared and edited.

Spanish and French translations of some articles can be accessed through the journal's homepage at www.cdc.gov/eid. Articles by authors from non-English-speaking countries can be made simultaneously available in English and in the author's native language (electronic version of the journal only).

Instructions to Authors

Manuscript Preparation

Follow "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Ann Int Med 1997:126[1]36-47) (http://www.acponline.org/journals/resource/unifreqr.htm).

Begin each of the following sections on a new page and in this order: title page, abstract, text, acknowledgments, references, tables, figure legends, and figures.

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). Also provide address for correspondence (include fax number and e-mail address).

Abstract and key words. Avoid citing references in the abstract. Include up to 10 key words; use terms listed in the Medical Subject Headings from Index Medicus (http://www.nlm.nih.gov/mesh/meshhome.html).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Type only on one side of the paper and number all pages, beginning with the title page. Indent paragraphs 5 spaces; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use Courier font size 10 and ragged right margins. Italicize (rather than underline) scientific names when needed.

Electronic formats. For word processing, use WordPerfect or MS Word. Send graphics in either (TIFF), or .EPS (Encapsulated Postscript) formats. The preferred font for graphics files is Helvetica. Convert Macintosh files into one of the suggested formats. Submit slides or photographs in glossy, camera-ready photographic prints.

References. Follow the Uniform Requirements style. Place reference numbers in parentheses, not in 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 in full. List the first six authors followed by "et al."

Tables and figures. Create tables within the word processing program's table feature (not columns and tabs within the word processing program). For figures, use color as needed; send files, slides, photographs, or prints. Figures, symbols, lettering, and numbering should be clear and large enough to remain legible when reduced. Place figure keys within the figure.

Manuscript Submission

Include a cover letter verifying that the final manuscript has been seen and approved by all authors.

Submit three copies of the original manuscript with three sets of original figures and an electronic copy (on diskette or by e-mail) to the Editor, Emerging Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd., MS C-12, Atlanta, GA 30333, USA; e-mail eideditor@cdc.gov.

Types of Articles

Perspectives, Synopses, Research Studies, and Policy Reviews: Articles should be approximately 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch.

Perspectives: Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases or related issues. Perspectives may also 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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Synopses: 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. Use of subheadings in the main body of the text is recommended. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text. Photographs and illustrations are encouraged.

Research Studies: These articles report laboratory and epidemiologic results within a public health perspective. Although these reports may be written in the style of traditional research articles, they should explain the value of the research in public health terms and place the findings in a larger perspective (e.g., "Here is what we found, and here is what the findings mean").

Policy Reviews: Articles in this section report public health policies that are based on research and analysis of emerging disease issues.

Dispatches: These brief articles are updates on infectious disease trends and research. The articles 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. Dispatches (1,000 to 1,500 words) need not be divided into sections. Provide a short abstract (50 words); references, not to exceed 10; figures or illustrations, not to exceed two; and a brief biographical sketch.

Book Reviews: Short reviews (250 to 500 words) of recently published books on emerging disease issues are welcome.

Letters: This section includes letters that give preliminary data or comment on published articles. Letters (500 to 1,000 words) should not be divided into sections, nor should they contain figures or tables. References (not more than 10) may be included.

News and Notes: We welcome brief announcements (50 to 150 words) of timely events of interest to our readers. (Announcements can be posted on the journal web page only, depending on the event date.) In this section, we also include summaries (500 to 1,500 words) of conferences focusing on emerging infectious diseases. Summaries may provide references to a full report of conference activities and should focus on the meeting's content.