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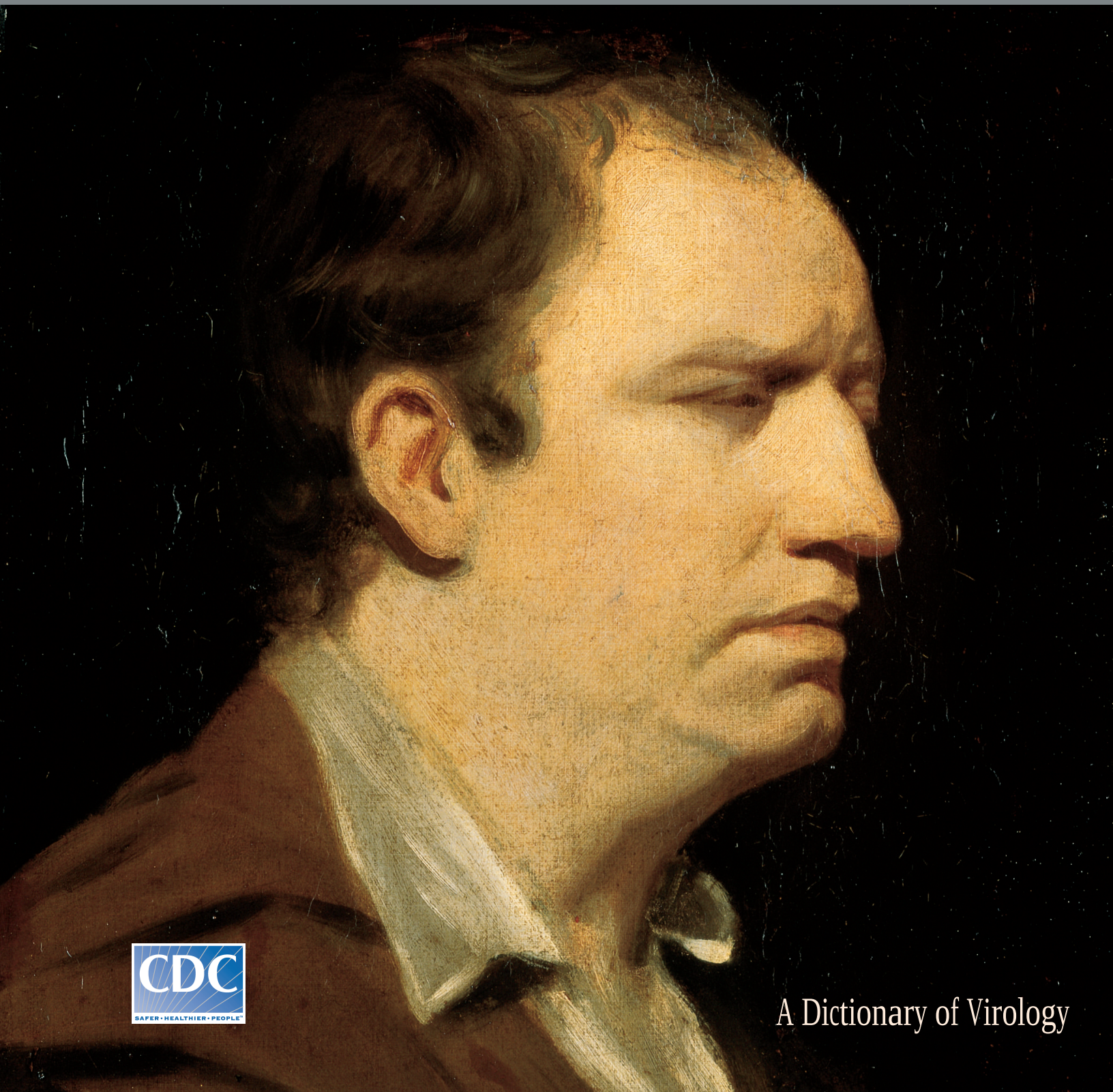

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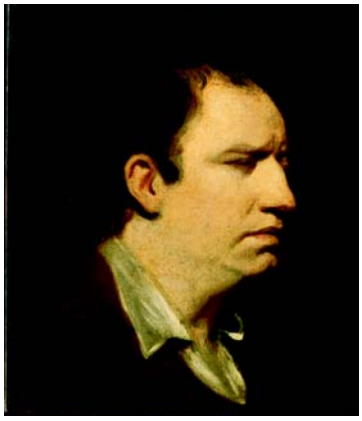

On the Cover: Samuel Johnson (c. 1769) (oil on canvas, 451mm X 387mm). Reduced copy, after Sir Joshua Reynolds (1723-1792).

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EMERGING INFECTIOUS DISEASES

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Clinical Epidemiology of Malaria in the Highlands of Western Kenya

Simon I. Hay,*† Abdisalan M. Noor,† Milka Simba,† Millie Busolo,‡ Helen L. Guyatt,*† Sam A. Ochola,‡ and Robert W. Snow*†‡

Malaria in the highlands of Kenya is traditionally regarded as unstable and limited by low temperature. Brief warm periods may facilitate malaria transmission and are therefore able to generate epidemic conditions in immunologically naive human populations living at high altitudes. The adult:child ratio (ACR) of malaria admissions is a simple tool we have used to assess the degree of functional immunity in the catchment population of a health facility. Examples of ACR are collected from inpatient admission data at facilities with a range of malaria endemicities in Kenya. Two decades of inpatient malaria admission data from three health facilities in a high-altitude area of western Kenya do not support the canonical view of unstable transmission. The malaria of the region is best described as seasonal and meso-endemic. We discuss the implications for malaria control options in the Kenyan highlands.

The temperate highlands of western Kenya were regarded by colonial settlers as safe havens from the surrounding malarious areas of Uganda and Kenya (1,2). After World War I, malaria encroached into these highland communities as a result of wide-scale population settlement linked to transport and agricultural development (2–6), and malaria epidemics were frequently reported by the early 1930s (7–11). These epidemics in the highlands caused concern to those in the colonial administration because of the economic importance of agricultural exports. During the 1950s and 1960s, control efforts such as indoor residual house-spraying, mass drug administration, or chemoprophylaxis effectively contained or prevented epidemics in some of these high-altitude areas (12–15).

In the late 1980s and early 1990s, a series of malaria "epidemics" were reported in Kenya and other communities located at high altitudes in the subregion (11,16–26). Some authors have labeled these resurgences as a new typology variant, "highland malaria," demanding special attention in the new global commitment to Roll Back Malaria (27–29). A generally accepted view has been that the transmission of *Plasmodium falciparum* in high-altitude communities is limited by low ambient temperature. Small changes in climate may therefore provide transiently suitable conditions for unstable transmission in populations that have acquired little functional immunity (8,9,30).

The highlands of Kenya constitute a densely populated, politically significant area, which serves as a major source of revenue and foreign exchange from agricultural exports. The Kenyan government has recently defined 15 districts in the highlands (31,32) as being prone to epidemics, meriting close inspection, preparation, and intervention (33). We examine a time series of age-structured clinical malaria data derived from three hospitals with inpatient admission facilities in the highlands of western Kenya. These data provide an empirical basis for understanding the epidemiology of malaria and consequent strategic approaches to disease management and prevention in this area. A companion paper investigates the epidemiologic and statistical problems associated with defining true epidemics in these high-altitude locations and tests a variety of epidemic surveillance algorithms on the monthly malaria admissions series abstracted from these facilities (32).

Methods

The location of the three hospitals that provided inpatient clinical care and were identified for use in this study, along with details about the collection of clinical data and the local weather conditions, are provided in our companion paper (32).

Providing a precise catchment area of the population for admission's data was not possible as such information is not routinely collected in the hospitals; we assumed therefore that most inpatients came from the immediate surrounding high-elevation catchment area. Typically, long-term, facility-based data are difficult to interpret without some estimate of the populations served and how the population may have changed over time. To provide demographic information on the number of people served by the hospitals, we used population estimates from Kenyan national censuses in 1979 (34), 1989 (35), and 1999 (36). District and lower level administrative boundaries changed with each census, so population growth rates were defined for three contiguous administrative areas, as close to the hospital as possible, which had not been subjected to boundary redefinition. Intercensal population growth rates (r) were calculated by using the formula $r=\log_e(t_2-t_1)$, where t_1 is the population estimate of the first census and t_2 the population estimate of the second.

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Data manipulation and statistical transformation were performed in Excel 2000 (Microsoft Corp., Seattle, WA) unless otherwise stated. Monthly mean adult (≥15 years) and child (<15 years) admissions were calculated and displayed as spider plots. The time series of admissions at each site was also plotted with a 25-point (month) moving average of the series to show more clearly the long-term movement in these data. For each hospital, we performed trend analysis through linear regression of the malaria admission data against a trend variable (observation month number/12). The coefficient of trend therefore indicates the annual trend (positive = increasing in time, negative = decreasing in time, zero = a stationary series). Such regression models are sensitive to seasonal variation, outliers, and heteroscedasticity (a term which refers to situations in which the variability of the residuals is not constant). To show the long-term trend unambiguously, seasonality was removed from each series by using an additive seasonal decomposition procedure (37,38) and the residuals were checked for normality and heteroscedasticity in SPSS version 11 (SPSS Inc., Chicago, IL).

Furthermore, we applied a proximate measure of transmission stability through a comparison of the numbers of adults to children with malaria admitted to the three hospitals. This adult:child ratio (ACR) of cases was calculated from total adult and child admissions for the duration of the available records. In areas of stable transmission, we assumed that the risks for complicated malaria in adulthood would be significantly lower than the risks in childhood; this assumption was based on expectations of the age distribution of malaria cases under varying transmission intensities (39–41). Conversely, areas of infrequent parasite exposure lend themselves to equivalent risks in adults and children. As such, an ACR derived from hospital admissions approaching unity would suggest an increasing tendency toward unstable transmission, assuming that the typical age-structured population pyramid for developing country and rural communities prevailed (36) and that there were no age-dependent biases in attendance rates.

Results

Time series of malaria admissions from 1980 to 1999, 1987 to 2000, and 1981 to 2000 were recorded for Kilgoris, Kisii, and Tabaka hospitals, respectively. These clinical data represent 171,312 admissions with a primary, coprimary, or coincidental diagnosis of complicated malaria over a total of 54 admission years. The Kilgoris, Kisii, and Tabaka hospitals managed an average of 2,243; 9,191; and 3,929 malaria admissions per year, respectively, for the duration over which records were available (Table 1). Throughout the study period, the frequency of childhood admissions was on average twice that of adult admissions (Table 1; Figure 1a,c,e). The average ACR calculated for all months was 0.46 for Kilgoris (14,079/30,793), 0.52 for Kisii (44,043/84,648), and 0.42 for Tabaka (23,692/55,871). The ACRs derived from monthly admissions are relatively constant throughout the duration of observation (Figure 1b,d,f). Several anomalies in these data were evident, however, particularly at Tabaka in 1985 and Kisii in 1999; we did not identify any obvious explanation for these exceptions in the time series, although they did not occur during periods of major epidemics at the sites (32). In further analysis, we focused on the primary pediatric clinical case data. We considered data from children to be more likely to give an accurate picture of local malaria transmission, as they are less likely to have developed functional immunity or to have traveled and acquired infections elsewhere.

The long-term data used in this analysis indicate that clinical cases of malaria occur every month at each hospital; acute seasonal peaks occur in June and July (Figure 1a,c,e). On average, one third of the total annual child malaria admissions were concentrated in these 2 months (35%, 32%, and 27% for Kilgoris, Kisii, and Tabaka, respectively).

The trends and interannual variation in pediatric malaria admissions at each facility are shown in Figure 2a–c. These graphs demonstrate clear, substantial between-year variation in child malaria admissions. The 2 years of highest case presentations were 1994 and 1998 for Kilgoris, 1996 and 1997 for Kisii, and 1997 and 1996 for Tabaka (the moving average line in Figure 2a–c clearly shows these years). Although Kisii and Tabaka showed similarities, little coherence occurred in peak years of child admissions between these sites and Kilgoris, despite their close geographic proximity. At each facility, pediatric malaria admissions rose substantially over the period of observation (Table 2). In Kilgoris, deseasonalized child malaria admissions rose from 56 in January 1980 to 200 in December 1999, an increase of 256% over 20 years ($p<0.001$). Similar trends were observed at Kisii (32% increase from January 1987 through December 1999; $p=0.019$) and Tabaka (91% increase from January 1980 through December 1999; $p<0.001$).

In parallel with these significant rises in number of cases, estimates of the annual rate of natural population growth in the communities around the hospitals suggest that child populations have increased by 215%, 49%, and 77% during the same period at Kilgoris, Kisii, and Tabaka, respectively.

Discussion

We examined longitudinal, age-structured, clinical data on the frequency of admission for severe and complicated *P. falciparum* malaria at the three hospitals located above 1,600 m in the highlands of western Kenya. These data provided an opportunity to explore in more detail several generally accepted positions about the clinical epidemiology of malaria at high altitude in East Africa.

In these time series, the increased malaria admission at each of the three hospitals was concentrated in children <15 years of age (approximately two thirds of all admissions). Given the equivalent sizes of at-risk population below and above 15 years of age (36), one must assume that adults have developed a degree of functional immunity to the severe

Table 1. Mean monthly child and adult admissions at the three study hospitals, Kenya[a]

	Mean monthly admissions												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Kilgoris (1980-1999)													
Child	96	154	164	127	151	242	296	158	60	34	27	34	1,543
Adult	49	81	73	61	68	94	112	60	41	26	16	21	702
Kisii (1987–2000)													
Child	401	465	515	483	632	913	1,000	539	336	248	233	281	6,046
Adult	239	300	285	267	285	357	387	271	193	190	173	200	3,147
Tabaka (1981–2000)													
Child	197	201	229	231	231	385	371	230	193	178	158	156	2,760
Adult	87	85	97	91	93	160	154	101	87	74	69	71	1,169

[a]Children are defined as <15 years of age. Adults are ≥15 years.

consequences of *P. falciparum* infection. The hypothesis that communities located at high altitude are prone to unstable, infrequent parasite exposure limiting the development of functional immunity before adulthood (8,9,30) is, therefore, not reflected in our data.

Complicated malaria warranting intensive clinical management is a problem every year at each hospital. Previous cross-sectional estimates of the prevalence of *P. falciparum* infection in children from birth to 10 years of age in homesteads in Kisii Central during 1990 suggested infection rates between 4.5% and 13% (42). More recently (July 2000), the prevalence of *P. falciparum* infection was 10.3% in children from birth to 9 years of age (HL Guyatt, unpub. data). Neither the clinical epidemiology nor estimates of the prevalence of

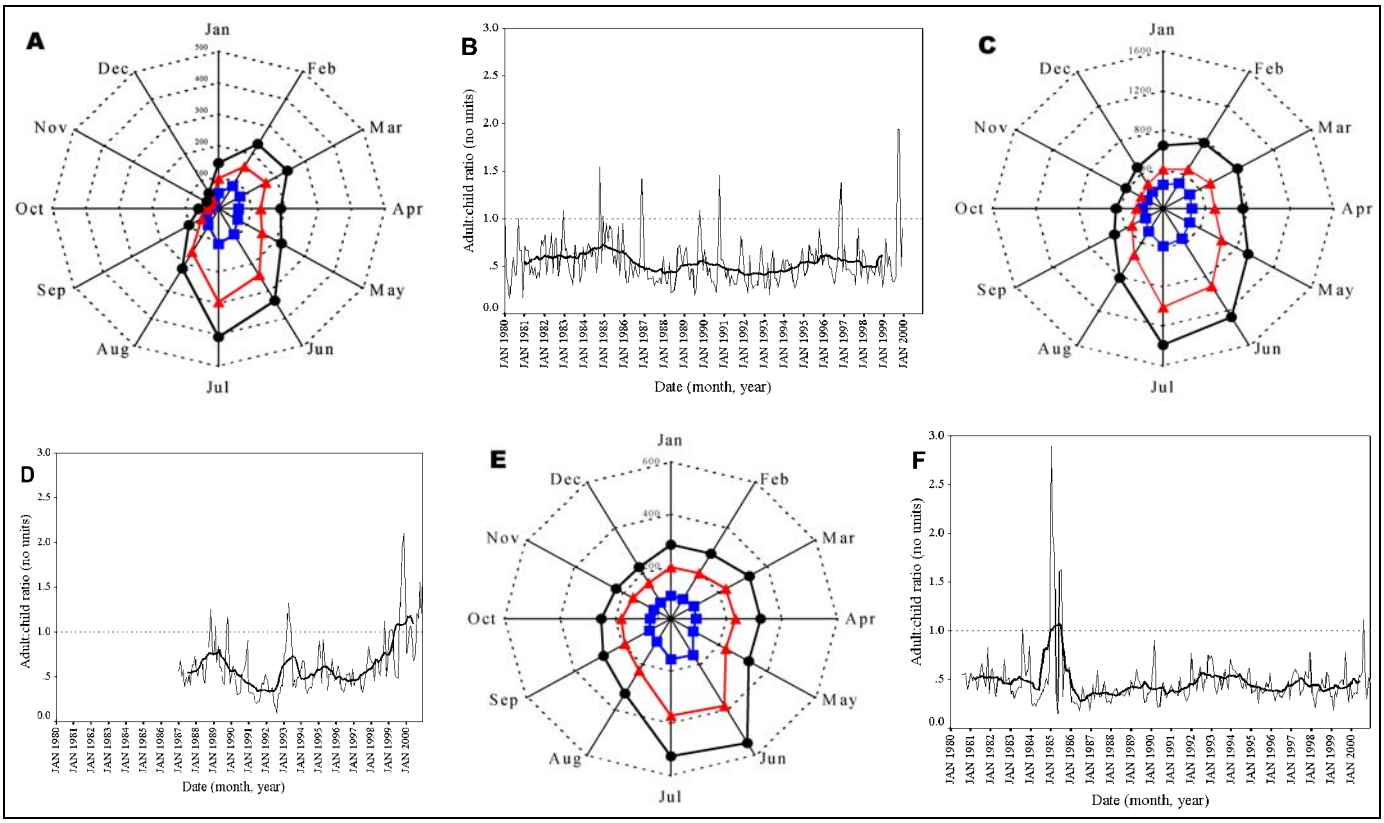

Figure 1. Spider plots of adult, child, and total admissions and time series of adult:child ratio for three study hospitals in Kenya. Spider plots of malaria admissions in Kilgoris (A), Kisii (C), and Tabaka (E). The data are monthly averages for the 1980–1999, 1987–2000, and 1981–2000 time periods, respectively. Adult cases (≥15 years of age) are shown in blue, child cases (<15 years) are shown in red, and total cases in black. Time series plots of the monthly adult:child ratio data are also shown for Kilgoris (B), Kisii (D), and Tabaka (f) as the continuous black line. The dashed line represents the value of 1 where adult and child admissions are equal, as is to be expected in true epidemic conditions (39–41). The bold line is a 25-point (month) moving average of the adult:child ratio.

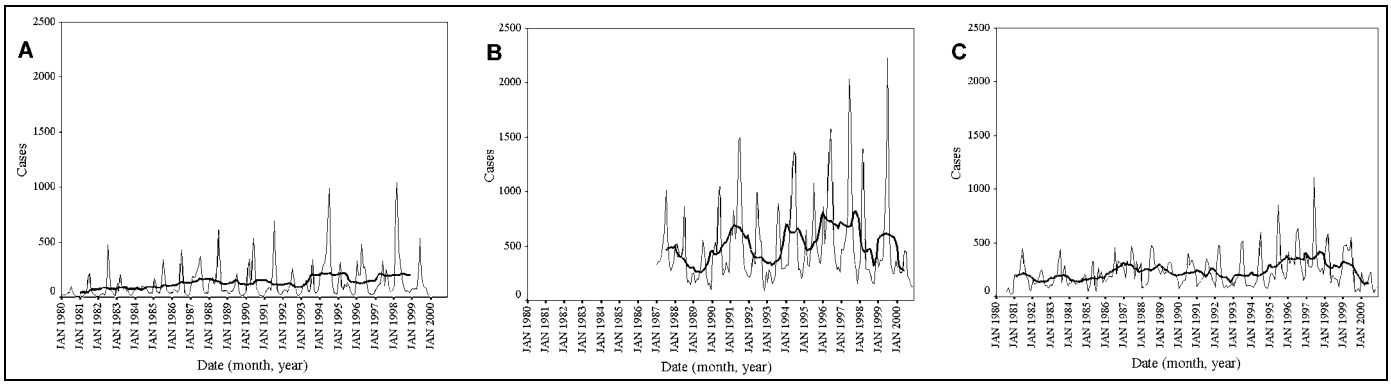

Figure 2. Time series of child admissions for the three study hospitals, Kenya. Time series of child admissions (<15 years of age) for Kilgoris (a), Kisii (b), and Tabaka (c) for 1980–1999, 1987–2000, and 1981–2000 time periods, respectively. The bold line is 25-point (month) moving average of the same data for child admissions.

infection in the community corroborate the view that the high-altitude areas served by the hospitals in our study support unstable transmission. Transmission is better characterized as seasonal and meso-endemic (43).

"Highland" malaria is either a new phenomena (16–18,23–25,30) or a reemergence of a previous prevailing epidemiology (21,44). Our data confirm significant surges in malaria cases, requiring intensive clinical management during specific years of the 1990s because of substantial overall increases in the number of cases at each hospital. To provide a series of explanations for these increases is tempting, invoking arguments for and against climate change, drug resistance, and land use changes; various authors discuss these arguments elsewhere (16–18,20,23–26,30,45–48). We emphasize in these arguments, however, the importance of considering population growth as the simplest explanation and note the close correspondence between the percentage increases in the population's growth rates in the districts served by each facility and the percentage rises in malaria cases. Characteristic of much of sub-Saharan Africa over the last 3 decades, including the highlands of western Kenya, has been a high rate of increase in population size, resulting from high fertility rates and increasing child survival. In the populations served by the hospitals in our study, annual growth rates averaged 3.9%. Under such circumstances, without any change in disease incidence, the increase in disease would be expected to have doubled over approximately an 18-year period. Clearly, without a concomitant investment in essential clinical services, beds, staff, and supporting infrastructure, the changing requirements for clinical management will have been perceived by most district-level public health officials as a crisis.

Defining true epidemics is difficult (32). For most public health workers, epidemics represent exacerbations of disease out of proportion to the normal level to which that facility is subject; these increases overwhelm the facility's ability to cope. Therefore, a slow but pervasive epidemic of clinical malaria may have emerged in the highlands of western Kenya, where lack of investment in the physical capacity to manage an increasing population has resulted inevitably in more malaria cases that require a basic clinical service. In addition to this demographic-to-service determinant, the western highlands are subject to acute seasonal transmission, as evidenced by the temporal distribution of cases (Figure 1a,b). These seasonal peaks in clinical disease exhibit marked between-year variations, and several years exhibit dramatic rises in severe and complicated disease (Figure 2a,b,c). Moreover, years of exceptional cases can be very different between health centers separated by no more than 10 km. With limited resources and bed capacities, these acute rises in disease incidence within a given year will undoubtedly put a considerable strain on any clinical service and represent a crisis (32).

We used a crude measure of transmission stability based largely on our understanding of patterns of acquired functional immunity (8,49). The ACR was derived from hospitalized patients diagnosed with malaria. Many of the cases would not have been confirmed with any degree of reliability through microscopy or careful clinical exclusion of alternative causes for fever (50). Our data and approach must therefore be

Table 2. Deseasonalized child admissions at the three study hospitals, Kenya[a]

Site	Data span	Constant	Slope	Change	t value	Significance	Adjusted r^2
Kilgoris	1980–1999 (n=20)	56.2	7.2	144	6.956	p<0.001	0.165
Kisii	1987–2000 (n=14)	433.8	10.0	140	2.362	p=0.019	0.027
Tabaka	1981–2000 (n=20)	166.4	7.6	152	6.980	p<0.001	0.174

[a] The data span shows the range of complete years for which data are available (parenthesis indicate the number of observation years). The adjusted r^2 (sometimes called the coefficient of determination) is goodness-of-fit measure of a linear regression model and varies between 0 and 1. The measure is the proportion of variation in the dependent variable (in this case, malaria admissions) explained by the regression model (in this case, the trend line). The t value is the value of a t-test used to determine if the adjusted r^2 value is significantly different from 0. The result is shown in the significance column where p values <0.05 are significant.

interpreted with this caveat. Nevertheless, in other areas of Kenya where stable transmission is well established (51), notably coastal Kwale (ACR = 4,181/6,692 = 0.63 based on admissions data, 1984–1999) and lakeside Homa Bay (ACR = 18,686/35,703 = 0.52 based on admissions data,1982–1999), many more children than adults are admitted to hospital with a malaria diagnosis, resulting in ACRs similar to those described in the highlands (R. Snow, unpub. data). Conversely, in an arid area of northeastern Kenya (Wajir), where a major malaria epidemic occurred in 1998, more adults than children were admitted to the hospital (ACR = 2,704/1,369 = 1.96 based on admissions data, 1988–2000) (52). Despite poor malaria diagnosis in many routine clinical facilities, we believe that the ACR is one possible tool to rapidly assess the extent to which a community has sufficient parasite exposure to invoke some degree of clinical immunity early in childhood. This tool should be explored further within the context of malaria classification for epidemic-prone areas of Africa.

In high-altitude zones of western Kenya, clinical malaria has an acutely seasonal distribution, is comparatively concentrated in the pediatric population, and is a substantial public health problem every year. Occasional, but exceptional, temporal surges of disease occur in some years. We can assume that parasite transmission in this area of Kenya is stable and a degree of functional immunity is acquired during early childhood. Low levels of parasite challenge have been found to be sufficient for early development of functional immunity (53). We argue that large parts of the western highlands, located at a similar altitude, have ecologies similar to many other areas with low, stable, but seasonal malaria in Kenya. Treating the highland districts as special cases; demanding intensive investment in early detection, warning, and forecasting systems; and frequent complex-emergency responses by government or nongovernmental organizations (33) may not be the most appropriate and cost-effective use of limited resources. Investment in sustainable approaches to vector control (spraying households with residual insecticide), promoting individual protection (insecticide-treated bed nets), and effective case management are perhaps more likely to achieve long-term reductions in disease.

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Dr. Hay is a research fellow, funded by the Wellcome Trust, in the Department of Zoology at the University of Oxford. He is also a member of the World Health Organization Roll Back Malaria Technical Support Network on Malaria Epidemic Prevention and Control. His research involves applying satellite technologies to the study and control of vector-borne diseases, with a particular emphasis on epidemic warning for malaria and dengue hemorrhagic fever.

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Perspectives. 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 of first author—both authors if only two.

Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and 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.

Geographic Association of *Rickettsia felis*-Infected Opossums with Human Murine Typhus, Texas

Ardys Boostrom,* Magda S. Beier,† Jacqueline A. Macaluso,† Kevin R. Macaluso,† Daniel Sprenger,* Jack Hayes,‡ Suzana Radulovic,† and Abdu F. Azad†

Application of molecular diagnostic technology in the past 10 years has resulted in the discovery of several new species of pathogenic rickettsiae, including *Rickettsia felis.* As more sequence information for rickettsial genes has become available, the data have been used to reclassify rickettsial species and to develop new diagnostic tools for analysis of mixed rickettsial pathogens. *R. felis* has been associated with opossums and their fleas in Texas and California. Because *R. felis* can cause human illness, we investigated the distribution dynamics in the murine typhus–endemic areas of these two states. The geographic distribution of *R. felis*-infected opossum populations in two well-established endemic foci overlaps with that of the reported human cases of murine typhus. Descriptive epidemiologic analysis of 1998 human cases in Corpus Christi, Texas, identified disease patterns consistent with studies done in the 1980s. A close geographic association of seropositive opossums (22% *R. felis*; 8% *R. typhi*) with human murine typhus cases was also observed.

Murine typhus is a common infectious diseases in south Texas. Often the disease is mild and unrecognized; however, it can be severe and even fatal. The severity of murine typhus infection has been associated with old age, delayed diagnosis, hepatic and renal dysfunction, central nervous system abnormalities, and pulmonary compromise. Up to 4% of hospitalized patients die (1–3). Murine typhus, which is endemic in many coastal areas and ports throughout the world, is one of the most widely distributed arthropodborne infections. Sporadic outbreaks of murine typhus have been reported in Australia and more recently in China, Greece, Israel, Kuwait, and Thailand (4–6).

Recent serosurveys have demonstrated a high prevalence of antibodies to typhus group Rickettsiae in humans living in Asia and southern Europe. In the United States, thousands of human cases were reported annually in the 1940s (1,2). A major public health measure consisting of a combination of environmental modification, rat, and vector-control programs greatly reduced human cases in the United States to <100 reported cases of murine typhus/year. As a result, most states no longer report murine typhus. However, murine typhus has been a reportable disease in Texas for the past 40 years.

Interest in this disease has been rekindled because of the resurgence of human cases of murine typhus in south Texas from 1980 through 1984, when 200 cases were reported to the Texas Department of Health. Twenty-eight percent of the patients resided in Nueces County, where the highest annual incidence rate, 4.2 patients/100,000 residents, was reported. Although onset of symptoms occurred throughout the year, 40% of cases were reported in April, May, and June. These studies (Boostrom et al., unpub. data; 7–8) also showed that the maintenance and transmission of *Rickettsia typhi*, the etiologic agent of murine typhus, did not occur by the classic cycle involving rats (*Rattus rattus* and *R. norvegicus*) and the rat flea, *Xensopsylla cheopis*. Detailed investigations of murine typhus in the Nueces County/Corpus Christi area have shown a cardinal role for the opossum (*Didelphis virginiana*) and the cat flea (*Ctenocephalides felis*) in the *R. typhi* life cycle (7,8). In addition to *R. typhi*, sampled opossums and their fleas were also infected with *R. felis* (formerly known as ELB agent [9–12]). Furthermore, in 1994 *R. felis* was detected by polymerase chain reaction (PCR) in a blood sample from a patient diagnosed with murine typhus. The presence of *R. felis*, clinically masquerading as dengue fever, was documented recently in patients from Yucatan, Mexico, and four patients with fever and rash in France and Brazil (13–15). Our published data and these recent reports not only support the pathogenic role of *R. felis* but also demonstrate its wide geographic distribution.

In this study, we report the presence of *R. typhi* and *R. felis* in opossums and their fleas collected during 1998 in south Texas. Data from our 1998 studies show that the rate of seropositive opossums and infected fleas, as well as the *R. typhi*/*R. felis* ratio, are comparable with those in our 1993 studies. In addition, we analyzed the reported cases of murine typhus in Corpus Christi in relation to opossum distribution and seroprevalence. We found a positive correlation between 1998 human murine typhus cases and the geographic distribution of seropositive opossums and their fleas.

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Materials and Methods

Review of Human Murine Typhus Cases

Historical data on cases of murine typhus are available through the Texas Department of Health and the Corpus Christi-Nueces County Health Department. Extant data fit the confirmed case definition of a fourfold rise in indirect immunofluorescence assay (IFA) titer or a single titer of ≥1:128 with clinical symptoms. The 1997 data were extracted from cases reported to the Texas Department of Health. In 1998, data included passive and active surveillance of Spohn Hospital System records. In addition, a board-certified infectious disease specialist contacted area physicians about a human typhus study, which was running concurrently with the opossum study. We also included murine typhus cases reported by area physicians during May through July 1998. Data were analyzed for trends in yearly case rate and incidence by age groups. The 1997 and 1998 data were analyzed for sex, age, symptoms, and geographic distribution of cases.

Opossum Collection

The sera analyzed in this study came from the opossums trapped by Corpus Christi residents during an 18-day period in mid-June 1998. A total of 149 opossums were given to animal control officers for euthanization. Opossums were removed from traps, tagged, and transported to the Vector Control facility, where they were numbered and anesthetized with a ketamine/xylazine mixture. The opossums were weighed, identified by age and sex, processed for ectoparasites, and bled by cardiac puncture. Fleas and ticks were removed with a flea comb. The ectoparasites were collected and placed in vials containing 70% ethanol.

Rickettsial Seroprevalence in Opossums

Over 95% of trapped opossums were used for a seroprevalence study of rickettsial infections. Initial screening of opossum serum samples of antibodies to *R. typhi, R. rickettsii, Coxiella burnetii,* and *Ehrlichia chaffeensis* was carried out at the University of Texas at San Antonio. Rickettsial diagnosis was performed with Multi-Test INDX R3E2 Dip-S-Ticks test strips (Integrated Diagnostics, Inc., Baltimore, MD). The assay uses a four-step enzyme-linked immunoassay dot technique for detecting both immunoglobulin (Ig) G and IgM antibodies. Serum samples from uninfected murine typhus patients were used as negative and positive controls. A titer >1:32 was considered positive for *R. typhi*. Eighty samples with equivocal results were retested by the kit manufacturer (Integrated Diagnostics, Inc.). In addition, opossum sera were tested by IFA for antibodies to *R. typhi* and *R. felis* by IFA. Briefly, *R. felis*-infected flea midguts (FleaData, Inc; Freeville, NY) were dissected and placed into individual wells of a 10-well Teflon-coated antigen slide at two midguts/well and allowed to air dry for 20 minutes. Slides were fixed in ice-cold acetone for 10 minutes, air dried, and incubated with individual opossum serum samples (diluted 1:64 and 1:128 in phosphate-buffered saline [PBS]), for 1 hour in a humidified chamber at 37°C. Serum was removed by aspiration, and wells were washed three times with PBS. Midguts were then incubated with secondary antibody (fluorescein isothiocyanate-conjugated goat anti-opossum IgG, [Bethyl Lab., Montgomery, TX], diluted 1:20 in PBS/0.01% Evan's blue) for 30 minutes at room temperature. After three PBS washes, slides were air dried and screened for seropositivity. *R. typhi*-infected Vero cells were also used for the serologic screening. Murine typhus convalescent-phase serum, *R.typhi*-positive opossum serum, negative control serum, and uninfected flea midguts (IFA and PCR negative) were used as positive and negative controls. The cat fleas, purchased from FleaData, Inc., were constitutively infected with *R. felis* (≥95% [15]) and used as positive controls and antigen sources for opossum serology. The IFA slides were screened by two readers for accuracy. Although attempts to isolate Rickettsiae from the serum samples during the acute phase of infection were unsuccessful, we extracted DNA from selected opossum serum samples. DNA was extracted from 200-µL serum samples by using QIAmp DNA Blood Mini Kit (Qiagen, Valencia, CA) and used for PCR with *Rickettsia*-specific primers.

Detection and Identification of Rickettsiae in Fleas

Detection and identification of rickettsial species in fleas collected from opossums were carried out using PCR and restriction fragment-length polymorphism analysis of PCR products. Detection of *R. felis* gene encoding 17-kDa protein antigen in fleas was done by PCR as described (9–11). Briefly, DNA from fleas was obtained by grinding the fleas with grinders containing 20 µL of sterile distilled H_2O and boiling the lysate for 10 minutes. After centrifugation, 5 µL of the supernatant containing DNA was used for PCR. The DNA template was added to a solution containing 18 µL of PCR Master mix (Roche, Mannheim, Germany) and 1 µL each of forward and reverse primers (100 µmol). In a PCR thermal cycler (Thermo Hybaid, Franklin, MA), each sample was heated to 94°C for 3 minutes, followed by 30 cycles of 94°C for 45 seconds, 55°C for 45 seconds, 72°C for 45 seconds, and an additional incubation period of 72°C for 5 minutes on the final cycle. The target PCR product was visualized by electrophoresis on a 1% agarose gel stained with ethidium bromide and excised; DNA was recovered from the gel with a StrataPrep DNA extraction kit (Stratagene, La Jolla, CA) according to manufacturer's protocol. Enzymatic digestion of cleaned PCR product was done by incubating 8 µL of DNA in 1X enzyme buffer (10 mM Tris-HCl [pH 7.5], 50 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/mL bovine serum albumin, and 50% glycerol), and 15 U of *Alu*I (Stratagene) for 1 hour at 37°C. Digested products were visualized on 8% TBE gels (Novex, San Diego, CA) stained with ethidium bromide. For sequencing, the purified 17-kDa fragments were subcloned in TOPO TA cloning vector (Invitrogen, San Diego, CA) and were sequenced by the dye terminator method on a model 373 automated fluorescence sequencing system (Applied Biosystems, Foster City, CA).

Sequence analysis was performed with the MacVector software package (Accelrys, Inc., Madison, WI), and the BLAST program (National Center for Biotechnology Information, Bethesda, MD) was used for comparison. Sequencing was carried out three times, in both directions, to ensure fidelity.

Results

Human Murine Typhus Cases, Corpus Christi, Texas

Since the 1970s, the number of murine typhus cases has fluctuated around 20 cases/year in south Texas. In 1997, however, a record number of cases, 72, were reported in Texas, resulting in a statewide incidence of 0.4/100,000 population. Sixty-nine of the 72 cases occurred in Region 11 of the Texas Department of Health; most cases occurred in three counties: Hidalgo, Cameron, and Nueces. These three counties consistently register the majority of murine typhus cases in Texas. Data from January 1985 through December 1997 show that Nueces County has averaged the most cases. Cases are reported year-round; however, peak incidence occurs during May and June, which leads local physicians to call murine typhus "the summer flu." Murine typhus cases from 1997 and 1998 (Figure 1), occurring in residents of Corpus Christi, were reviewed. Patients ranged from 5 to 79 years of age (mean 40 years). The 1997 and 1998 murine typhus patients were analyzed for race, ethnicity, history of fleabite, exposure to cats and opossums, and presence of symptoms. Fifty-five percent of patients were Hispanic, and 62.2% were female. Symptoms included headache (56%), fever (100%), rash (27%), nausea/vomiting (51%), malaise/fatigue (44%), arthralgia/myalgia (22%), and diarrhea (20%). Fewer than 15% of patients reported a history of fleabite, and exposure to cats or opossums at residences was associated with only 13% and 11% of cases, respectively. Nueces County/Corpus Christi had 14 of the 42 confirmed murine typhus cases reported in 1999 in Texas and 20 of the 52 reported cases in 2000.

Characteristics of Opossums Trapped for Typhus Studies

Opossums are nuisances for residents of Corpus Christi by inhabiting den sites in junk heaps, storage sheds, garages, and attics. Corpus Christi's opossum population is controlled primarily by private citizens using personal traps. Fifty traps are available at nominal rental through the Corpus Christi Animal Control Program. In contrast, anecdotal information from the nearby Flour Bluff and Calallen areas suggests that residents in these areas tolerate opossum presence. Most opossums that cause problems for residents in these areas are destroyed privately; occasionally, they are used for food. Nevertheless, from 1996 through 1998, Corpus Christi Animal Control trapped and euthanized >18,000 opossums. The mean number of trapped opossums during this 3-year period was 6,324/year. Although data regarding opossum population size, based on the average number of trapped opossums/year, are not available for the study area, the trapped population may represent 20% to 30% of the total yearly population. If this is the case

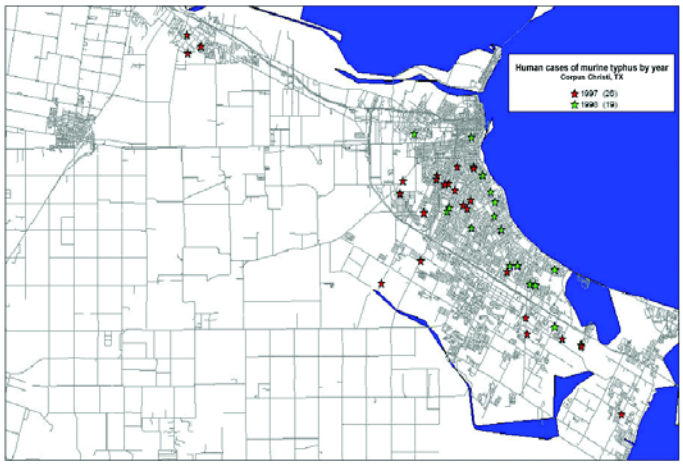

Figure 1. Geographic distribution of human murine typhus cases in Corpus Christi, Texas, 1997 and 1998.

and assuming equal distribution of opossums' ideal habitats throughout the city, the opossum population density in Corpus Christi could approach ≥75 opossums/square mile or approximately 1 opossum/0.013 square mile.

Although opossums are collected continuously in the Corpus Christi area, the 1998 study focused on opossums trapped within approximately a 3-week period during the traditional peak of human murine typhus cases. The characteristics of the 149 opossums trapped during June 8–25, 1998, were as follows: 51% female (n=76); 49.0% (n=73) male; and 47.7% juveniles and sexually immature (Table 1). Weight of the trapped opossums ranged from 5 oz to 8 lbs (mean weight 13 oz for juveniles; 4 lbs 14 oz for adults).

Rickettsial Seroprevalence in Opossums

In 1998, a seroprevalence study for *R. typhi* showed a geographic association between human cases of murine typhus and ranges of seropositive opossums (Figures 2 and 3). Six (31.6%) of the 19 patients lived within the minimum home range, 0.02 square mile, of a seropositive opossum. Another five patients (26.3%) were within the maximum home range, 0.1 square mile, of a seropositive opossum. Initial studies on seroprevalence of rickettsial infections in opossums carried out by enzyme-linked immunoassay showed no seroreactivity to *C. burnetti*, the agent of Q fever; *E. chaffeensis*, the agent of monocytic ehrlichiosis; and *R. rickettsii*, the agent of Rocky Mountain spotted fever. However, >25% of the 149 serum

Table 1. Rickettsial seroprevalence in 149 opossums, Corpus Christi, Texas

	Female	Male
Age	Positive/total (%)[a]	Positive/total (%)[a]
Juvenile	7/31 (23)	10/40 (25)
Adult	15/45 (33)	6/33 (18)
Total	22/76 (29)	16/73 (22)

[a] Enzyme-linked immunoassay (Integrated Diagnostics, Inc., Baltimore, MD)

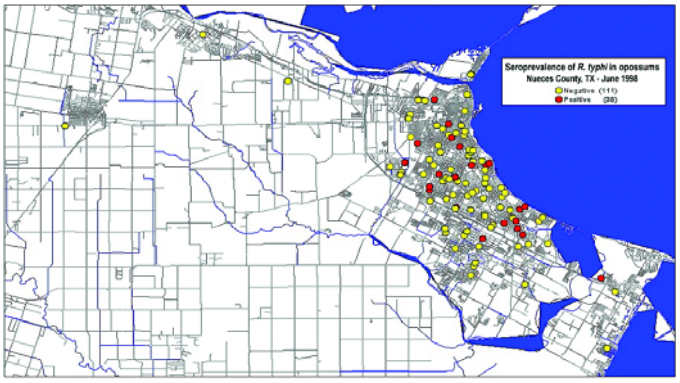

Figure 2. Geographic distribution of seropositive opossums in the residential areas of Corpus Christi, Texas, June 1998.

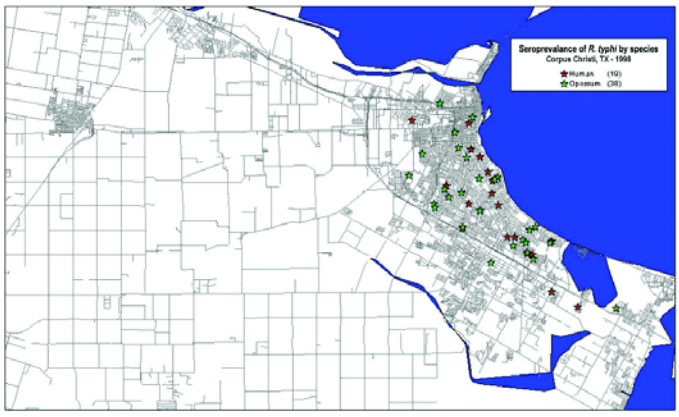

Figure 3. Geographic distribution of R. typhi by species in the residential areas of Nueces County, Texas, 1998.

samples reacted with *R. typhi* antigens (Table 2). Seventeen (23.9%) of juvenile and 21 (26.9%) of adult opossums were seropositive (Table 1). Reevaluation of the opossum serum samples by using IFA with both *R. typhi* (Wilmington strain) antigens and the *R. felis*-infected cat flea midguts, showed that 8% and 22% of opossum sera were reactive at ≥1:128 with *R. typhi* and *R. felis,* respectively (Table 2). Although *R. felis*-infected flea midguts were used to identify non-*R. typhi* seropositive opossums, these two rickettsial species could not be distinguished in some samples (n=6). Since both *R.typhi*- and *R. felis*-positive fleas were collected from opossums, the possibility of dual infections of opossum could not be ruled out, even though dual rickettsial infection in fleas has not been reported (16).

Detection and Identification of Rickettsiae in Cat Fleas

A total of 3,401 fleas were collected from 147 opossums. Over 99% of the fleas collected were identified as *Ctenocephalides felis*. The number of fleas per opossum ranged from 1 to 488 (mean 23 fleas/opossum). Initially, ricksttsial infection in fleas was assessed by using IFA with rat polyclonal anti-*R. typhi.* A total of 359 fleas collected from 50 opossums were sampled and tested individually by IFA; 20% of the fleas were positive. PCR was used to confirm rickettsial infection in fleas. Analysis of 529 individual fleas from 144 opossums showed an overall infection rate of 2.6% (14 confirmed positive). Restriction fragment-length polymorphism analysis of positive flea PCR products yielded a banding pattern representing 3 *R. typhi*- and 11 *R. felis*-infected fleas (Figure 4). The overall *R. felis* infection rates for 1998 samples were lower than 1993 infection rates (Table 3). Overall, 8% of the opossums had positive fleas when fleas were tested individually, compared with 21% when flea pools (50 pools; <20 fleas/pool/opossum) were used. The observed discrepancy between the results from pooled and individual flea samples reflects the variability in the DNA recoverable by PCR procedure. Although there was a positive correlation between the opossum age and the flea/opossum ratio, infected fleas came from both juvenile and adult opossums. Additionally, no correlation between the infected fleas and seropositive opossums existed.

Discussion

Since 1946, the Annual Summary of Notifiable Diseases in Texas has included murine typhus. Historical data identify 1,127 cases of murine typhus in 1946. However, the reported cases of murine typhus dropped rapidly with the advent of successful rodent and flea controls; by 1952, <100 cases/year in Texas were reported (1,2). Through 1960, the number of human cases steadily decreased, ranging from 12 to 50 and averaging 20 cases/year. The sudden increase in locally acquired cases in the 1990s presented a different reservoir-vector-rickettsia paradigm. Historically, murine typhus infection as an urban zoonosis has been maintained and transmitted in commensal rodents, in particular the Norway rat (*R. norvegicus*) and the oriental rat flea (*X. cheopis*) (1,2). However, in recent years the zoonotic cycle responsible for the documented human murine typhus cases in south Texas, as well as southern California, has been shown to involve opossums and cat fleas (7–10,17). The role of opossums and cat fleas in the transmission of *R. typhi* in suburban focus of murine typhus in Los Angeles County has been well documented (9,17). As in our study, a high proportion of opossums collected in Orange County, California, was seropositive for rickettsia (9,17). Opossums, as a peridomestic animal, are frequent visitors of human habitations, where they search for both harborage and food and thus expose the occupants to cat fleas and consequently to rickettsial pathogens. Cat fleas are frequently found in large numbers on opossums and are avid feeders on humans and household pets. In addition to *R. typhi,* the cat fleas also

Table 2. Seroprevalence of *Rickettsia typhi* and *R. felis* in 149 opossums collected in Corpus Christi, Texas, June 1998[a]

	Serologic assays Positive/total (%)	
Opossum serum samples	R. typhi	*R. felis*
EIA	38/149 (25)	N.D.
IFA[b]	10/125 (8)	28/125 (22)

[a] EIA, enzyme-linked immunoassay (Integrated Diagnostics, Inc., Baltimore, MD); N.D., not done; IFA, indirect immunofluorescence antibody assay.
[b]IFA with *Ctenocephalides felis* (FleaData *R. felis*-infected colony) midgut smears and *R. typhi* (Wilmington strain) as antigens at ≥1:128 titer.

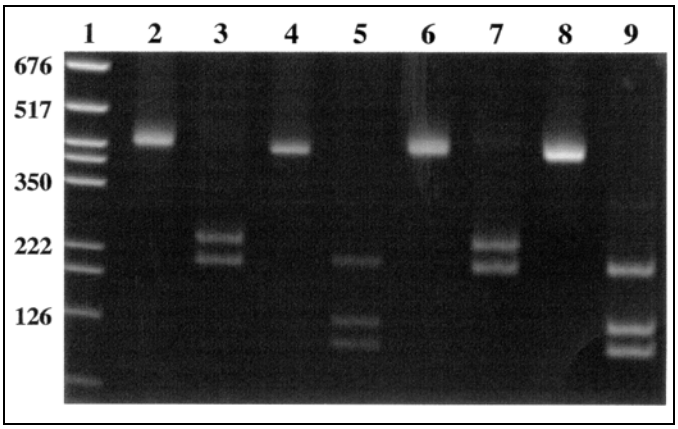

Figure 4. Representation polymerase chain reaction of a 434-bp amplification product of rickettsial 17-kDa protein gene and digestion with *Alu*I from fleas collected in Texas. Lane 1: D-15 DNA marker (Novex). Lanes 2 and 3: purified 17-kDa-fragment amplification product from *Rickettsia typhi*-infected Vero cells and *Alu*I digest, respectively. Lanes 4 and 5: purified 17-kDa-fragment amplification product from colony-raised *R. felis*-infected fleas and *Alu*I digestion, respectively. Lanes 6 and 7: purified 17-kDa-fragment amplification product from *R. typhi*-infected fleas collected in Texas and *Alu*I digestion, respectively. Lanes 8 and 9: purified 17-kDa-fragment amplification product from *R. felis*-infected fleas collected in Texas and *Alu*I digestion, respectively.

harbor *R. felis*. In fact, the cat flea infection with *R. felis* is more common than *R. typhi* (7,8,10). Both rickettsial species are readily maintained transovarially in fleas (11,12,18), but in contrast to commercial cat flea colonies that usually maintain >80% *R. felis*-infection rates, only 1% to 5% of wild-caught fleas are infected with this rickettsial species (7,8,10,18).

We have shown that cat fleas collected from opossums from Corpus Christi, Texas, were infected with either *R. typhi* or *R. felis,* and the infection rates remained <5% in both the 1993 and 1998 samplings. While we have found no evidence for dual infection in individual fleas, opossums fed on by infected fleas could have antibodies against both *R. typhi* and *R. felis*. Our initial opossum serosurvey results (Tables 1 and 2) using enzyme-linked immunoassay were directed against *R. typhi* only. However, IFA results from *R. felis*-infected fleas confirmed our earlier findings (7,8,10) that the cat flea/opossum cycle is responsible for the maintenance of both *R. typhi* and *R. felis* in Corpus Christi.

We have reported the importance of *R. felis* as a component of murine typhus transmission cycles (14,19,20). Both *R. typhi* and *R. felis* were found in fleas and opossum tissues from the murine typhus–endemic areas of southern California and south Texas (7,8,10). Additionally, a retrospective investigation of five murine typhus patients from Texas demonstrated that four of the patients were infected with *R. typhi* and the fifth had been infected with *R. felis* (7). This documented human infection with *R. felis* and its presence in opossums and their fleas, and possibly in other wildlife associated with human habitations, have raised concerns about *R. felis* spillover into human populations. In addition, cat fleas infected with *R. felis* have been identified not only in the United States (19) but also in Central and South America, Europe, and Australia (T. Kilminster, unpub. data).

Table 3. *Rickettsia typhi* and *R. felis* infections in opossums and their fleas, Corpus Christi, Texas

	Total	*R. typhi*	*R. felis*
Sample	Positive/total (%)	Positive/total (%)	Positive/total (%)
Opossum			
1993[a]	3/9 (33)	0/3 (0)	3/9 (33)
Cat flea[a]			
1993	18/399 (5)	3/399 (1)	15/399 (4)
1998	14/529 (3)	3/529 (1)	11/529 (2)

[a]Confirmed with polymerase chain reaction/restriction fragment-length polymorphism sequencing.

Together, our published data and these recent reports not only support the pathogenic role of *R. felis* but also demonstrate its wide geographic distribution. However, we know very little regarding the natural maintenance and transmission of this organism in areas of the world besides south Texas and southern California. The cat flea, known as an indiscriminate feeder, has an extremely broad host range. While it parasitizes cats, opossums, and other animals of the same size, the flea readily switches to different hosts, and it has been found on rats and mice. Because cat fleas are commonly found on household pets, we extended our studies to determine rickettsial seroprevalence in cats. Our pilot serologic studies showed >15% of 513 serum samples from the eastern USA were reactive at ≥1/64 with *R. felis*, as assessed by IFA (Higgins et al., unpub. data). Sorvillo et al. (17), in their Los Angeles study of a suburban focus of murine typhus, reported that 9 of 10 domesticated and 3 of 26 feral cats were seropositive to *R. typhi*. Thus, domesticated cats and cat fleas, as well as peridomestic animals, may play an important role in the maintenance cycle of *R. felis* and its transmission to humans. Our study further documents the involvement of the opossum/Rickettsia/cat flea triad in the flea-associated rickettsial transmission cycle of urban and suburban areas of south Texas and southern California. Similar host/parasite relationships may also operate in other parts of the world where recent *R. felis* human cases have been documented (13,15). Recent attention to *R. felis*, which already has resulted in reassignment of this organism to the spotted fever group rickettsiae (14,15), may further elucidate the other components involved in the maintenance of this rickettsiosis.

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Dr. Boostrom is the director of Family Health Services at the Corpus Christi-Nueces County Public Health District. Her research interests include communicable disease surveillance and the epidemiology of typhus in south Texas.

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Emerging Infectious Diseases Policy on Corrections

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Correction, Vol. 8, No. 3

In *Listeria monocytogenes* Infection in Israel and Review of Cases Worldwide, by Y. Siegman-Igra et al., an error appears in the discussion section. The corrected sentence appears below and online at http://www.cdc.gov/ncidod/EID/vol8no3/01-0195.htm .

The case-fatality rate in the collected data on nonperinatal infection was 36% (413 of 1,149 patients for whom this information was available).

We regret any confusion this error may have caused

Defining and Detecting Malaria Epidemics in the Highlands of Western Kenya

**Simon I. Hay,*† Milka Simba,† Millie Busolo,‡ Abdisalan M. Noor,†
Helen L. Guyatt,*† Sam A. Ochola,‡ and Robert W. Snow*†‡**

Epidemic detection algorithms are being increasingly recommended for malaria surveillance in sub-Saharan Africa. We present the results of applying three simple epidemic detection techniques to routinely collected longitudinal pediatric malaria admissions data from three health facilities in the highlands of western Kenya in the late 1980s and 1990s. The algorithms tested were chosen because they could be feasibly implemented at the health facility level in sub-Saharan Africa. Assumptions of these techniques about the normal distribution of admissions data and the confidence intervals used to define normal years were also investigated. All techniques identified two "epidemic" years in one of the sites. The untransformed Cullen method with standard confidence intervals detected the two "epidemic" years in the remaining two sites but also triggered many false alarms. The performance of these methods is discussed and comments are made about their appropriateness for the highlands of western Kenya.

Epidemics of all infectious diseases generate considerable public attention and are reported widely in the popular and scientific press. The definition of truly exceptional numbers of cases from commonly perceived "epidemics" is often difficult, however, particularly for widespread pathogens (1). *Plasmodium falciparum* malaria is extensive, prevalent, and increasing in sub-Saharan Africa (2–4). Stable endemic malaria predominates throughout the continent, but epidemics occur at the fringes of endemic areas, particularly among communities at the southernmost latitudes, across the arid regions of North Africa, and among the highlands of East, central, and Horn of Africa (5,6).

In the late 1980s and early 1990s, a series of malaria "epidemics" were reported in the western highlands of Kenya and other communities at high altitude in the subregion (5,7–17). A widely held view is that the transmission of *P. falciparum* in such communities is limited primarily by low ambient temperature and that small changes in temperature could therefore provide transiently suitable conditions for unstable transmission within populations that have acquired little functional immunity (18–21). Furthermore, the highlands of Kenya are densely populated and agriculturally productive. These factors have contributed to the Government of Kenya's decision to define 15 districts in the western highlands (Figure 1.; [22]) as being prone to epidemics and thus meriting special attention for surveillance to increase epidemic preparedness (23).

The World Health Organization's (WHO's) Roll Back Malaria's efforts to manage epidemic malaria in sub-Saharan Africa include supporting the establishment of early detection (surveillance), early warning, and forecasting systems to provide adequate preparation time to prevent or contain malaria

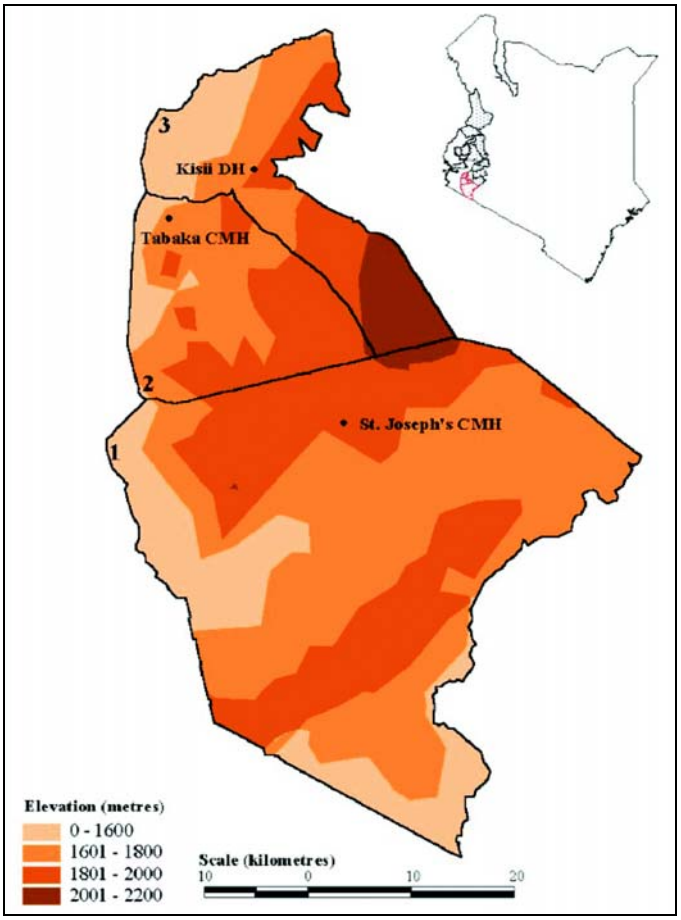

Figure 1. Locations of the three study hospitals and the administrative districts they serve in the highlands of western Kenya. The inset map of Kenya shows the 15 districts designated by the Government of Kenya as at risk from unstable, temperature-limited, and hence epidemic malaria. The three districts shaded in red are those in the large map. The St. Joseph's Catholic Mission Hospital (CMH) at Kilgoris, Tabaka CMH, and Kisii District Hospital are shown within their administrative boundaries of (1) Trans Mara, (2) Gucha and (3) Kisii Central District, respectively. The districts are shown against a backdrop of a digital elevation model for which a key is provided. North is to the top of the page.

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epidemics (24,25; URL: http://www.rbm.who.int/). The object of early detection (or epidemiologic surveillance) is to monitor a disease continually so that abnormal events can be identified rapidly, in the expectation that intervention efforts can be initiated in a timely manner (26,27). Extensive research on the optimization and comparison of surveillance algorithms exists (28–34); most published articles, however, are concerned with weekly reporting of rare infectious diseases in relatively wealthy countries. In technologically underdeveloped nations, governments have far fewer resources for disease prevention and medical care. Resource constraints in the health sector are often so severe that the time a health service employee may devote to surveillance will inevitably result in compromises elsewhere. In such circumstances, these cost-benefit considerations favor simple, robust surveillance systems (35).

We examined three simple techniques proposed for malaria epidemic detection (24) to evaluate what early warning information would have been provided if surveillance had been implemented using standard admissions records at three hospitals in the western Kenyan highlands during the late 1980s and 1990s. We did not explore the meteorologic correlates of temporal changes in malaria cases at these sites as a basis for malaria early warning (6,36–38), although this is the subject of ongoing research (39,40).

Methods

Study Area

Three hospitals providing inpatient clinical care were identified in the western Kenyan highlands (Figure 1). These hospitals were selected because malaria epidemics had been reported within the last 5 years where they were located, and complete clinical records, spanning more than 10 years, were available for review. The three hospitals were St Joseph's Catholic Mission Hospital at Kilgoris in Trans Mara District (latitude 1.068 S, longitude 34.958 E; altitude 1,683 m); Tabaka Catholic Mission Hospital (latitude 0.751 S, longitude 34.663 E; altitude 1,684 m) in Gucha District; and Kisii District Hospital (latitude 0.684 S, longitude 34.770 E; altitude 1,815 m) in Kisii Central District. The hospitals serve varying catchment populations and are within 40 km of one another.

Each facility is located above 1,600 m, an altitude above that defined as characterizing highland/epidemic-prone malaria (18–20), although such limits have been challenged (5). The average altitudinal limits of the wider area shown in Figure 1 range from 1,600 to 2,200 m.

Monthly temperature and rainfall data were extracted for January 1980 to December 1995 from an interpolated global climate surface at 0.5 x 0.5° spatial resolution (41,42), using georeferencing details from Tabaka Catholic Mission Hospital. The synoptic year (1980–1995) shows a remarkably stable mean monthly temperature of approximately 20°C (Figure 2a), with peak rainfall (approximately 200 mm) occurring in the months of April and May (Figure 2b), usually referred to as the "long rains."

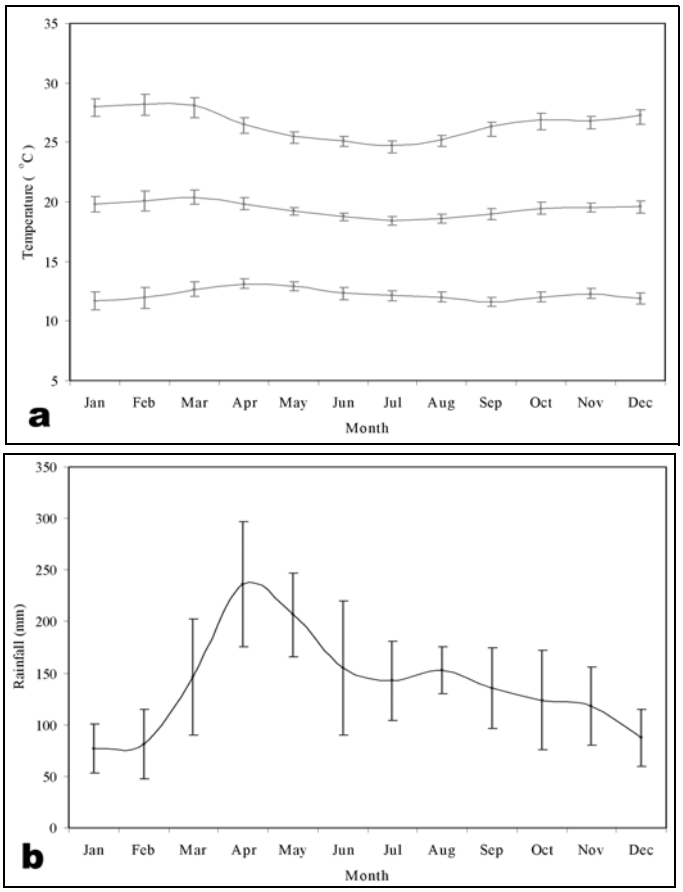

Figure 2. Meteorologic time-series for study hospitals. Temperature and rainfall profiles for a synoptic (1980–1995). (a) minimum (bottom), mean (middle), and maximum (top) monthly temperatures (°C); (b) average total monthly rainfall (mm). The error bars denote standard errors of the monthly means.

Clinical Data

Hospital admission registers for every ward at each facility were located and sequentially reviewed to identify patient age, date, and cause of admission. Month- and age-tallied cases of "clinical malaria" were compiled for each complete year. Criteria used to select malaria cases were based on whether malaria was made as a primary, coprimary, or coincidental diagnosis by the admitting physician. Not all diagnoses were microscopically confirmed, and discharge diagnoses may have been different from those defined on admission, following further clinical and laboratory investigations. Nevertheless, patients at each facility were treated for malaria during the initial 24 hours of admission and represent the monthly clinical commitment to malaria case management at each hospital. Such data are used routinely to define epidemics by local health authorities and serve as the basis for increasing demands for resources.

In these analyses we consider only the pediatric malaria admissions (patients <15 years of age), who constituted approximately two thirds of the patients at each facility (Kilgoris, 14,079 adults and 30,793 children; Kisii, 44,043 adults and 84,648 children; and Tabaka, 23,692 adults and 55,871 children during the study period). The rationale is that

children are more likely to give an accurate picture of local malaria transmission than adults, as they are less likely to have functional immunity or to have traveled and acquired the disease elsewhere. Cumulative monthly cases were also computed for each year to show the overall annual burden and acute, seasonal rises in malaria admissions. The years of exceptional malaria cases were defined simply as the 2 years of highest cases during the surveillance period.

Epidemic Detection Techniques

We assumed a minimum set of requirements for resource-constrained, district-level health services in Kenya: access to a computer, limited knowledge of a spreadsheet application, and availability of at least 5 years of admission records from a health facility. For this reason, we focused on a subset of those techniques advocated by WHO for application to malaria surveillance in resource-constrained environments (24).

Epidemic alerts can be based on simple incidence thresholds only, as is common with meningococcal meningitis at the district level in sub-Saharan Africa (43–46); when a threshold is exceeded, an alert is triggered. The value of the threshold is usually determined from expert opinion informed by an examination of retrospective case data over wide geographic areas. This technique is not applicable to a single facility where accurate population denominator data (necessary to calculate incidence) are often not available and therefore not considered further.

Many epidemic surveillance techniques aim to identify points in a disease time series outside the 95% confidence intervals of a normal distribution determined from the history of cases at that location. A method proposed by Cullen (47) uses the previous 5 years of data (in which epidemic years are arbitrarily excluded) to construct an admissions profile for an average year. The alert threshold for each month is then determined as the mean plus 2 times the standard deviation (strictly, the arithmetic mean plus 1.96 times the standard deviation should capture 95% of cases in normally distributed data [48]). This technique was successfully applied to cases of *Plasmodium vivax* malaria in northern Thailand during the 1980s (47). It has also been used for surveillance of *P. falciparum* malaria in the Madagascan highlands (49).

WHO has advocated the use of a conceptually similar method that triggers an alert when current cases exceed the upper 3rd quartile or the "upper normal limit" determined from 5 years of retrospective monthly case data (50). For 5 years of observations, quartile 0 is the minimum, quartile 1 the second lowest, quartile 2 the median, quartile 3 the second highest, and quartile 4 the maximum value of the series for any given month. If the current month's cases exceed quartile 3, an alert is triggered. This method has been implemented to detect highland malaria epidemics in Ethiopia (22).

The Centers for Disease Control and Prevention has developed a further cumulative sum (c-sum) method for detecting epidemics. It is based on the construction of an average or base year, determined by calculating the expected number of cases using the average for that month (and the previous and following month) during the past 5 years (n=15) (29,51,52). For example, the expected number of cases for March 2000 would be derived from the average of February, March, and April admissions from 1995 to 1999, inclusive. A ratio of present to past cases is then usually presented as a current to past history graph (53), with values greater than one representing disease increases.

Statistical Analysis

WHO, Cullen, and c-sum methods were tested on the series of pediatric malaria admissions data to evaluate their usefulness in the identification of epidemics, defined as the 2 years of highest numbers of cases. We modified the c-sum technique to provide 95% confidence intervals for the expected cases so that it could be evaluated against the other techniques. For each method, the expected cases in a given month were defined by the previous 5 years of data and sequentially updated for each new observation year in the series. "Epidemic years" were not excluded from the base years, as no objective criteria have been offered to define years that are epidemic and excluding these years would increase the likelihood of detecting epidemics**.** A skewness statistic that measures the degree of asymmetry in a distribution around the mean (Microsoft Excel 2000, Seattle, WA) was also applied to the data to test assumptions of normality in the admissions data. Positive or negative values indicate an asymmetric tail extending towards more positive or more negative values, respectively. The Cullen and c-sum techniques were then repeated by using $\log_{10}$ transformed childhood admissions data to investigate potential problems with the techniques that assume normally distributed data. Confidence intervals were determined for the Cullen and c-sum techniques on untransformed and $\log_{10}$ normalized admissions data by using the mean + (2x standard deviation) as well as the mean + (t value at 0.05 confidence interval x standard error), as is recommended for small sample sizes (48).

Results

Figure 3a-c shows pediatric admissions for the three study hospitals during the surveillance period. The graphs of cumulative cases (Figure 4a-c) show a distinct seasonality in admissions; the sharpest rise in case numbers occurred in June and July, immediately after the long rains in April and May (Figure 2b). The 2 years of highest case numbers were 1994 and 1998 for Kilgoris, 1996 and 1997 for Kisii, and 1997 and 1996 for Tabaka. In these so-called epidemic years, cases were often above normal in all months.

The child admissions data at each site were positively skewed with values of 2.88, 1.96, and 1.78 (skewness statistic = 0 for normal data series) for Kilgoris, Kisii, and Tabaka, respectively (Table 1). Log10 transformations of these data reduced the positive skew, thus helping normalize each series to values of -0.13, 0.34, and -0.08 for Kilgoris, Kisii, and Tabaka, respectively.

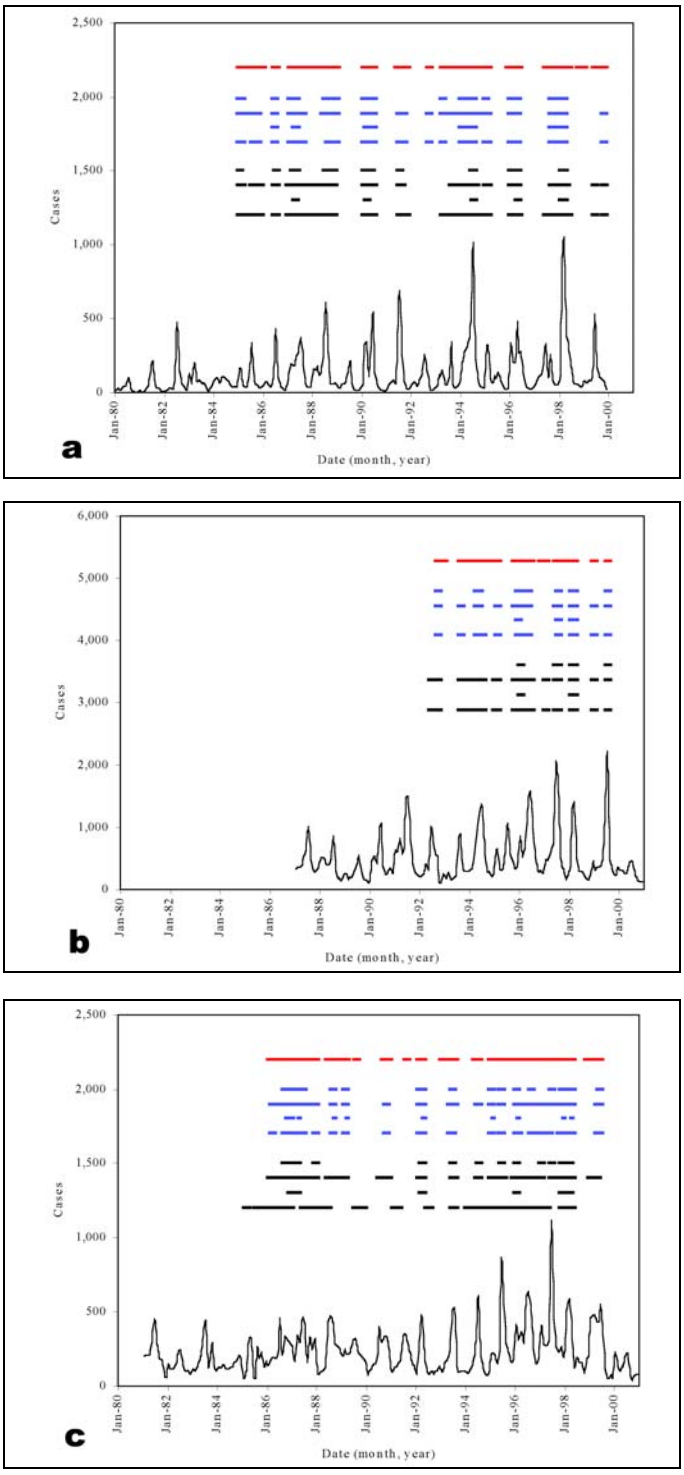

Figure 3. Time-series of child admissions and epidemic alerts for the three study hospitals.Time-series of child admissions (<15 years) for Kilgoris (a) Kisii (b), and Tabaka (c) for the 1980-1999, 1987–2000, and 1981–2000 time periods, respectively. The results of the "epidemic" prediction techniques are shown for the World Health Organization, Cullen, and c-sum techniques in red, blue, and black lines, respectively. For the Cullen and c-sum methods, the top line represents untransformed data with standard confidence intervals; the second line is untransformed data with confidence intervals adjusted for small sample sizes; the third line shows $\log_{10}$ transformed data with standard confidence intervals; and the fourth line shows $\log_{10}$ transformed data with confidence intervals adjusted for small sample sizes.

WHO methods concluded that 41.7%, 31.5%, and 42.8% of months in the surveillance period were epidemic for Kilgoris, Kisii, and Tabaka, respectively (Table 2; Figure 3a-c). The Cullen method showed fewer than half of these months to be epidemic, 14.4%, 10.2%, and 12.8%, respectively. The c-sum method indicated fewer still at 9.4%, 5.6%, and 10.6 %, respectively. Log_{10} transforming the child admissions data further reduced the proportion of months detected as epidemic. Adjusting the confidence intervals for small sample sizes had the opposite effect (Table 2). The WHO method and Cullen and c-sum techniques using the Kirkwood confidence intervals predicted approximately one third of all months during the surveillance period as epidemic (average 31.7%, range 14.8% to 42.8 %) (Table 2; Figure 3a-c). Strict statistical evaluation between the remaining techniques is difficult because of the problem of retrospectively determining what months were true epidemics; thus such evaluation was simply on the criteria of identifying the 2 years of highest cases (Figure 4). All techniques identified these 2 epidemic years in Kilgoris, but only the untransformed Cullen method with standard confidence intervals detected both epidemic years in Kisii and Tabaka as well.

Discussion

Reports of epidemics in the highlands of western Kenya increased in frequency in the early 1990s (10,12,54,55); as a consequence, detection and control of epidemics became a priority for the recently launched national malaria strategic plan (23). This initiative forms part of a broader international effort to develop surveillance and warning systems for epidemic detection in Africa as part of the WHO Roll Back Malaria initiative (24,56). The definition of epidemics continues to confuse many public health practitioners specializing in common diseases such as malaria. Epidemics are more often defined in response to political necessity rather than by examining empirical data. Little critical examination of long-term clinical data against proposed methods for epidemic interpretation in nominally epidemic-prone areas of sub-Saharan Africa has occurred. To address this, we examined time series of pediatric malaria admission data during the late 1980s and 1990s from three hospitals located in districts of the western highlands of Kenya identified by the Ministry of Health as prone to epidemics.

Application of three primary epidemic detection methods indicated alert signals in most years of the test period with or without modifications. Rather than representing an inadequacy in the methods, this reflected the restricted utility of these approaches in areas of acutely seasonal malaria case burdens, characterized by a large degree of between-year variability in the timing of seasonal onset and a gradual increasing trend in admissions. Clearly, having such frequent epidemic alert signals makes the usefulness of such techniques in this particular area of the western Kenyan highlands questionable.

A further characteristic of this area is between-year variability in malaria incidence. During the 1990s, at least two

important and dramatic seasonal rises in malaria occurred at each of the three hospitals (Figure 4). Sharp rises occurred during the months of February, and more commonly April or May (with the onset of the rains [Figure 2b]). Plotting monthly cumulative cases provided a more informative tool than traditional time-series plots to show seasonal deviations from previous years and simultaneously represented overall annual malaria cases. For the two exceptional years at each of the hospitals, the most sensitive of the "epidemic" detection methods shown in Figure 3 was the nontransformed Cullen technique that used standard confidence intervals. This technique, however, would also have given rise to a substantial number of false alarms during the observation period.

Applying the statistical techniques we have outlined highlights several methodologic issues that deserve comment, particularly for the Cullen and c-sum techniques, and should be considered by those advocating further application of these tools to common vector-borne diseases. First, mosquito-borne diseases that are sensitive to climate and hence are often seasonal, can show a skewed non-normal distribution in time. Methods that depend on arithmetic means and standard deviations (with their assumptions of data normality) to define alerts may require data transformation. Simple $\log_{10}$ transforms achieved data normalization and decreased the sensitivity of the techniques at all three facilities in this study. Second, each technique recommends using 5 years of retrospective admissions data so that standard deviations and hence alert thresholds for an average month are based on only five samples. A more appropriate formula for calculating the standard deviation in such situations has been proposed (48), although applying such modifications to these health facilities made the epidemic detection techniques substantially more sensitive. Third, when cases are increasing over the duration of the study, it is important to take a 5-year moving average to adjust the magnitude of the base year accordingly. Testing for the sensitivity of these techniques to the duration of moving average used was beyond the scope of this research but requires future investigation. Fourth, exclusion of "epidemic years" is an undefined procedure. For example, how many months detected as epidemic are needed in any year to prompt that year's exclusion from the moving average, and after exclusion, what data are used to define the confidence intervals for alerts? This exercise demonstrates that many factors need to be more fully considered before widely advocating such techniques.

Our analyses used records of severe and complicated malaria admissions to tertiary-level health facilities, where diagnosis is often supported by microscopy. We have not applied the epidemiologic surveillance tools to patients with mild, ambulatory cases of malaria treated as outpatients. These latter data may provide a more robust tool for early detection, but they are also subject to imprecise clinical case definitions, where diagnosis is almost always made presumptively without microscopy. Improvements in the provision of microscopy in the diagnosis of outpatient malaria may facilitate improvements of these surveillance tools.

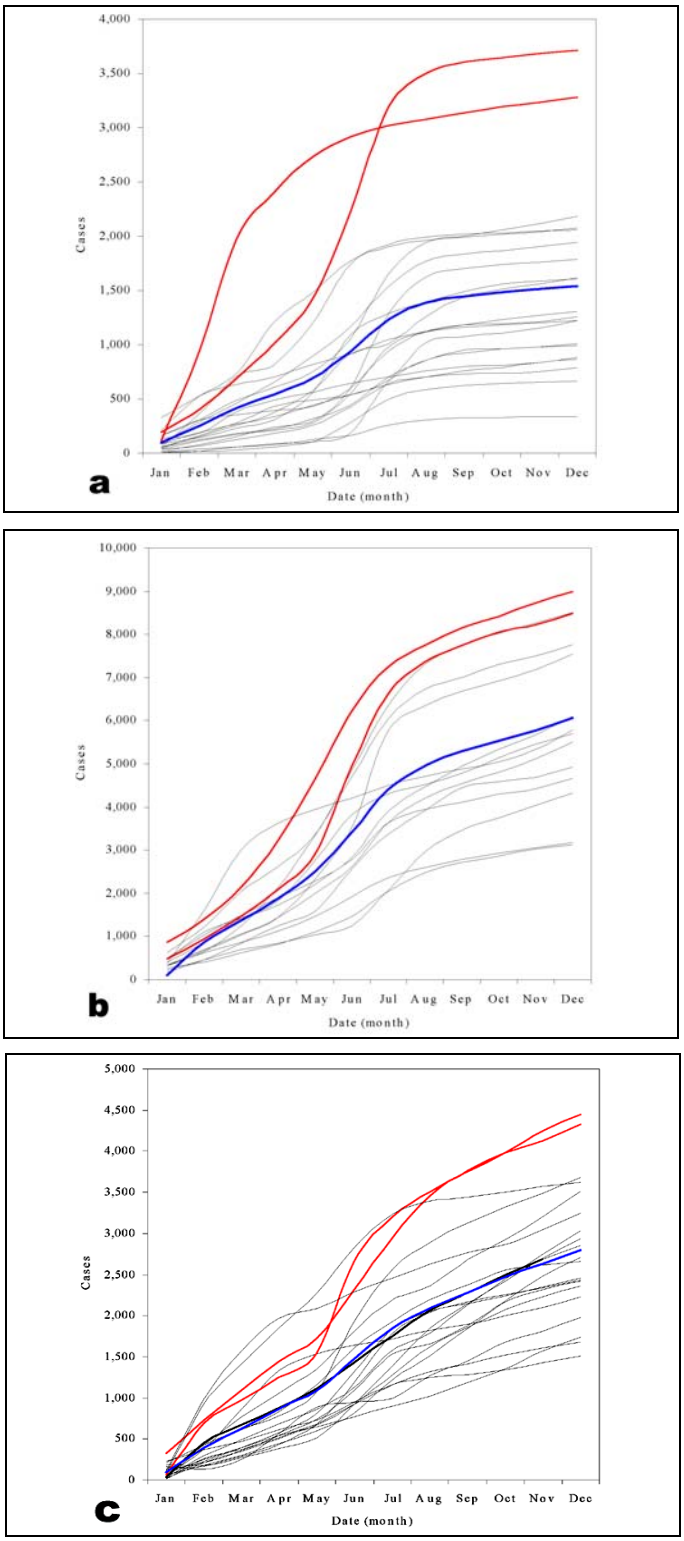

Figure 4. Cumulative case graphs for child admissions in the three hospitals. Cumulative child admissions (<15 years) in Kilgoris (a), Kisii (b), and Tabaka (c). All years for which data were available are shown, 1980–1999, 1987–2000, and 1981–2000 time periods for Kilgoris, Kisii and Tabaka, respectively. Black dashed lines are all "normal" years. The blue line shows mean average cumulative child admissions over all years. Red lines show epidemic years, defined as the 2 years of highest total admissions. For Kilgoris these exceptional years are 1994 and 1998, for Kisii they are 1996 and 1997, and for Tabaka they are 1997 and 1996.

Table 1. Descriptive and skewness statistics for child admissions at three study hospitals, western Kenya

	Kilgoris (1980–1999)		Kisii (1987–2000)		Tabaka (1981–2000)	
Transformation	Normal	Log_{10}	Normal	Log_{10}	Normal	Log_{10}
Mean	128.30	1.86	503.85	2.61	232.80	2.29
Minimum	3.00	0.48	95.00	1.98	35.00	1.54
Maximum	1,043.00	3.02	2,229.00	3.35	1,110.00	3.05
Sum	30,793.00	446.00	84,647.00	438.00	55,871.00	549.00
Count	240.00	240.00	168.00	168.00	240.00	240.00
Standard deviation	157.46	0.48	375.87	0.28	147.80	0.26
Standard error	10.16	0.03	29.00	0.02	9.54	0.02
Skewness	2.88	-0.13	1.96	0.34	1.78	-0.08

Table 2. Comparison of total number of epidemic months detected by the World Health Organization (WHO), Cullen, and cumulative-sum techniques for three study hospitals, western Kenya[a]

Technique	Method	Kilgoris 1998–1999 N=180 (%)	Kisii 1992–2000 N=108 (%)	Tabaka 1986–2000 N=180 (%)
WHO	Not transformed	75 (41.7)	34 (31.5)	77 (42.8)
Cullen	Not transformed, SCI	26 (14.4)	11 (10.2)	23 (12.8)
	Not transformed, KCI	47 (26.1)	16 (14.8)	45 (25.0)
	Log_{10} transformed, SCI	13 (7.2)	4 (3.7)	12 (6.7)
	Log_{10} transformed, KCI	39 (21.7)	15 (13.9)	36 (20.0)
C-sum	Not transformed, SCI	17 (9.4)	6 (5.6)	19 (10.6)
	Not transformed, KCI	55 (30.6)	27 (25.0)	64 (35.6)
	Log_{10} transformed, SCI	6 (3.3)	3 (2.8)	8 (4.4)
	Log_{10} transformed, KCI	66 (36.7)	30 (27.8)	76 (42.2)

[a]Figures are number of months defined as epidemic in the monitoring period. Brackets are the percentage of the total months defined as epidemic.

A further important problem that needs to be addressed is what constitutes an epidemic. Epidemic malaria was precisely described by MacDonald as "… an acute exacerbation of disease out of proportion to the normal to which the community is subject….Epidemics are common only in zones of unstable malaria, where very slight modification in any of the transmission factors may completely upset equilibrium, and where the restraining influence of immunity may be negligible or absent, and they therefore show a very marked geographic distribution" (57,58).

The term epidemic is applied more liberally today for malaria in the Kenyan highlands; it is essentially used for any occurrence of cases in excess of normal. Much of the confusion around defining epidemics spatially or temporally relates to knowing what is (or should be) expected routinely. Endemic malaria, for example can show considerable expected temporal variation. This can relate to climate-driven variation, seasonality, interepidemic periods resulting from population dynamics, or long-term trends (39). These factors can all operate simultaneously and are not epidemics, although they may have substantial public health implications. Deviations from any of these expected variations are true epidemics if they result from a disturbance of the normal epidemiologic equilibrium (50). Such considerations are crucially important in the determination of the normal situation against which epidemics are measured.

The highlands of western Kenya is an area where so-called malaria epidemics have been increasingly reported. The area was recently highlighted by the government of Kenya as epidemic prone. Considerable international efforts are also being made to develop and promote early warning and improved case-detection systems for epidemic-prone areas (24,56,59). These results indicate that the simple epidemic detection techniques recommended to date require substantial refinement before they can be considered operationally robust, since they lack the required sensitivity in detecting aberrant case burdens. The further question as to whether these techniques are appropriate for facilities that have pronounced and acutely seasonal transmission of malaria is still open. The dual goals of technique development and a more comprehensive description of the local malaria epidemiology in this region are the subjects of ongoing research. A related article in this issue outlines the implications of these data for interpreting the epidemiology of *P. falciparum* malaria in this highland region of western Kenya (60).

Acknowledgments

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Waterborne Outbreak of Norwalk-Like Virus Gastroenteritis at a Tourist Resort, Italy

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In July 2000, an outbreak of gastroenteritis occurred at a tourist resort in the Gulf of Taranto in southern Italy. Illness in 344 people, 69 of whom were staff members, met the case definition. Norwalk-like virus (NLV) was found in 22 of 28 stool specimens tested. The source of illness was likely contaminated drinking water, as environmental inspection identified a breakdown in the resort water system and tap water samples were contaminated with fecal bacteria. Attack rates were increased (51.4%) in staff members involved in water sports. Relative risks were significant only for exposure to beach showers and consuming drinks with ice. Although Italy has no surveillance system for nonbacterial gastroenteritis, no outbreak caused by NLV has been described previously in the country.

Norwalk virus is the prototype of the genus Norwalk-like virus (NLV) in the *Caliciviridae* family, which includes a large number of genetically related strains that together represent the most important cause of gastroenteritis outbreaks worldwide (1–2). NLV accounts for up to 96% of outbreaks of nonbacterial gastroenteritis in the United States (3) and has been implicated in 43% of all foodborne outbreaks in England, 67% in Sweden, and 80% in the Netherlands (4–6).

Outbreaks of NLV gastroenteritis more frequently affect adults and children >5 years of age. Because of the low infectious dose of the agent (10–100 viral particles can induce symptoms), outbreaks are characterized by a high secondary attack rate (7). In most documented outbreaks, the incubation period has been reported as 24–48 hours; the average duration of symptoms is 12–60 hours. During an outbreak, >50% of infected persons have symptoms of vomiting, most often in combination with diarrhea (8). The main source of infection is usually contaminated food or water (9–13), while the usual mode of transmission is direct person-to-person contact with saliva, vomit, or aerosols. Transmission may also occur through contact with contaminated objects and surfaces such as showers, sinks, mats, and floors (3).

In Italy, which has no surveillance system for nonbacterial gastroenteritis, the impact of NLV infection is unknown, and no previous outbreaks of confirmed NLV infection have been reported. We describe a large outbreak of gastroenteritis caused by NLV at a resort in Italy.

Methods

The outbreak occurred at a tourist resort in the Gulf of Taranto, southern Italy, during July 7–31, 2000 (Figure 1). The resort has an area of 122 hectares with 456 guest rooms in 19 buildings, in addition to staff quarters. The buildings are situated around a central area where a restaurant, a swimming pool, and the resort management office are located. The resort can accommodate 1,000 guests, who usually arrive on a Saturday and depart 1 or 2 weeks later, resulting in approximately 50% turnover of guests each weekend.

The resort's water tank is supplied via a 1-km pipe connected to the main public water supply (Figure 2). On July 13, a break in this water pipe was observed (Figure 2, point 2). Inspection also showed a bypass connecting the tank to an unused irrigation system (Figure 2, points 4 and 5).

On July 18, 2000, the local health unit and the Institute of Hygiene of the Faculty of Medicine in Bari were notified about an outbreak of gastroenteritis at the resort. An epidemiologic investigation was initiated the same day to identify the agent and the mode and vehicle of transmission and to

Figure 1. Map of Italy, showing location of tourist resort on Gulf of Taranto.

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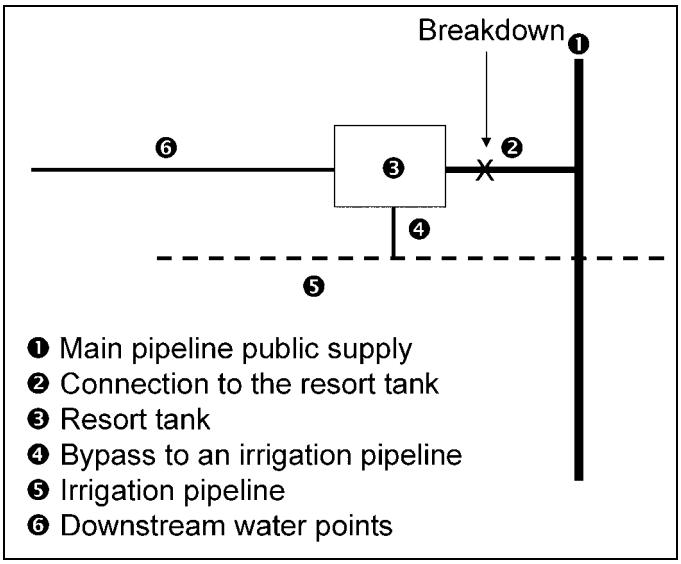

Figure 2. Water supply system in tourist resort, Italy.

implement control measures. By July 20, when the local health unit notified the Istituto Superiore di Sanità in Rome, the outbreak had already been in progress for approximately 2 weeks and >150 persons were ill.

Outbreak Investigation

A case was defined as illness in any guest or employee who stayed at the tourist resort during the period July 1–31 and who had diarrhea (three or more loose stools in any 24-hour period) or vomiting (at least one episode) or both, in the same period. Case finding was done by checking records of the resort medical center; after July 20, a door-to-door search was initiated. Demographic data and information on symptoms were collected in face-to-face interviews by the medical staff of the local health unit and the University of Bari.

Because of the high number of cases in resort staff members, a retrospective cohort study was performed to assess risk factors associated with illness in this group. Persons eligible for the study were staff members employed at the resort from July 1 to 31. Standard questionnaires were sent to all 224 staff members in the first week of August. Information requested included name, date of birth, sex, room number, job type, date of onset and type of symptoms, and water and food preferences. A month had elapsed between onset of symptoms and distribution of the questionnaires. We did not inquire about actual food history and activities of staff members during the outbreak but rather about their food preferences and usual activities.

Statistical Analysis

The questionnaires from guests and staff members were returned to the Istituto Superiore di Sanità, where the data were analyzed by using SPSS Base 10.0 (SPSS Inc., Chicago, IL) and Epi-Info 6.04 (Centers for Disease Control and Prevention, Atlanta, GA). Information collected on cases was used to construct the epidemic curve and describe the clinical presentation of the disease. Attack rates, denominator data, personal characteristics, and clinical symptoms of cases were compared between guests and staff members by chi square or Fisher exact test when appropriate; the Mann-Whitney U-test was used for comparisons of age. The room location of ill persons was plotted on a map of the resort that included water pipelines in an attempt to identify any clustering of cases along the pipeline. Statistical test for clustering was performed by the cluster k-means method with SPSS Base 10.0 (SPSS Inc.).

In the cohort study, the attack rate was calculated for the total staff and also by specific job type. Relative risks and 95% confidence intervals were also calculated for job type, behaviors and activities, and food preferences.

Laboratory Investigations

From July 18 to 28, samples (28 fecal and 2 vomit specimens) were collected from 30 participants whose illness met the case definition. Part of each specimen was stored at -20°C until examination for viral particles and free fecal cytotoxins, and the rest was refrigerated and processed within 12 hours of collection.

Ova and parasites were detected by direct microscopy, and *Salmonella, Shigella, Campylobacter*, *Yersinia enterocolitica, Staphylococcus aureus*, and enteropathogenic *E. coli* were sought by standard methods (14). The presence of *Clostridium perfringens* enterotoxin (CPE) was determined either by assaying the cytopathic effect on Vero cells or by reverse passive latex agglutination (RPLA) test (Oxoid Italia Spa, Garbagnate Milanese, Milan) according to the manufacturer's instructions (14).

Stool and vomit suspensions were examined by NLV-specific reverse transcription/polymerase chain reaction (RT/PCR) with generic primers JV12–JV13 to a consensus sequence on the RNA polymerase segment of the genome shared by most NLV strains (15). For confirmation of the diagnosis, gels were further analyzed by Southern blot with a mixture of NLV-specific probes (15). The 327-bp amplification product was subjected to sequence analysis with PCR primers, and the sequences obtained were aligned with those in the European Molecular Biology Laboratory Nucleotide Data Bank.

After July 13, water samples were repeatedly collected from the main public water supply and from various points outside and inside the resort. Samples from food in the kitchen and the refrigerators were collected and sent to the University of Bari on July 18. Water and food samples were subjected to culture tests for enteric bacterial pathogens, according to standard methods.

Results

Descriptive Epidemiology

Of 344 cases identified from July 1 to 31, 69 (20%) were in staff members. Information on personal characteristics and clinical presentation was available for 248 ill persons (Table 1). Diarrhea, vomiting, and abdominal pain were observed in

Table 1. Personal characteristics of ill persons and clinical symptoms, tourist resort, Italy, July 2000

Characteristics	Guests	Staff members	Total
Information available (n)	179	69	248
Age (years); mean (range)	23 (1–88)	26 (19–53)	24 (1–88)
Female (%)	52	54	53
Diarrhea (%)	93	92	93
Vomiting (%)	84	72	80
Fever (%)	53	58	54
Abdominal pain (%)	63	83	70
Hospitalization (%)	2.2	1.4	2.0

>70% of all cases. Five patients were hospitalized; all recovered rapidly and were discharged within a few hours. None of the patients had any further sequelae. Attack rates did not differ by age, sex, or symptoms for cases in guests or staff members. For cases in guests, the median interval from the arrival date at the resort and onset of symptoms was 4 days, and symptoms developed in 77% within 5 days.

The epidemic curve shows three distinct peaks in each of the 3 weeks, beginning on July 12 (70 cases), July 18 (26 cases), and July 27 (55 cases). Over the total outbreak period, 275 cases occurred in guests and 58 in staff (Figure 3). Fifty-seven percent of cases in staff members occurred before July 15. The outbreak lasted 24 days, and no cases were observed after July 31.

Because of the rapid turnover at the resort, attack rates for guests were calculated separately for each week: an attack rate of 102 (10.5%) of 970 was observed in week 1; 66 (8.7%) of 760 in week 2; and 105 (10.1%) of 1,034 in week 3. Ill guests occupied 157 of the resort's 456 rooms. No significant evidence of either clustering by the cluster k-means methods (p=0.392) or increased frequency of cases in rooms near the water pipeline was observed. Attack rates by sex, age group, and week of stay were similar.

Analytical Epidemiology

For the analysis of risk factors in the cohort study, 181 questionnaires from 224 staff members were completed and analyzed. The attack rate in this group was 69 (38.1%) of 181. The lowest attack rates were observed in staff members who worked in the kitchen or the office, and the highest were in waiters, sports trainers, entertainers, and cleaning staff (i.e., staff members who have close contact with guests) (Table 2). Staff members who took showers on the beach or consumed drinks with ice were more likely to become ill than those who did not. No association was found between disease and eating any particular type of food or with being at work on July 8–11 (the first days of the outbreak) (Table 3).

Microbiologic Results

Stool samples from 28 patients were negative for ova and parasites and bacterial enteropathogens. Of the 28 stool samples examined by NLV-specific RT-PCR, 22 had an amplified DNA of the size expected for NLV. The 327-bp amplification product was also confirmed for all samples by Southern blot hybridization with NLV-specific probes. Vomit specimens from two other subjects were negative.

A readable common sequence of 290 bp was obtained with sequence analysis and found to be the same for eight samples, indicating a single outbreak virus strain. The sequence was analyzed against the European Molecular Biology Laboratory Nucleotide Data Bank, yielding a best fit with the RNA polymerase sequence of the Lordsdale strain of NLV (16). Nucleotide identity between the two strains was 93.1% (270/290 residues), indicating that the outbreak NLV strain belongs to GGII.

When the stool supernatants stored at -20°C were examined by the Vero cell assay for free bacterial toxins, a CPE consistent with that of *C. perfringens* enterotoxin was induced by seven samples. The RPLA test confirmed the presence of *C. perfringens* enterotoxin in all seven samples. The positive specimens had been collected July 18–21, and all were also positive for NLV (Table 4).

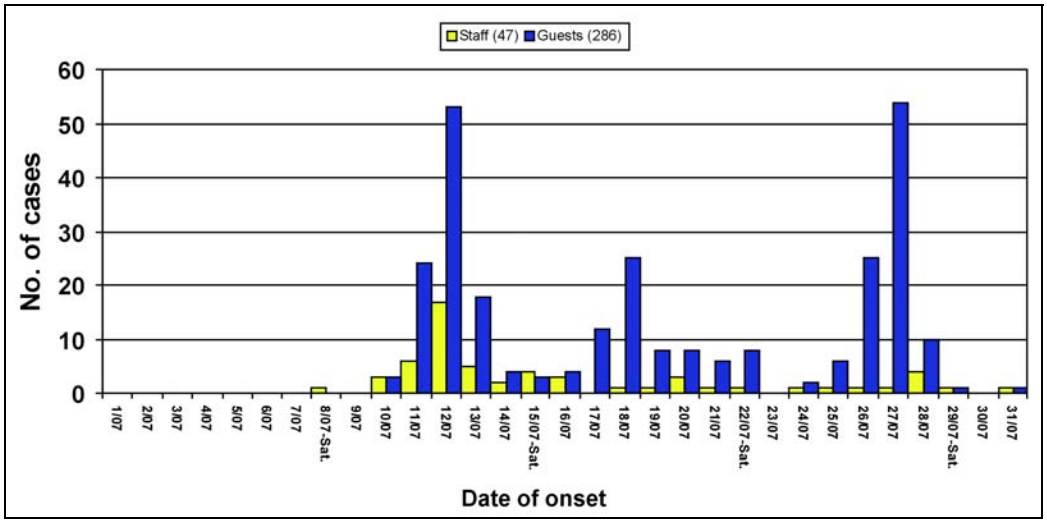

Figure 3. Cases of gastroenteritis with known date of onset (n=333) in guests and staff members at a tourist resort, Italy, July 2000.

Table 2. Attack rates and relative risks for staff members (n=69) according to type of work, tourist resort, Italy, July 2000

Type of work	Attack rate (%)	Relative risk	95% CI [a]
Kitchen staff	4/34 (11.8)	Referent	
Office staff	3/14 (21.4)	1.8	0.5–7.1
Bar staff	3/11 (27.3)	2.3	0.6–8.8
Shop assistant	7/21 (33.3)	2.8	0.9–8.5
Cleaning staff	16/36 (44.4)	3.8	1.4–10.2
Sports trainers and entertainers	19/37 (51.4)	4.4	1.6–11.5
Waiters	17/28 (60.7)	5.2	2.0–13.6

[a]CI, confidence interval.

All food samples tested were negative for enteropathogenic bacteria. Water samples collected on July 13 from faucets in the bar, the kitchen, and a guest room (Figure 2, point 6) had high levels of coliforms (up to 130 CFU/mL) and fecal streptococci (up to 22 CFU/mL). The same level of contamination was observed in water samples from the pipe connecting the resort to the public water supply (Figure 2, point 2); samples collected from the public water supply outside the resort (Figure 2, point 1) were always negative. After July 15, when chlorine was added to the tank, the level of contamination of tap water inside the resort steadily decreased; no contamination was detectable after superchlorination on July 22.

Discussion

Although NLV gastroenteritis epidemics likely occur as frequently in Italy as in the rest of Europe, to our knowledge this is the first outbreak of NLV infection to be confirmed in the country. It affected many guests and employees at a summer vacation resort and involved high attack rates in all age groups. The actual number of cases has likely been underestimated since persons with a mild illness may not have sought medical attention. In fact, the retrospective investigation of staff members showed an attack rate three times higher than in guests.

This outbreak had an unusual pattern, with three regular peaks occurring at constant intervals for 3 weeks. This pattern, which is compatible with a point-source infection (3), may be explained by the rapid turnover of guests and their periodic replacement with susceptible persons in the presence of a constant exposure to infection. Most guests arrived at the resort on a Saturday and stayed 1–2 weeks. Guests who became ill did so a few days after their arrival, suggesting that exposure to a source of infection was relatively constant during the whole period. Moreover, a large proportion of staff members had onset of illness in the first week of the outbreak. The hypothesis of a common source of infection is further supported by the identical nucleotide sequence detected in viruses from eight patients during the outbreak.

Water was the likely source of this outbreak. Environmental inspection identified a breakdown in the water system of the resort, and tap water samples from different places in the resort showed contamination with fecal bacteria. Although microbiologic testing for NLV could not be performed on drinking or recreational water, the presence of fecal bacteria suggests that the water system may have been the actual source of NLV. Despite the possible passage of the virus through several hosts during the outbreak, the genome segment used for diagnosis showed complete stability, suggesting that a very high number of human passages may be required to produce the known nucleotide variability for NLV, at least in the RNA polymerase region.

Table 3. Attack rates and relative risks according to usual behaviors and activities of staff members, tourist resort, Italy, July 2000

Exposure	No. (n=69)	No. exposed	Attack rate (%)	Relative risk	95% CI [a]
Shower on the beach	22	14	63.6	1.8	1.2–2.6
Swimming in the pool	45	22	48.9	1.4	0.9–2.0
Drinking tap water	104	47	45.2	1.4	0.9–2.2
Drinks with ice	128	55	43.0	1.8	1.0–3.2
Swimming in the sea	72	31	43.0	1.2	0.8–1.7
Eating at resort restaurant	159	64	40.2	1.5	0.5–3.9
Eating ice cream	140	56	40.0	1.1	0.6–1.9
Eating meat	151	60	39.7	1.2	0.6–2.4
Eating salad	123	48	39.0	1.0	0.6–1.6
Eating fruit	139	54	38.8	1.0	0.6–1.8
Eating pasta	142	55	38.7	1.2	0.6–2.1
Consuming drinks on draught	91	35	38.5	1.0	0.7–1.4
Eating fish	112	40	35.7	0.7	0.5–1.1
Eating seafood	85	28	32.9	0.7	0.5–1.1

[a]CI, confidence interval.

Table 4. Presence of Norwalk-like virus and *Clostridium perfringens* enterotoxin in stool samples from ill persons, Italy, July 2000

Sampling date	No. examined	No. positive for NLV [a]	No. positive for CPE	No. positive for NLV + CPE
07/18/01	12	9	0	0
07/20/01	1	1	1	1
07/21/01	8	7	6	6
07/27/01	2	2	0	0
07/28/01	5	3	0	0
Total	28	22	7	7

[a]Abbreviations used: NLV, Norwalk-like virus; CPE, *Clostridium perfringens* enterotoxin.

Some specimens showed evidence of simultaneous infection with NLV and enterotoxigenic *C. perfringens.* The food item(s) that could have been the source of infection by *C. perfringens* remained unknown. However, the presence of *C. perfringens* enterotoxin in a small, defined cluster of patients (7 of 28 stool samples) and the concomitant presence of NLV in the 7 positive stools suggests that *C. perfringens* played only a minor role, if any, in the outbreak.

Control measures to limit the spread of the infection had no effect, probably because they did not address the point source and failed to prevent person-to-person transmission. After July 15, 2000, the consumption of tap water was banned, and only bottled mineral water was served in the resort restaurant and used to wash vegetables. Water from the main tank, however, continued to be used for showers, to make ice for consumption (through July 28), and for irrigation. Furthermore, on July 22, the bypass pipe was removed, the water inside the resort tank underwent superchlorination, and the pipe connecting the resort to the public water supply was shut down. However, NLV do survive high levels of chlorination (3,8), and the treatment was performed only once, at a late stage of the outbreak, and at only one point upstream of the resort water system. Finally, although the resort was serviced by a mobile tank truck that provided water from the main public water supply, the resort main tank was never emptied and cleaned before treatment. Therefore, after July 22, contaminated residual water could have been gradually diluted by refilling the resort tank with clean water from mobile tanks.

In the cohort study of staff members, having showers on the beach was identified as a risk factor, while consuming drinks with ice was only weakly associated with illness. No exposure to other water sources, including drinking tap water (the use of which was forbidden after July 15) was significant. Our analysis found no evidence that contaminated food was the source of infection: no food preference was associated with an increased risk of being ill, and personnel working in the kitchen had the lowest attack rate.

In addition to water contamination, person-to-person transmission may have played a role in this outbreak. Typically staff members of tourist resorts share the same living quarters and have frequent contact with guests during meals, sport training, entertainment, and other activities. Person-to-person transmission may also explain the fact that the time between arrival and onset of symptoms in guests was longer than the incubation period expected for NLV. Person-to-person transmission of NLV infection is well documented (8), and secondary cases may occur. Airborne and fomite transmission also may facilitate the spread of the virus during outbreaks. Such hypothesis is confirmed by the higher attack rate in the cleaning staff and in staff members working in close contact with guests.

This investigation had several limitations. Since the cohort study was carried out after the outbreak had ended, we could inquire only about food preferences and usual activities rather than actual food histories and activities before the outbreak. Recall bias may have occurred, which may have led to nondifferential misclassification of exposure and underestimation of the observed relative risks. If NLV had spread through water and person-to-person transmission had occurred, virtually everyone in the resort would have been exposed to the agent and any epidemiologic association would be difficult to find. Finally, no test specific for NLV was performed on water samples, and the hypothesis of water as the actual source of infection cannot be confirmed.

In conclusion, this event confirms that large outbreaks due to NLV may be occurring in Italy, but without the use of appropriate diagnostic methods this pathogen may go unrecognized. This occurrence highlights the need for a surveillance system of such outbreaks in cooperation with laboratories capable of diagnosing viral gastrointestinal infections.

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Dr. Boccia is a microbiologist with the National Public Health Institute in Rome. Her main research interest is the epidemiology of antimicrobial resistance, and she has been frequently involved in outbreak investigations caused by viral and bacterial agents.

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Medical Care Capacity for Influenza Outbreaks, Los Angeles

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In December 1997, media reported hospital overcrowding and "the worst [flu epidemic] in the past two decades" in Los Angeles County (LAC). We found that rates of pneumonia and influenza deaths, hospitalizations, and claims were substantially higher for the 1997–98 influenza season than the previous six seasons. Hours of emergency medical services (EMS) diversion (when emergency departments could not receive incoming patients) peaked during the influenza seasons studied; the number of EMS diversion hours per season also increased during the seasons 1993–94 to 1997–98, suggesting a decrease in medical care capacity during influenza seasons. Over the seven influenza seasons studied, the number of licensed beds decreased 12%, while the LAC population increased 5%. Our findings suggest that the capacity of health-care systems to handle patient visits during influenza seasons is diminishing.

In December 1997, television and newspaper media reported that high numbers of patients seeking treatment for respiratory illnesses had overwhelmed the capacity of emergency departments and outpatient facilities in Los Angeles County (LAC). The situation was described as a looming health-care disaster (1) and the worst influenza epidemic in the previous 2 decades (2,3).

Influenza viruses infect all age groups and cause annual or near-annual winter epidemics. The health impact of seasonal epidemics is variable, averaging >20,000 excess deaths (i.e., deaths above an expected baseline of deaths in the absence of influenza) and >110,000 excess hospitalizations per year in the United States (4). Severe influenza seasons can result in >40,000 excess deaths and >200,000 excess hospitalizations.

Global pandemics of influenza, which occur when novel influenza viruses emerge, happen unpredictably and less frequently (e.g., 1918–19, 1957–58, and 1968–69 in the 20th century) than seasonal epidemics (5). However, the resulting elevation in the number of illnesses and deaths can be much greater than during regular influenza epidemics (4,6). The Centers for Disease Control and Prevention (CDC) projected that a pandemic similar in impact to the 1957 pandemic, widely considered to be a "medium" pandemic, might result in approximately 300,000 to 750,000 excess hospitalizations and 18 million to 42 million excess outpatient visits in the United States (7).

In December 1997, the LAC Acute Communicable Disease Unit, the California Department of Health Services, and CDC conducted a preliminary investigation of situations in which patients were diverted from one emergency facility to another in LAC. Because findings suggested that approximately 65% of LAC health-care facilities had diverted patients to other hospitals because of overcrowding and concerns about the hospitals' ability to respond to seasonal and pandemic influenza, we studied the impact of the 1997–98 influenza season on LAC hospitals and emergency services.

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Methods

Study Population

The entire population of LAC was used as the denominator to estimate death rates from pneumonia and influenza (P&I) and the number of licensed beds per 100,000 persons. Six publicly funded LAC hospitals and six Kaiser Permanente Southern California (KPSC) hospitals were used to study hospitalization patterns. KPSC is a not-for-profit group model health maintenance organization in southern California. Rates of hospitalizations in LAC-funded facilities and KPSC were calculated by using population estimates for persons at or below the poverty level (8) and the KPSC enrollee population, respectively. LAC-funded hospitals and KPSC hospitals ranged in size from approximately 100 to 2,000 licensed beds and from approximately 100 to 600 beds, respectively.

Study Periods

Seven influenza seasons (from 1991 to 1998) were studied. A broad 24-week influenza season was defined as the last 12 weeks of 1 year and the first 12 weeks of the following year. Within each season, we further defined a peak influenza period as the 4-consecutive-week period in which the greatest total number of influenza isolates and antigen detections were reported to the U.S. World Health Organization (WHO) influenza laboratories in Region IX (California, Washington, Oregon, and Hawaii). The peak influenza periods were defined independently of hospitalization, KPSC claims, or emergency medical services (EMS) data.

P&I death data for LAC were obtained from the California Department of Health Services Vital Statistics Section. A P&I death was defined with a code 480–487, International Classification of Diseases, Ninth Revision (ICD-9). These ICD-9 codes include viral pneumonia, pneumococcal pneumonia, other bacterial pneumonia, pneumonia due to other specified organisms, pneumonia classified elsewhere, bronchopneumonia, pneumonia-organism unspecified, and influenza.

P&I hospitalization data were obtained directly from the six LAC-funded hospitals and KPSC. For LAC-funded hospitals, a P&I hospitalization was defined as one in which one of the first three discharge codes included ICD-9 codes 480–487 (no principal diagnosis was available). For KPSC, a P&I hospitalization was defined as a hospitalization for which the principal discharge diagnosis was assigned an ICD-9 code of 480–487. Hospital data limitations prevented us from using an identical P&I hospitalization definition for both the LAC and KPSC facilities. Hospitalization data included the facility name and the patient's sex, age, discharge date, and disposition at discharge.

Data from claims related to P&I hospitalizations (ICD-9 480–487) were obtained from KPSC**.** A claim is a bill or charge generated by an outside facility when a KPSC patient receives a medical service (e.g., a radiograph or administration of a medication) while hospitalized at a non-KPSC facility. Claims usually are generated when a KPSC facility is diverting patients or when a KPSC patient is too sick to be transported to another KPSC facility. Since a separate claim is generated for each service, one hospitalization usually results in multiple claims. These data, therefore, were analyzed separately from the hospitalization data.

A hospital was considered to be on EMS diversion when its emergency department could not receive incoming patients transported by an Advanced Life Support (ALS) unit. The number of hours that LAC emergency rooms were on diversion for March 1993 to March 1998 was obtained through the LAC Local Emergency Medical Service Agency (LEMSA). Data were not available for periods before March 1993. Under California statute, all ALS diversions require LEMSA approval. LEMSA may deny such requests during periods when regional patient volume is high and extended transport time may have a negative health effect on patients (9). This study analyzed only EMS diversions because of emergency department saturation.

We obtained the total number of general acute-care facilities in LAC and licensed beds in each facility, for 1991 to 1997, from data compiled by the Office of Statewide Health Planning and Development, California Department of Health Services (unpub. data). Licensed beds are those licensed to a particular facility regardless of availability for patient care; the total was obtained from the number of beds that appeared on each facility's license on the last day of the calendar year. Staffed beds are those available for patient care based on current staffing levels. Reliable numbers on staffed beds were unavailable.

Statistical Methods

Rates of P&I deaths, P&I hospitalizations at LAC-funded facilities and KPSC hospitals, and KPSC claims were calculated for each influenza season from 1991–92 to 1997–98. Rates of P&I deaths, hospitalizations, and KPSC claims during the 4-week peak influenza period were compared with the rates for the remaining 20 weeks of the influenza season by the Mantel-Haenszel common odds ratio (OR) in Stat-Xact 3.0 (10). In addition, rates of P&I-associated deaths, hospitalizations, and KPSC claims during the peak influenza period of 1997–98 were compared with the rates in the other six peak periods (1991–92 through 1996–97). Person-week denominators were calculated for peak and nonpeak influenza seasons by using LAC population for each given year and the number of weeks in the period studied (for peak period, 4 weeks; for nonpeak periods, 20 weeks).

Results

In 1997–98, the 4-week period with the most influenza detections was 52–2 (i.e., weeks 52 and 53 in 1997; weeks 1 and 2 in 1998), when a total of 445 viral isolations and positive antigen tests were reported (Table 1). Most of the other peak periods also occurred in late December and early January.

During each of the seven influenza seasons studied, rates of P&I deaths were consistently higher in the 4-week peak influenza period than the other 20 weeks of each influenza season (OR 1.57; 95% confidence interval [CI] 1.50 to 1.64; $p<0.001$) (Table 2). The rate of P&I deaths during the 1997–98 peak influenza period (16.3 deaths per million person-weeks) was significantly higher ($p<0.001$) than the rates in the other six peak periods (6.9–12.3 deaths per million person-weeks).

At the LAC-funded facilities, rates of P&I hospitalizations were also consistently higher during the 4-week peak influenza period than in the other 20 weeks in the influenza season (OR 1.47; 95% CI 1.39 to 1.55; $p<0.001$) (Table 2). The rate of P&I hospitalizations during the 1997–98 peak period (75.3 hospitalizations per million person-weeks) was significantly higher than the rates during the six other peak periods (20.6–31.8 hospitalizations per million person-weeks) (OR 1.62; 95% CI 1.44 to 1.83; $p<0.001$).

The rates of P&I hospitalizations and claims in the Kaiser facilities during the 4-week peak periods were significantly higher than rates of P&I hospitalizations and claims during the nonpeak periods (OR 1.63; 95% CI 1.55 to 1.71; $p<0.001$ for P&I hospitalizations; OR 2.05; 95% CI 1.97 to 2.13; $p<0.001$ for P&I claims) (Table 2).

Similar to LAC-funded facilities, the rate of P&I hospitalizations in the Kaiser facilities during the 1997–98 peak influenza period was higher than the rate of P&I hospitalizations during the previous six peak periods (OR 1.48; 95% CI 1.3 to 1.64; $p<0.001$ hospitalizations; OR 4.01; 95% CI 3.75 to 4.29; $p<0.001$ for P&I claims) (Table 2). In the 1997–98 season, the rate was 84 hospitalizations per million person-weeks, compared with 40–75 hospitalizations per million person-weeks for the previous years studied. The number and rate of KPSC

Table 1. Peak period of influenza detections[a] reported to the World Health Organization's influenza laboratories, U.S. Region IX, 1991–1998

	Influenza season						
	1991–92	1992–93	1993–94	1994–95	1995–96	1996–97	1997–98
Peak no. of influenza detections/ 4-wk period (% positive)	192 (28)	57 (10)	173 (26)	61 (13)	164 (21)	117 (22)	445 (27)
Peak 4-wk period week no.	2–5	53[b]–3	52–3	9–12	51–2	51–2	52[b]–2

[a] Includes viral isolations and positive antigen tests.
[b]Year extended over 53 weeks.

claims were also higher during the peak influenza period of 1997–98 (266 claims per million person-weeks) compared with previous years (range 6–135 claims per million person-weeks) (OR 4.0; 95% CI 3.75 to 4.29; p<0.001).

In the 1997–98 peak influenza period, the largest number and percentage of P&I hospitalizations occurred in persons >60 years of age (n=491, 45%), followed by persons <5 years of age (n=166, 15%). This general age distribution was observed in all seven peak influenza periods, but the absolute number of patients was greater for the 1997–98 peak period than for any of the other peak periods.

The months with the highest number of EMS diversion hours were December 1997 (10,109 hours) and January 1998 (11,388 hours). These months coincided with the peak of the 1997–98 influenza period. From the 1993–94 to the 1997–98 influenza seasons, the number of hours that all LAC hospital emergency departments were on EMS diversion during each December to February (months encompassing all but one of the 4-week influenza peaks) increased from 15,844 hours to 25,584 hours (Figure 1). For comparison, an average of 3,715 EMS diversion hours per month occurred during noninfluenza months in years 1993–1996. Influenza hospitalizations (KPSC and county-funded facilities) and peaks in influenza detections in Figure 1 show the relationship between EMS diversions and influenza activity.

From 1991 to 1997, the number of acute-care hospitals and licensed acute-care hospital beds in LAC decreased from 137 to 130 hospitals and 29,987 to 26,244 licensed beds, respectively. The drop in licensed beds corresponds to a decrease of 334 beds per 100,000 persons to 227 beds per 100,000 persons (Figure 2). Accurate counts of staffed beds were not available.

Table 2. Rates of pneumonia and influenza (P&I) deaths, hospitalizations, and claims[a]

	P&I deaths			LAC hospitalizations[b]			KPSC hospitalizations[c]			KPSC claims		
Influenza period by season	Count	Person-wks[d]	Rate	Count	Person-wks[d]	Rate	Count	Person-wks[d]	Rate	Count	Person-wks[d]	Rate
Peak 4-wk period												
91–92	345	36	9.5	157	8	20.6	262	5	52.4	32	5	6.4
92–93	287	37	7.8	172	8	21.7	235	5	48.5	148	5	30.5
93–94	457	37	12.3	277	9	31.8	355	5	75.2	257	5	54.5
94–95	257	37	6.9	214	9	23.6	190	5	40.5	239	5	51.0
95–96	348	37	9.3	246	9	27.4	330	5	67.3	564	5	115.0
96–97	333	38	8.8	192	9	22.6	308	5	58.1	715	5	134.8
97–98	624	38	16.3	648	9	75.3	482	6	84.3	1,0	6	265.9
Nonpeak 20-wk period												
91–92	1,177	182	6.5	570	38	15.0	1,065	25	42.6	78	25	3.1
92–93	1,044	184	5.7	796	40	20.1	976	24	40.3	542	24	22.4
93–94	1,252	186	6.7	737	44	16.9	955	24	40.5	618	24	26.2
94–95	1,081	187	5.8	924	45	20.4	915	23	39.0	947	23	40.4
95–96	1,126	187	6.0	710	45	15.8	931	25	38.0	1,419	25	57.9
96–97	1,319	189	7.0	808	43	19.0	882	27	33.3	2,338	27	88.2
97–98	1,453	192	7.6	1,610	43	37.4	927	29	32.4	2,548	29	89.1

[a]Abbreviations used: LAC, Los Angeles County; KPSC, Kaiser Permanente Southern California.
[b]Represents six LAC-funded hospitals.
[c]Represents six KPSC hospitals.
[d]Represents 1 million person-weeks.

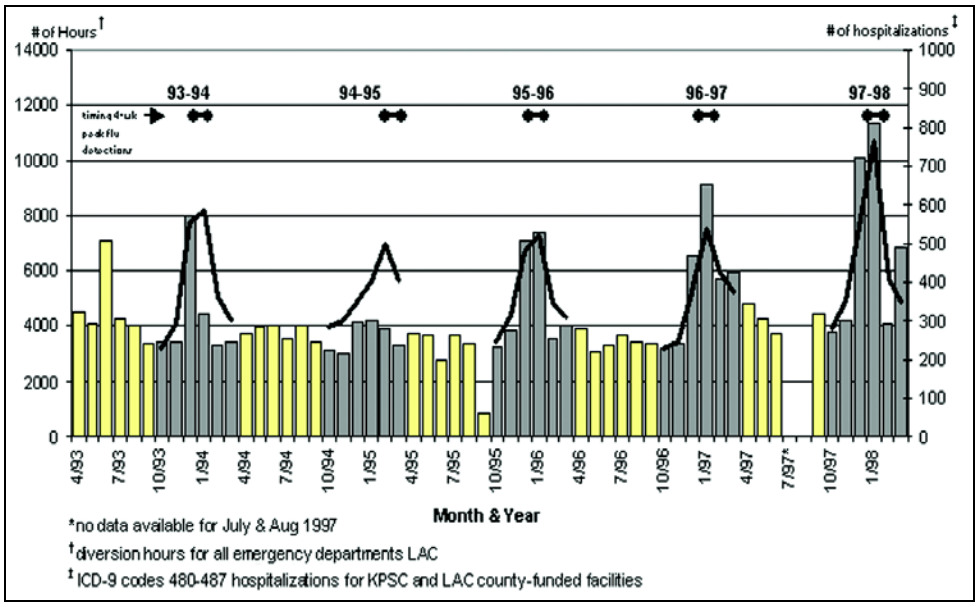

Figure 1. Emergency department diversion hours, influenza hospitalizations, and detection peaks, Los Angeles County, April 1993–March 1998.

Discussion

Several important patterns were observed in this study of LAC hospitalizations and EMS diversion during influenza seasons in LAC from 1991 through 1998. The impact of the influenza season on LAC hospitals was more severe in 1997–98 than in the preceding 6 years. However, although more severe, the elevated levels of hospitalizations and deaths were not unique. In six of seven winters from 1991–92 through 1997–98, similar peaks of P&I hospitalizations and P&I deaths were observed. These peaks correlated with elevated levels of circulating influenza viruses, suggesting that this infection is a key factor leading to increased demands on medical systems in the winter. The study also demonstrated that the number of hours that LAC hospitals were on EMS diversions peaked at approximately the same time that respiratory deaths and hospitalizations peaked. Most importantly, the number of hours that LAC hospitals were on EMS diversion during the peak influenza periods increased substantially over the period of the study. During this period, the LAC population increased, while the number of licensed hospital beds in LAC decreased. If an increasing trend in EMS diversion hours reflects the inability of hospitals to handle critically ill patients, then these contemporaneous patterns call into question the capability of current medical systems to handle regular influenza seasons, as well as more stressful events, such as pandemic influenza.

Our evidence suggests that influenza infections were the major precipitating cause for the annual winter upsurge in patient visits. In this study, we defined the 4-week peak influenza periods on the basis of the number of influenza isolates reported to WHO laboratories in Region IX, not on the basis of hospitalization or death patterns. During these peak influenza periods, levels of respiratory-related deaths, hospitalizations (at both the LAC-funded and KPSC hospitals), and claims for KPSC patients were substantially higher than during the periods when influenza viruses were not in circulation. The patients most frequently hospitalized for P&I-related illnesses (ICD-9 codes 480–487) during the peak influenza weeks were the elderly and the young, a pattern consistent with the epidemiology of influenza. Finally, the number of EMS diversion hours in the 1994–95 season, characterized by light influenza activity, was notably low.

One of the characteristics of influenza epidemics is a highly variable impact on populations. The degree of impact depends on several factors, including prevalence of infections, levels of protective immunity in the population, demographic and health characteristics of the population, and circulating strain. The increased severity of the 1997–98 influenza season was likely due to the appearance of influenza A/Sydney/5/97-like (H3N2) viruses in the United States. This virus, a drift variant of the previously predominant influenza A/Wuhan/359/95 (H3N2) virus strain, first emerged in the spring of 1997 and quickly became the predominant influenza strain in the United States and worldwide (11). During the 1997–98 influenza season, this strain accounted for >90% of the influenza virus isolates from Southern California. However, because of

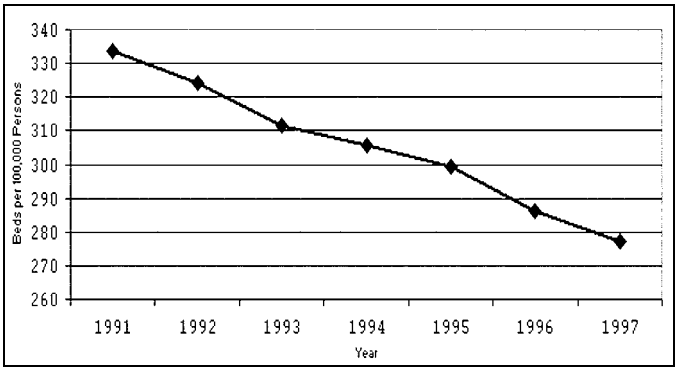

Figure 2. Number of licensed beds per 100,000 persons, Los Angeles County, 1991–1997.

the timing of its emergence, this strain was not included in the 1997–98 influenza vaccine (12).

The most important observation in our study was the increasing trend in EMS diversion hours during peak influenza periods. One factor in this trend appeared to be a steady erosion in hospital bed capacity despite a growing population in LAC. From 1991 to 1997, the bed capacity in LAC decreased by 17%, when population growth was taken into account. Concomitantly, from 1993–94 through 1997–98, EMS diversion hours combined during the months of December, January, and February increased from 15,844 to 25,584 hours. Since EMS diversion hours reflect times when hospitals are unable to receive critically ill patients, such hours serve as a marker of general overcrowding in emergency rooms and time during which patients are at risk for long waits and poor outcomes (13).

Our findings suggest that decreasing bed capacity was an important underlying cause for hospitals' inability to handle the upsurges in patients and the increasing numbers of hours spent on EMS diversions during the influenza seasons studied. However, we did not examine other potentially important factors, such as nursing shortages, staff illnesses, or limitations in the availability of equipment or intensive-care unit capacity. In a recent study in the emergency rooms in the United States, 14 common causes of emergency room overcrowding were identified, of which 43% were directly related to resource shortages, such as beds and staff (13). In a similar survey of emergency department directors in California, 96% of the directors reported overcrowding as a problem and identified increasing numbers of severely ill patients, hospital bed shortages, delays in receiving laboratory results, and nursing shortages as underlying causes (14).

These findings have two important implications. First, the near-annual peaking of both P&I hospitalizations and EMS diversions during the influenza season suggests that hospitals and medical systems can and should develop plans to handle the upsurges in patient visits for respiratory illnesses. Second, the increasing number of EMS diversion hours suggests the need to further identify reversible factors responsible for the ongoing erosion in the ability of LAC hospitals to handle upsurges in patient visits.

One important aspect of this study was our decision to restrict the analysis of hospitalizations, KPSC claims, and deaths to ICD9 codes 480–487—codes often used to monitor influenza trends (15). In further analyses (not shown), we found that the rates of hospitalizations increased twofold when additional respiratory codes were added and almost threefold when congestive heart failure was included. Other studies have shown that hospitalizations for other respiratory conditions (e.g., bronchitis, chronic airway obstruction) and congestive heart failure increase during influenza season (16). These considerations suggest that our analysis of the magnitude of the problem was conservative.

In response to the 1997–98 influenza season, the California Department of Health Services, in collaboration with Kaiser Permanente and the CDC, augmented active influenza surveillance in California. Methods for influenza surveillance were expanded to include monitoring of influenza-related hospitalizations and use of influenza antiviral medications. In addition, surveillance of influenza-like illnesses in outpatient settings and collection of respiratory virus isolation data from several major laboratories throughout the state were also implemented.

Emergency room crowding and diversion is a year-round problem caused by multiple factors within the medical system (13,17). Our study shows that influenza places an additional stress on an overburdened system. Although we did not study hospitals outside LAC, we think that the situation in LAC is not unique. During the 1997–98 influenza season, media in northern California suggested a similar pattern there (18). Furthermore, in January 2000, the New York Times reported emergency rooms were "flooded" secondary to influenza-like illnesses (19).

The ability to handle upsurges in patients is compromised in at least some parts of the country. Action is needed to reverse this situation. Because of the 1997–98 influenza season, the Healthcare Association of Southern California, a health-care industry organization, made the following recommendations for hospitals during periods of heavy influenza activity: 1) reduce or eliminate elective surgery; 2) relax staff-versus-patient ratios by working with state licensing agencies; 3) develop methods of identifying and mobilizing additional staff during the winter; 4) establish walk-in influenza clinics to triage and treat patients at lower cost; and 5) develop methods for identifying additional equipment (20). This health-care association also recommended influenza immunization programs for staff members and their families early in the season. Implementing such recommendations could alleviate some of the stresses experienced by medical systems in years of increased influenza outbreaks.

Ontario, Canada, implemented a program in 2000–01 that offered influenza vaccines to all its residents to alleviate pressures on its hospitals (21). The results of this approach may not be known for several years. The importance of vaccinating persons at high risk for influenza-related complications cannot be overemphasized**.** This step should be widely implemented to reduce hospitalizations and other serious complications of influenza (22). Finally, hospitals should also work closely with local and state health departments to obtain up-to-date information about the local circulation of influenza viruses. Such information could be used by hospitals to trigger the implementation of predesignated policies.

Conclusion

Rebuilding the medical care capacity to handle such patient upsurges will be difficult and expensive. Hospitals in LAC regularly and increasingly exceed their capacity to handle respiratory illness cases. This lack of capacity, along with concerns about the next influenza pandemic and potential terrorist events, suggests that it is time to start this process.

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Drought-Induced Amplification of *Saint Louis encephalitis virus*, Florida

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We used a dynamic hydrology model to simulate water table depth (WTD) and quantify the relationship between *Saint Louis encephalitis virus* (SLEV) transmission and hydrologic conditions in Indian River County, Florida, from 1986 through 1991, a period with an SLEV epidemic. Virus transmission followed periods of modeled drought (specifically low WTDs 12 to 17 weeks before virus transmission, followed by a rising of the water table 1 to 2 weeks before virus transmission). Further evidence from collections of *Culex nigripalpus* (the major mosquito vector of SLEV in Florida) suggests that during extended spring droughts vector mosquitoes and nestling, juvenile, and adult wild birds congregate in selected refuges, facilitating epizootic amplification of SLEV. When the drought ends and habitat availability increases, the SLEV-infected *Cx. nigripalpus* and wild birds disperse, initiating an SLEV transmission cycle. These findings demonstrate a mechanism by which drought facilitates the amplification of SLEV and its subsequent transmission to humans.

Florida is vulnerable to epidemic transmission of *Saint Louis encephalitis virus* (SLEV). Five epidemics (>20 human cases each) of SLEV have been recorded in south Florida since 1952 (1). The most recent epidemic occurred in 1990 when 226 cases were reported throughout south-central Florida. The ability to accurately forecast SLEV epidemics is needed to minimize human health risks and focus vector control efforts. The development of such forecasting capabilities, however, requires complete understanding of the mosquito vector and amplification-host interactions that result in virus transmission to humans.

The annual SLEV transmission cycle in south Florida can be divided into four phases: January–March, maintenance; April–June, amplification; July–September, early transmission; and October–December, late transmission (2). The amplification phase involves the epizootic cycling of SLEV between mosquito vectors and avian amplification hosts. Amplification is necessary to achieve mosquito infection rates sufficient to cause human epidemics (3). In Florida, resident juvenile and nestling wild birds serve as the primary amplification host of SLEV (4). Nestling and juvenile birds are excellent amplification hosts because of their inefficient, poorly developed immune systems; their sparse feather coverage, which allows large numbers of mosquitoes to feed; and their lack of defensive behavior toward blood-feeding mosquitoes (4). Evidence also suggests that young birds may have elevated and extended viremias compared with their adult conspecifics (3), further facilitating SLEV amplification.

Others have proposed that SLEV epidemics may result from a specific combination of biotic and abiotic conditions that favor early season virus amplification followed by transmission (1). Several meteorologic variables have been associated with the amplification and transmission of SLEV and with vector abundance (5,6). High temperature accelerates the rate of pathogen and vector development, and high humidity increases vector flight and host-seeking behaviors (6).

Particular attention has been focused on precipitation, which is necessary for the formation of mosquito breeding habitats. In Florida, *Culex nigripalpus* Theobald is the epidemic and epizootic vector of SLEV (7–9). Provost (10) suggested that droughts during the *Cx. nigripalpus* breeding season, followed by heavy rainfall and high humidity, may favor SLEV transmission. More recent studies have shown that summer and autumn rainfall patterns are correlated with SLEV transmission (11), blood feeding (12), oviposition (13), and abundance (2). The association of rainfall with virus transmission provides a working model for the prediction of SLEV transmission to humans in Florida.

The availability of mosquito breeding habitats, however, can be more directly assessed by using current hydrologic modeling techniques to track temporal variations in water table depth (WTD). Such techniques have been used to predict mosquito abundance in temperate settings (14). We expanded on this approach, applying these methods to predict both *Cx. nigripalpus* abundance and SLEV transmission dynamics in Florida.

For this study, we used a dynamic hydrology model (15) to simulate daily WTD in the Vero Beach area of Indian River County, Florida, which was the epicenter of the 1990 Florida SLEV epidemic (16). We then evaluated the association of WTD with SLEV transmission to sentinel chickens from 1986 to 1991. Modeled daily WTD was also compared with field collections of *Cx. nigripalpus* taken in Indian River County during the same time period.

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Modeling Overview and Methods

Variations of WTD in space and time determine where and when pools of water form at the land surface, thus creating potential mosquito breeding habitats. WTD, however, is not merely a function of precipitation. Other meteorologic variables, as well as soil and vegetation type and antecedent conditions, must be considered if evapotranspiration, water movement within the soil column, and river runoff are to be quantified. Topography must also be constrained if the flow of water across the land surface, runoff rates, and the local convergence of water in lowlands (surface pooling) are to be modeled accurately.

We combined a soil column model, which simulates the vertical movement of water and heat within the soil and between the soil surface, plus vegetation and the atmosphere, with the TOPMODEL (TOPography-based hydrology MODEL) approach (17–20), which incorporates topographic data to track the horizontal movement of shallow groundwater from the uplands to the lowlands. TOPMODEL formulations permit dynamically consistent calculations of both the saturated fraction within the watershed (partial contributing area) and the groundwater flow that supports this area, from knowledge of the mean depth of the water table and a probability density function for soil moisture deficit derived from topographic statistics. Using the model, we can produce a three-dimensional picture of soil moisture distribution within a catchment. This approach to modeling the land surface has been validated at several catchments, ranging in scale from the Red Arkansas Basin (570,000 km^2) (21) to the Black Rock Forest catchment (1.34 km^2) (22).

Data Collection and Analysis

SLEV Transmission Data

Sentinel chickens were used to measure SLEV transmission. The annual timing and distribution of SLEV transmission to sentinel chickens have been strongly correlated with SLEV in humans (1). Data derived from five sentinel flocks maintained in Indian River County were used in this study. Figure 1 is a map of the region of study and flock locations.

Sentinel chicken flocks were maintained by personnel from the Indian River Mosquito Control District. From 1986 to1990, flocks with six birds each were placed in the field by mid-June and removed at the end of December. In 1991, the year after the SLEV epidemic, surveillance was year-round. A 1.0-mL blood sample was drawn once a week from each bird during peak transmission periods (July through November) and twice a month during the rest of the year. Blood samples were assayed for hemagglutination inhibition antibodies to SLEV at the Florida Department of Health and Rehabilitative Services, Tampa Branch Laboratory. Individual chickens testing positive for hemagglutination inhibition antibodies were replaced with fresh sentinels, and the entire flock was replaced each spring.

We defined SLEV transmission intensity for each sentinel flock as the number of seropositive chickens per weekly sample. We also defined SLEV transmission incidence for each flock as a categorical data set: one, if one or more chickens were SLEV seropositive per weekly sample; or zero, if no chickens were seropositive. Data from all sentinel sites were also pooled, and SLEV transmission intensity and incidence were similarly determined.

Mosquito Data

Western Indian River County is dominated by citrus groves intermixed with hammock "islands" of southern live oak and cabbage palm (23). Dense ground cover makes these hammocks an excellent daytime resting site for *Cx. nigripalpus* of both sexes and female *Cx. nigripalpus* in all gonotrophic stages (2). During 1986 through 1991, at least three times per week, one 20-minute collection was made approximately 2 hours after sunrise with a portable ground aspirator along a transect at a hammock site 6.4 km southwest of Vero Beach (27 38′ N, 80 27′ W, see Figure 1). Collected mosquitoes were sorted by species, categorized by sex and gonotrophic condition, and counted.

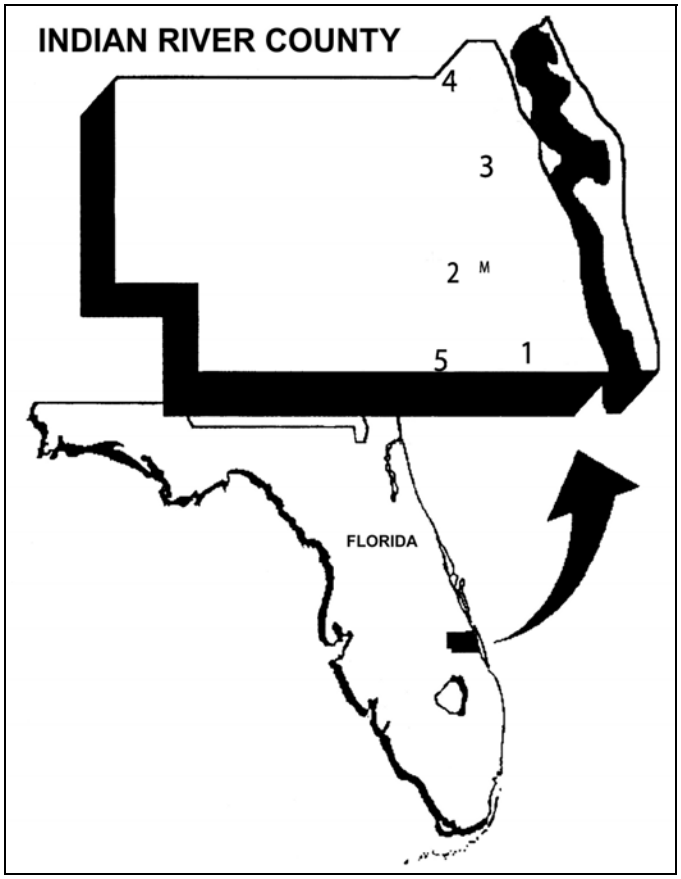

Figure 1. Map of Indian River County, Florida, with numbered locations of the five sentinel chicken flocks. The location of the mosquito collection site is denoted by "M."

Model Input and Validation Data

Hourly meteorologic data were assembled from National Climate Data Center archives for Vero Beach, Florida. Gaps in the record were filled with hourly data from National Climate Data Center archives for Melbourne and West Palm Beach. Solar radiation data were provided by the Northeast Regional Climate Center from analysis of the National Climate Data Center data by using the Northeast Regional Climate Center solar energy model (24). Topographic statistics for the Vero Beach area were generated from a 10-m cell U.S. Geological Survey National Elevation Dataset Digital Elevation Model of south-central Florida, using TarDEM version 4 routing freeware (25). Soil and vegetation types were derived from U.S. Department of Agriculture sources and personal inspection of the Vero Beach landscape.

The hydrology model was run from 1984 through 1995 and provided a daily series of mean WTD for the study area. Because of the channelization and water control in south Florida, the model was validated by using groundwater well measurements and surface (canal) water levels, provided by the St. John's Water Management District. The partitioning of runoff and evapotranspiration matched bulk estimates taken from U.S. Geological Survey and St. John's Water Management sources.

Statistical Analysis

Univariate and bivariate logistic regressions were used to associate the probability of SLEV transmission incidence with single time lags of modeled WTD and combinations of two time lags of WTD. Whole model goodness-of-fit was measured by log-likelihood ratio and the pseudo r-squared (uncertainty) coefficient. Individual parameter estimates were made by Wald's chi-square test.

Results

All five sentinel flocks had SLEV transmission recorded during the study period (1986–1991). Figure 2 provides a time series of SLEV transmission intensity and weekly averaged modeled WTD. Modeled WTD was lowest in 1989 and 1990, matching a period of drought in Vero Beach (based on Palmer Drought Severity Index records, data not shown). Three instances of SLEV transmission (the late summer and early fall of 1986, 1989, and 1990) were recorded. All three episodes occurred during a wetting period (rising of the water table) that followed a drought (low WTD). The two larger instances of SLEV transmission intensity in the Vero Beach area, 1989 and 1990, were recorded during the wet conditions that followed a prolonged drought. This sequence of hydrologic conditions, antecedent and coincident with SLEV transmission, is similar to the scenario suggested by Provost (10).

Univariate logistic regression was performed to explore the relationship between SLEV transmission incidence and time lagged modeled WTD. A range of time lags (0–29 weeks) was tested for the individual sites and for all five sites combined. Table 1 provides a list of the best-fit logistic regression

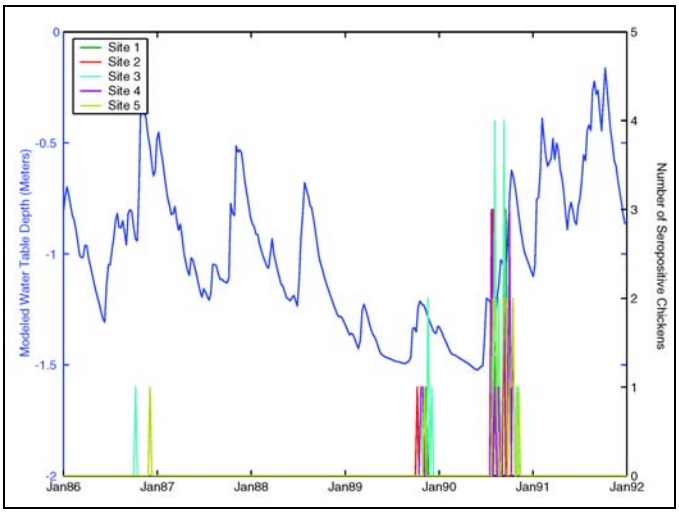

Figure 2. Time series of weekly seroconversion of sentinel chickens (transmission intensity) and weekly averages of modeled mean water table depth (WTD). All five sentinel flocks had *St. Louis encephalitis virus* (SLEV) transmission during the study period (1986–1991).

results produced by this analysis, and Figure 3 presents these results graphically. All logistic regression models were highly statistically significant ($p<0.0001$); in fact, a range of time lag values (generally 10–25 weeks) produced statistically significant models ($p<0.001$, data not shown). All five sites show the same trend: SLEV transmission incidence was strongly associated with low WTD 16 to 25 weeks before onset of SLEV transmission.

A second, bivariate logistic regression analysis was performed to explore the effects of both antecedent drought and coincident wetting conditions. Modeled WTD time lags of 10 to 25 weeks were paired with modeled WTD time lags of 0, 1, 2, or 3 weeks and used together in bivariate analysis of SLEV incidence. Table 2 provides a list of the best-fit model equations resulting from this analysis. The optimal range of fits among sites is more tightly constrained when two variables are used (range 12–17 weeks before transmission for antecedent

Table 1. Best fit results of univariate logistic regression analysis, Florida[a]

	Univariate Best Fits					
Site	Time lagged WTD	Intercept	Slope	Whole model fit p-value	Intercept p-value	Slope p-value
1	16	66.70	44.22	0.015	0.016	0.0001
2	17	22.99	14.63	0.0093	0.015	0.0001
3	18	28.72	18.82	0.0050	0.0069	0.0001
4	16	74.10	49.01	0.016	0.017	0.0001
5	25	20.35	13.22	0.0004	0.0010	0.0001
All five sites	19	18.55	12.49	0.0001	0.0001	0.0001

[a]WTD, water table depth; the probability of SLEV transmission incidence is represented as a function of single time lags of weekly averaged modeled WTD. Whole model goodness-of-fit was assessed by log-likelihood ratio and the pseudo r-squared (uncertainty) coefficient. Individual parameter estimates were made by Wald's chi-square test.

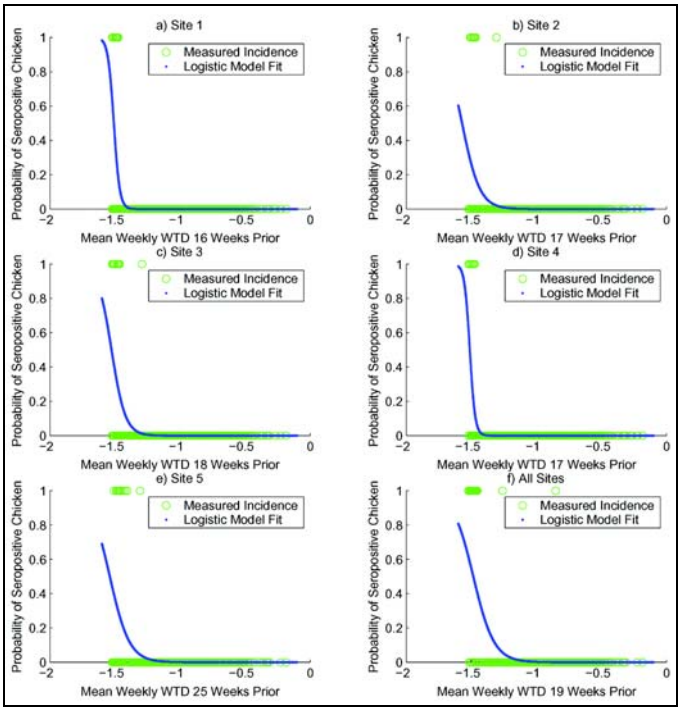

Figure 3. Best fit, univariate logistic regression results. a) site 1; b) site 2; c) site 3; d) site 4; e) site 5; f) all five sites, Florida.

drought, 1–2 weeks before transmission for coincident wetting). The bivariate models are also more statistically significant than their univariate counterparts (based on log-likelihood ratio whole model goodness-of-fit), and the parameter estimates of both explanatory variables are statistically significant ($p<0.01$; $p=0.068$ for site 2).

Figure 4 presents the bivariate model fit of SLEV incidence for all five sites combined. Figure 4a shows the logistic regression fit for a continuous range of modeled WTDs 2 weeks before transmission and fixed values of modeled WTD 17 weeks before transmission. This figure shows that antecedent drought conditions are necessary for SLEV transmission; only with a modeled WTD of <1.2 m 17 weeks before transmission is there any probability of SLEV transmission. This probability, however, is modulated by a rise in the WTD 2 weeks before transmission. This moderating effect is also shown in Figure 4b, which fixes values of modeled WTD 2 weeks before transmission but allows the conditions 17 weeks before transmission to vary. Combined, these two explanatory variables (modeled WTD 17 weeks before transmission and 2 weeks before transmission) offer a strong prediction of SLEV transmission.

The results from analysis of SLEV transmission incidence with modeled WTD were highly statistically significant but did not fully explain why the sequence of drought and wetting fosters SLEV transmission. However, a probable mechanism is suggested by mosquito collection data taken in the area.

Figure 5 shows the distribution of total female *Cx. nigripalpus* versus mean modeled WTD for each calendar year from 1986 through 1991. Total collected female *Cx. nigripalpus* display a bimodal distribution with respect to mean modeled WTD for 3 years (1987, 1989, and 1990). Similar bimodality was evident in the yearly distributions of *Cx. nigripalpus* males and the individual female age-grades (data not shown). For 1989 and 1990, the driest years, one of the maxima of *Cx. nigripalpus* developed sharply at WTDs <1.4 m. None of the other years, including 1987, had this level of drought or this sharp bimodality. Two inferences may be drawn from these data: either the mosquito population increased at both the driest and wettest times of the year, or during the drought of 1989 and 1990, mosquitoes congregated in the hammock collection site. The latter inference is consistent with field observations that the hammocks in Indian River County and throughout south Florida provide refuge for mosquitoes during periods of drought (23). This "hammock" effect masks the true population dynamics; however, it illustrates an effect previously reported (26), namely, that drought concentrates large numbers of mosquitoes in selected refuges that also harbor large numbers of avian amplification hosts.

Discussion

Our findings suggest the following sequence of events for SLEV transmission in Indian River County. Springtime drought restricts *Cx. nigripalpus* activity to densely vegetated hammock habitats where nestling, juvenile, and adult wild

Table 2. Best fit results of the bivariate logistic regression analysis, Florida[a]

	Bivariate best fits							
Site	Antecedent WTD (wks)	Coincident WTD(wks)	Whole model fit p value	Intercept	Antecedent slope	Antecedent slope p value	Coincident slope	Coincident slope p value
Site 1	12	1	0.0001	29.49	26.80	0.0005	-8.98	0.0085
Site 2	16	2	0.0001	17.83	14.13	0.0008	-3.62	0.068
Site 3	17	2	0.0001	22.11	18.64	0.0001	-5.26	0.0071
Site 4	15	1	0.0001	28.92	24.63	0.0011	-7.26	0.0096
Site 5	15	2	0.0001	21.60	23.19	0.0002	-11.37	0.0012
All five sites	17	2	0.0001	19.03	18.06	0.0001	-6.21	0.0005

[a]The probability of SLEV transmission incidence is represented as a function of two time lags of weekly averaged modeled water table depth (WTD). Whole model goodness-of-fit and individual parameter estimates were assessed as per the univariate analysis.

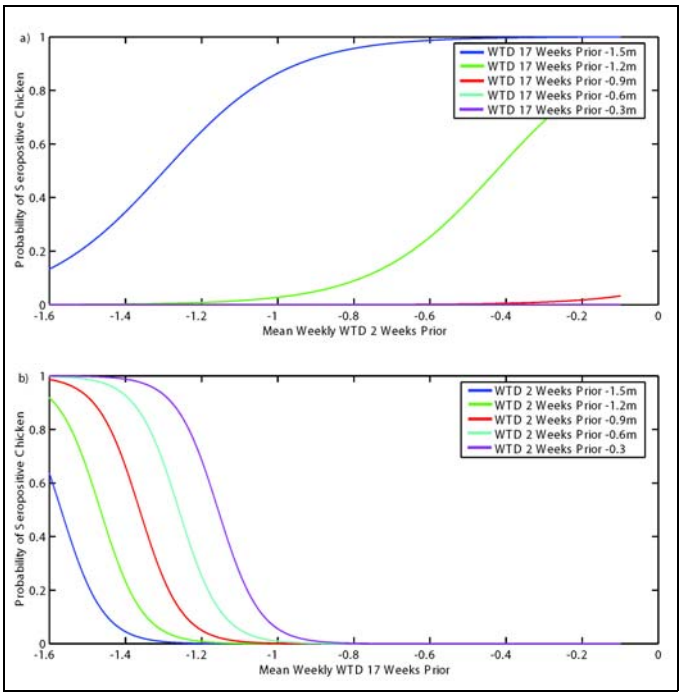

Figure 4. Best fit bivariate logistic regression model of *St. Louis encephalitis virus* (SLEV) incidence at all five sites combined. a) Plotted for a continuous range of modeled water table depths (WTDs) 2 weeks before transmission and fixed values of modeled WTD 17 weeks before transmission; b) plotted for a continuous range of modeled WTDs 17 weeks before transmission and fixed values of modeled WTD 2 weeks before transmission.

birds are found. This forced convergence of mosquito vectors and avian amplification hosts provides an ideal environment for the rapid epizootic amplification of SLEV. When the drought ends and water resources increase, infected mosquitoes and birds disperse from the hammocks, initiating the early transmission phase of the Florida SLEV cycle.

The relationship reported here between modeled WTD and SLEV incidence has only two explanatory variables and provides a simple predictive framework for forecasting SLEV transmission. To be sure, additional factors influence the dynamics of SLEV transmission. Pre-drought conditions may moderate hammock amplification by increasing or decreasing the overall abundance of mosquito vectors and avian amplification hosts. Data from other Florida counties and previous epidemics will have to be examined to elucidate how such population variability affects SLEV amplification and transmission. Future validation of the model should also include census of wild bird populations and sampling of seropositivity rates in the wild birds. Such data were not available for this study.

Whether a critical period of drought is necessary for maximum epizootic amplification also requires exploration. The 1986 data, for which the drop in WTD was short-lived and SLEV transmission was limited, suggest that the longer droughts of 1989 and 1990 were necessary for adequate amplification to produce the mosquito infection rates needed for epidemic transmission. However, if a drought persists for too long, the vectors may die, thus precluding SLEV transmission. Certainly, the biological cycles of virus, vector, and amplification hosts must be coordinated to produce an SLEV epidemic.

The mechanism of drought-induced amplification described here for Indian River County may also operate in regions outside south-central Florida that have similar epidemic SLEV transmission. In fact, the development of SLEV epidemics after drought has long been noted in many regions of the United States (6,7). Future research will attempt to quantify this relationship between drought, vector, and SLEV transmission for such regions. Differences in vector species composition, resting habitat availability, and zoonotic host prevalence will no doubt affect transmission rates and the findings of such studies.

Comparable drought-induced amplification may also occur in other arboviruses. The recent sporadic outbreak of *Eastern equine encephalitis virus* and *West Nile virus* in northern Florida, which came on the heels of a drought broken by the landfall of Hurricane Allison, suggests that these other disease systems warrant similar study.

Modeling the hydrologic cycle permits quantification of the relationship between drought and SLEV transmission and enables real-time monitoring and forecasting of SLEV transmission incidence. Using the hydrology model in conjunction with climate forecast projections, we are developing an arboviral forecast for Florida.

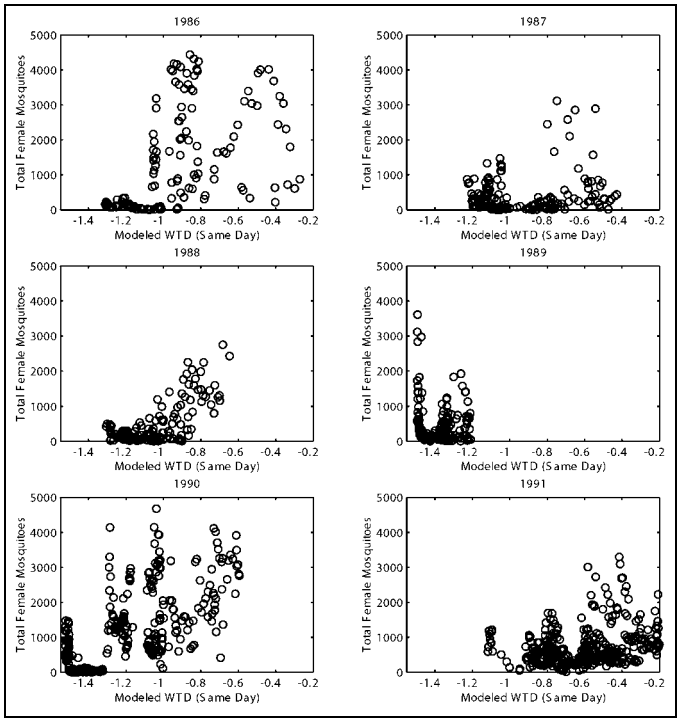

Figure 5. Total collected female *Culex nigripalpus* plotted as a function of modeled water table depth (WTD) (same day). Individual plots represent individual years.

Acknowledgment

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Epidemiologic Differences Between Cyclosporiasis and Cryptosporidiosis in Peruvian Children

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We compared the epidemiologic characteristics of cyclosporiasis and cryptosporidiosis in data from a cohort study of diarrhea in a periurban community near Lima, Peru. Children had an average of 0.20 episodes of cyclosporiasis/year and 0.22 episodes of cryptosporidiosis/year of follow-up. The incidence of cryptosporidiosis peaked at 0.42 for 1-year-old children and declined to 0.06 episodes/child-year for 5- to 9-year-old children. In contrast, the incidence of cyclosporiasis was fairly constant among 1- to 9-year-old children (0.21 to 0.28 episodes/child-year). Likelihood of diarrhea decreased significantly with each episode of cyclosporiasis; for cryptosporidiosis, this trend was not statistically significant. Both infections were more frequent during the warm season (December to May) than the cooler season (June to November). Cryptosporidiosis was more frequent in children from houses without a latrine or toilet. Cyclosporiasis was associated with ownership of domestic animals, especially birds, guinea pigs, and rabbits.

The coccidian protozoal parasites *Cyclospora cayetanensis* and *Cryptosporidium parvum* are recognized diarrheal pathogens among children in developing countries (1–4), but longitudinal data, especially for cyclosporiasis, are sparse. *Cyclospora cayetanensis* is more closely related genetically to *Eimeria* species than to *Cryptosporidium* species (5), and the two organisms have biological differences. For example, *C. parvum* is infectious when excreted and can be transmitted directly from person to person; *Cyclospora cayetanensis* requires a period of time in the environment to sporulate into the infectious form (3), decreasing the likelihood of direct person-to-person spread. *Cryptosporidium parvum* infects both humans and a variety of mammals (6), and evidence is mounting that non-*parvum* zoonotic *Cryptosporidium* species can also infect immunocompetent humans (7,8). Conversely, natural or experimental infection of animals with *Cyclospora cayetanensis* has not been convincingly demonstrated (9–11). Thus, cryptosporidiosis is transmitted through a variety of routes, including contaminated water or food, from person to person, or from animal to person. In contrast, the only major known risk factors for cyclosporiasis are consumption of contaminated water or produce (12–14).

Surveillance data suggest that both organisms are associated with diarrheal illness and asymptomatic infection but differ in their seasonality and susceptible age groups (15). The reasons for these differences are not well understood. Cohort studies of children in Peru provided an opportunity to better understand the characteristics of endemic cryptosporidiosis and cyclosporiasis. The objectives of the analysis were to provide a detailed description of the longitudinal epidemiology of the two organisms and to seek risk factors for infection.

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Materials and Methods

Study Participants

Field work was conducted in the periurban *pueblo joven* (shantytown) of Pampas de San Juan de Miraflores, 25 km from the center of Lima, Peru. In the 1980s this community (pop. approximately 40,000) was heavily settled by immigrants from rural areas. Immigration to the community has slowed, and general living conditions have improved. In 1995, 97% of houses had electricity, 48% had toilets, and 64% had a household water connection (Asociación Benéfica PRISMA, Lima, Peru, unpub. data, 1995).

Our analysis was based on longitudinal data from two cohort studies conducted simultaneously from February 1995 to December 1998. The birth cohort study included all children born during the recruitment period whose mothers consented to participate; its major objectives were to elucidate the relationship between diarrheal disease and nutritional status (16) and to study the epidemiology of viral gastroenteritis. The objective of the other cohort study was to examine the epidemiology of cyclosporiasis. Children from 1 month to 10 years of age were chosen at random from the complete census of the community. Siblings of birth cohort children could be enrolled in the cyclosporiasis cohort if they were chosen by random selection. Twenty sibling pairs, a number too small to allow analysis of household clustering, were included in the analysis. Excluding at random one member of each sibling pair had no

effect on results, and both siblings are included in the analysis presented here.

The same epidemiologic data and specimens were collected from children in both cohort studies. At the time of recruitment, field workers collected data regarding household characteristics, including type of housing, sanitary facilities, water source, and presence of animals. Field workers visited each household daily throughout the follow-up period to compile a daily record of the presence or absence of diarrhea in the child in the primary caretaker's opinion, number of bowel movements, and consistency of stools (liquid, semiliquid, or formed).

Stool specimens were collected weekly from all children, on the first day of a diarrheal episode, and, when one of the pathogens of interest was detected, daily until negative. Stool specimens were transported without preservative and arrived in the laboratory within 24 hours of collection. Each specimen was processed by a standard ether concentration procedure and examined microscopically for *Cryptosporidium* species on modified acid-fast Ziehl-Neelsen stained slides and for *Cyclospora cayetanensis* on wet mount by direct examination and epifluorescence (17,18) in the pathology laboratory of the Universidad Peruana Cayetano Heredia.

Epidemiologic Analysis

We defined a day with diarrhea as a 24-hour period during which the child was reported to have three or more liquid or semiliquid stools and, in addition, was thought by his or her primary caretaker to have diarrhea. An episode of diarrhea was considered to end when the child had at least 3 consecutive days that did not meet the criteria for a day with diarrhea. An episode of cryptosporidiosis or cyclosporiasis was defined by one or more stool specimens positive for the respective parasite. An episode of infection was considered to end on the last day of oocyst detection, followed by at least three negative stools and no oocyst detection for at least 28 days. An episode of infection was associated with diarrhea if at least 1 day met the definition for a day with diarrhea during the infection episode or within 1 week of the beginning or end of the episode.

We included children in the epidemiologic analysis if they had been monitored for at least 6 months and at least 24 stool specimens had been submitted for analysis. All statistics were calculated with SAS for Windows, version 8.0. We tested for seasonality and trends associated with diarrhea and infection order by Poisson regression analyses in SAS Proc Genmod (SAS, Inc., Cary, NC), incorporating generalized estimating equations to account for correlation between multiple observations from the same person. We assumed an exchangeable correlation structure. Relative risks were assessed for significance by the chi-square test. All statistical results were evaluated at the 0.05 level of significance.

Results

Of 533 children originally recruited for the cohorts, 368 children (201 [55%] boys) met our inclusion criteria. The 165 excluded children were comparable in age and sex distribution with study children; for 25 children, no stool specimen was submitted, while <6 months of surveillance was completed for the others. At the time of entry into the study, 256 children (70%) were <1 year, 45 (12%) were 1–4 years, and 67 (18%) were 5–11 years of age. The 368 children contributed a total of 889 child-years of surveillance data; nearly half the data were from children <2 years of age. Children were followed for a mean of 2.4 years and had an average of 1.95 episodes of diarrhea/child-year of follow-up. The highest incidence of diarrhea, 3.3 episodes/child-year, was recorded in children 12–23 months old; after the age of 3 years the incidence of diarrhea declined to <1 episode/child-year. The median duration of diarrheal episodes was 2 days (range 1–27). A total of 44,042 stool specimens were screened for coccidian parasites, a median of 124 stools/child (range 24–227); 897 (2%) of the stool specimens were collected during diarrheal episodes.

Children had an average of 0.20 episodes of cyclosporiasis/year and 0.22 episodes of cryptosporidiosis/year of follow-up (Table 1). Of the 368 children, 123 (33%) had at least one detected episode of *Cyclospora* infection, 30 children had two infections, and 10 children had ≥3 infections. A total of 143 children (39%) had at least one *Cryptosporidium* infection; 34 children had two infections, and 9 had ≥3 infections. Rates varied by age: the incidence of cryptosporidiosis peaked at 1 year and then fell sharply, but cyclosporiasis incidence remained fairly constant during the 1- to 9-year age period. For the 189 children who were enrolled in the study before the age of 3 months, the mean age at first infection was older for

Table 1. Incidence of coccidial infections and association with diarrhea by age group, Peru, February 1995 – December 1998

Age[a] (years)	Child-years of follow-up	Cryptosporidiosis			Cyclosporiasis		
		Infections	Infections/ child-year	With diarrhea no. (%)	Infections	Infections/ child-year	With diarrhea no. (%)
<1	230.6	47	0.20	20 (43)	16	0.07	4 (25)
1	192.0	80	0.42	32 (40)	40	0.21	11 (28)
2–4	243.2	58	0.24	10 (17)	67	0.28	18 (27)
5–9	170.3	10	0.06	2 (20)	47	0.28	6 (13)
10–12	53.5	1	0.02	0 (0)	4	0.07	1 (25)
≤12	889.5	196	0.22	64 (33)	174	0.20	40 (23)

[a]Age on the first day of parasite detection.

cyclosporiasis than for cryptosporidiosis (1.69 versus 1.36 years; p<0.01). After an initial episode of cyclosporiasis, the likelihood of diarrhea decreased significantly (p=0.049) with each subsequent infection (Table 2). For cryptosporidiosis, this trend was less consistent and did not reach statistical significance.

In a regression analysis in which data were controlled for concurrent cyclosporiasis, the expected mean duration of oocyst shedding was longer for cryptosporidiosis associated with diarrhea than for cryptosporidiosis not associated with diarrhea (expected mean 9.4 versus 4.8 days; p=0.0002). In the analogous regression analysis controlled for concurrent cryptosporidiosis, a similar relationship was found for *Cyclospora cayetanensis* shedding with and without diarrhea (15.7 days versus 6.2 days; p=0.004). Diarrheal episodes associated with cryptosporidiosis, but not cyclosporiasis, lasted longer than diarrheal episodes not associated with coccidian parasites (expected mean duration 4.67 days for cryptosporidiosis versus 2.55 days with no coccidia; p<0.001; mean 2.96 days for cyclosporiasis versus 2.55 days with no coccidia; p=0.35).

Both parasitic infections were more frequent during December to May than June to November, but the effect was more marked for cyclosporiasis (relative risk [RR] 3.3; p<0.0001) than for cryptosporidiosis (RR 1.9; p<0.0001). After data were adjusted for seasonality and age, the risk for cyclosporiasis or cryptosporidiosis did not differ by household water supply at the time of entry into the study (Table 3). Using a field rather than a toilet or latrine for defecation was associated with a higher risk of cryptosporidiosis but not cyclosporiasis. No association with exposure to animals could be demonstrated for cryptosporidiosis, but cyclosporiasis was more common in children in households with avians, guinea pigs, rabbits, or any domestic animal.

Discussion

Our analysis confirms that cryptosporidiosis and cyclosporiasis are common infections in this community, with distinct age-related patterns of occurrence. As noted (4,15), cyclosporiasis affected cohort children at later ages than cryptosporidiosis. The reasons for this epidemiologic pattern are not clear. One possible explanation might be that early infections afford less effective immunity for *Cyclospora cayetanensis* than for *Cryptosporidium parvum*. However, our data appear to contradict this hypothesis: first episodes of cyclosporiasis, but not cryptosporidiosis, protect against later symptomatic infection with the same organism. The development of better laboratory tools will be essential to elucidate the immune mechanisms involved. Another possibility is that the differences in age-specific incidence are related to predominant modes of exposure. This hypothesis is consistent with the assumption that *Cyclospora cayetanensis* is usually transmitted by exposure to contaminated environmental sources, from which young infants are usually relatively protected, while cryptosporidiosis can be transmitted by many routes, including from one toddler to another.

The ability of cryptosporidiosis to cause multiple symptomatic episodes may also be related to genetic heterogeneity. In a previous study of specimens from the same cohort, most cryptosporidiosis in this shantytown was caused by the *Cryptosporidium parvum* human genotype. However, children also had infections with *C. parvum* bovine and dog genotypes, *C. meleagridis*, and *C. felis* (8). Heterologous immunity may be less effective than homologous immunity. Although some polymorphism has been demonstrated in *Cyclospora cayetanensis* isolates (19), genetic studies are still in the early stages, and, to date, this parasite appears to be less heterogeneous than *Cryptosporidium* species. Nevertheless, immunity to both organisms occurs in this highly endemic setting, since immunocompetent adults rarely experience symptomatic infections (4).

For both coccidia, we found a high proportion of infections without diarrhea: 67% of cryptosporidiosis and 77% of cyclosporiasis episodes were not associated with diarrhea. These results differ from those reported in studies with different designs and reflect our methods, which aimed to detect as many coccidial infections as possible, independent of symptoms. Each child had a stool specimen screened nearly every week, so that 98% of the stool specimens were not collected during diarrheal episodes. Nevertheless, serologic data suggest that even intensive stool surveillance may miss a substantial proportion of cryptosporidiosis episodes (20). Data from the same Peruvian community showed that cryptosporidiosis

Table 2. Coccidial infections and their association with diarrhea in Peruvian children, February 1995 – December 1998

	Cryptosporidium episodes			*Cyclospora* episodes		
Infection order	Total	With diarrhea[a]	Without diarrhea[a]	Total	With diarrhea[b]	Without diarrhea[b]
First	143	52 (36)	91 (64)	123	33 (27)	90 (73)
Second	43	9 (21)	34 (79)	38	6 (19)	32 (81)
Third	9	3 (33)	6 (67)	10	1 (10)	9 (90)
Fourth	1	0 (0)	1 (100)	2	0 (0)	2 (100)
Fifth	0			1	0 (0)	1 (100)
All episodes	196	64 (33)	132 (67)	174	40 (23)	134 (77)

[a]Test for trend in proportion with diarrhea by infection order, p=0.17.
[b]Test for trend in proportion with diarrhea by infection order, p=0.049.

Table 3. Associations[a] of environmental exposures and infection with coccidian parasites in 368 Peruvian children, February 1995 – December 1998

		Cyclosporiasis				Cryptosporidiosis			
	Child-years of follow-up	No. of episodes	Incidence density[b]	RR (95% CI)[c]	p	No. of episodes	Incidence density[b]	RR (95% CI)	p
Water truck	45.2	11	0.24	0.85 (0.58, 1.26)	0.2	7	0.22	1.01 (0.77, 1.33)	0.94
Water source outside house	268.7	44	0.16	1.01 (0.56, 1.82)	0.98	65	0.24	0.84 (0.49, 1.45)	0.54
Water connection in house	548.1	114	0.21	Referent		119	0.16	Referent	
Defecates in field	298.7	36	0.22	1.00 (0.69, 1.47)	0.99	47	0.29	1.26 (0.97, 1.67)	0.08
Latrine	163.7	54	0.18	0.86 (0.69, 1.47)	0.45	59	0.20	0.96 (0.71, 1.32)	0.85
Flush toilet	380.7	78	0.21	Referent		84	0.22	Referent	
Any animal	665.9	147	0.22	1.72 (1.08, 2.72)	0.02	140	0.21	0.97 (0.74, 1.26)	0.82
No animals	223.6	27	0.12	Referent		56	0.25	Referent	
Chickens	357.7	76	0.21	1.10 (0.81, 1.49)	0.52	75	0.21	1.0 (0.77, 1.29)	0.99
No chickens	531.9	98	0.18	Referent		121	0.23	Referent	
Ducks	245.0	52	0.21	1.07 (0.78, 1.48)	0.64	47	0.19	0.89 (0.67, 1.21)	0.47
No ducks	644.5	122	0.19	Referent		149	0.23	Referent	
Any avian[d]	477.6	108	0.23	1.34 (0.96, 1.87)	0.08	97	0.20	0.91 (0.71, 1.17)	0.48
No avians	412.0	66	0.16	Referent		99	0.24	Referent	
Dog	316.9	68	0.22	1.19 (0.88, 1.59)	0.26	70	0.22	0.93 (0.71, 1.21)	0.58
No dog	572.6	106	0.19	Referent		126	0.22	Referent	
Guinea pig	37.5	12	0.32	1.56 (0.99, 2.44)	0.05	10	0.27	1.63 (0.89, 2.99)	0.11
No guinea pig	852.0	162	0.19	Referent		186	0.22	Referent	
Rabbit	49.6	20	0.40	2.13 (1.23, 3.69)	0.007	11	0.22	1.05 (0.56, 2.0)	0.87
No rabbit	840.0	154	0.18	Referent		185	0.22	Referent	

[a]The analyses were adjusted for seasonality and age of child.
[b]Episodes of infection per child-year of follow-up.
[c]RR=relative risk; CI=95% confidence intervals.
[d]Any avian=one or more of the following birds: chickens, ducks, turkeys, pigeons, parrots, or parakeets.

without diarrhea had a substantial effect on childhood growth (21,22); because such infections may have long-term sequelae it is somewhat misleading to call them asymptomatic.

Cryptosporidium species infect a wide range of mammalian hosts and can be zoonotic infections (6), while *Cyclospora cayetanensis* has never been convincingly demonstrated to infect a nonhuman host (9–11). However, the animals most commonly associated with zoonotic *C. parvum* were rare in this urban setting: no families had calves, only one family had goats and lambs, and six families had adult sheep. This finding may explain why we could not show an association of cryptosporidiosis with animal exposure. Children in households with animals, especially birds, guinea pigs, and rabbits, appeared to be at higher risk of cyclosporiasis. The finding of an association with avians is consistent with results of a *C. cayetanensis* case-control study in Guatemala (14), but this association is still unexplained. Possibly the presence of domestic animals is a marker for some other unmeasured risk factor. Cyclosporiasis appears to be much more common in Lima than in the mountains of Peru (Asociación Benéfica PRISMA, Lima, Peru, unpub. data), and most residents of the study community migrated from the mountains to Lima. Raising domestic animals, especially poultry, may be more common among recent rural migrants with less exposure and therefore higher susceptibility to *C. cayetanensis*.

Our exposure data were collected at the beginning of longitudinal surveillance, and the time that elapsed between their collection and the occurrence of an infection could have been as long as several years, which may have decreased our ability to detect associations. Studies specifically designed to identify risk factors close to the time of an infection and use of molecular techniques to distinguish genotypes or strains may help clarify some of these issues.

As more longitudinal data become available for these organisms, we are gaining a clearer picture of the overall epidemiology of cyclosporiasis and cryptosporidiosis in endemic settings. Both organisms cause a spectrum of disease, from apparently asymptomatic infection to prolonged episodes of diarrhea that may have a profound effect on a child's well-being. Our findings for cryptosporidiosis are consistent with the view that the organism infects children very early in life and has multiple routes of transmission, but mysteries remain concerning the cycle that maintains cyclosporiasis as an endemic infection. In-depth study of the transmission of *C. cayetanensis* will be key to designing effective strategies for intervention.

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Population-Based Study of Acute Respiratory Infections in Children, Greenland

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Acute respiratory infections (ARI) are frequent in Inuit children, in terms of incidence and severity. A cohort of 294 children <2 years of age was formed in Sisimiut, a community on the west coast of Greenland, and followed from 1996 to 1998. Data on ARI were collected during weekly visits at home and child-care centers; visits to the community health center were also recorded. The cohort had respiratory symptoms on 41.6% and fever on 4.9% of surveyed days. The incidence of upper and lower respiratory tract infections was 1.6 episodes and 0.9 episodes per 100 days at risk, respectively. Up to 65% of the episodes of ARI caused activity restriction; 40% led to contact with the health center. Compared with studies from other parts of the world, the incidence of ARI appears to be high in Inuit children.

In children of the Inuit, the aboriginal Eskimo population of the Arctic, acute respiratory infections (ARI) are frequent, measured in terms of incidence and severity. Infant death and disease from ARI are higher than in Denmark, United States, and Canada (1–3); many Inuit children have severe lower respiratory tract infections (LRI) early in life (4). Childhood otitis media, with an occurrence rate among the highest in the world (5–7), is characterized by early age at onset and a high chronicity (6–9). The causes of the high rates of otitis media are largely unknown, but nasopharyngeal carriage of potentially pathogenic bacteria and viruses in Greenlandic children in combination with frequent upper respiratory tract infections (URI) may be important (10).

To determine the incidence of ARI on the basis of population, we established a cohort of children <2 years of age in Sisimiut, a community on the west coast of Greenland. The goals of this study were to determine the epidemiology of acute respiratory tract infections in children on a prospective and longitudinal basis and to identify risk factors for such disease.

Materials and Methods

Study Area

Sisimiut is the second largest town in Greenland (pop. 5,117, January 1996). Of these inhabitants, 88% were born in Greenland and 12% outside Greenland, primarily in Denmark (11). In our study population, each household had a median of three rooms (interquartile range [IQR] three to four) and four persons (IQR four to five). Eighty percent of children lived in nuclear families with two parents, and 70% attended child-care centers during the study period.

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All health services in Sisimiut, except for a dental clinic, are located at the community health center, which serves as general practice facility, birth clinic, and regular hospital. All births in the town take place at the center. All health services in Greenland, including prescribed medication, are free of charge.

Study Population

The cohort consisted of all children <2 years living in Sisimiut from April 1, 1996, to June 1, 1998, including all children born there and all children who moved there during that period. To ensure that we included all Sisimiut children in the cohort, we obtained information on inhabitants in Sisimiut at regular intervals from the local authorities and from the Civil Registration System of Greenland (12), in which all citizens of Greenland are registered. Parents of children eligible for inclusion in the study were contacted by letter or in person. Using a standardized interview, trained project staff visited the home to obtain written informed consent and collect background information. Families who declined participation were asked their reasons by open-ended questions. Because visiting nurses see newborns in Sisimiut for the first 5 weeks, most children were enrolled after 6 weeks, although 12 children were, for the mothers' convenience, enrolled before that time. The Commission for Scientific Research in Greenland, the scientific ethical board for research, approved the study.

Illness Surveillance

From July 30, 1996, to August 13, 1998, children were monitored through weekly visits in their homes or child-care centers. Children absent from child-care centers at the scheduled time were visited at home. At all visits a standardized medical history based on the presence of respiratory symptoms (nasal secretion, cough, earache, ear discharge, hoarseness, sore throat, rapid or difficult breathing, or chest indrawing),

fever, diarrhea (loose or watery stools >3 times a day), and signs of general malaise since the last information was obtained from the parents. Symptoms were recalled for the prior 1 to 2 weeks, and the exact days that symptoms occurred were recorded. If the parents reported one or more respiratory symptoms for the preceding week, a clinical examination focusing on the respiratory system was done including non-pneumatic otoscopy and tympanometry (MicroTymp2 tympanometers, Welch-Allyn, NY). The presence of diarrhea or fever alone did not prompt a clinical examination.

At the end of the study period, the children's inpatient and outpatient charts kept at the community health center were reviewed, and doctors' diagnoses or reporting of characteristic clinical signs of URI (common cold, pharyngitis, tonsillitis, or otitis media) and LRI (croup, bronchitis, bronchiolitis, or pneumonia) were noted.

Case Definitions

An ARI episode, first reported as respiratory symptoms by the parents, was characterized as a URI or LRI, if confirmed by clinical examinations made by medical students as part of the study or by local doctors at the health center. The medical students used a modified set of signs proposed by the Board on Science and Technology for International Development (BOSTID) studies (13). A diagnosis of URI included one or more of the following clinical signs: purulent nasal discharge; cough; a red, bulging tympanic membrane with loss of normal landmarks and abnormal tympanometry; purulent ear discharge; and pharyngo-tonsillar erythema or exudate without signs of LRI. A diagnosis of LRI was made if one or more of the following clinical signs was present: respiratory rate >50/min and labored breathing or cough; rales; stridor; wheezing; cyanosis; or chest indrawing. During data analysis the presence of clear nasal discharge as the only finding was omitted from clinical definitions. Health center visits for rhinitis, pharyngitis, tonsillitis, or otitis media were regarded as URI, and visits for croup, bronchitis, bronchiolitis, or pneumonia were included as LRI. Episodes of respiratory infections, during which more than one clinical examination was conducted, were classified as LRI if one of the examinations met the criteria for LRI.

The minimum interval between two episodes of respiratory symptoms was considered to be 7 consecutive days. We defined prevalence as the number of days with a given symptom reported positive, divided by total number of days of observation, and we calculated incidence as the number of new episodes divided by person time at risk (14). Time at risk was defined as the number of days with no recorded symptoms including the first day of any episode, but excluding the 7 consecutive days without symptoms following an episode. The number of days of a given episode was considered to be its duration. If the last day of an episode was not followed by 7 consecutive days with no symptoms recorded (for example, in the case of missing information), this time period was truncated at the last day with recorded symptoms.

Severity was assessed by using the following measures: duration of illness, activity restriction, and visit or admission to health center. We used parental reporting to note activity restriction, including the child's general condition, confinement to bed, absence from the child-care center, change in sleeping and eating patterns, and presence of fever.

Statistical Methods

Where appropriate, chi-square test and Fisher's exact test were used to test differences in distribution. Assuming a Poisson distribution for the number of episodes, we estimated 95% confidence intervals (CI); likelihood-ratio tests were used to test differences in incidence with respect to sex, age, and calendar period. The distribution of episode duration was estimated by using the nonparametric Kaplan-Meier estimator, thereby taking into account that some episodes were truncated. All statistical analyses were performed by using SAS v. 6.12 (SAS Institute Inc., Cary, NC). The GENMOD procedure was used for the Poisson regression analysis and the LIFETEST procedure to calculate Kaplan-Meier estimates (15,16).

Results

Study Population

Of 356 children eligible for study, consent was obtained for 312 (87.6%), and 44 (12.4%) refused. For those who refused, reasons given were lack of time, various non-disease-related reasons, and medical issues not related to ARI; 15 gave no reason. Of the group of 312 children, 17 children passed their second birthday before the initial visit was made and were not included. One child was excluded during the study period for laryngomalacia, leaving 294 children as the study population (Table 1). Of the study population, 242 (82.3%) children participated for the scheduled period; 52 (17.7%) withdrew. Of those who withdrew, 37 (71.2%) moved out of Sisimiut, 14 (26.9%) declined to continue, and 1 died of aspiration pneumonia caused by febrile convulsions. Of those who declined to continue, none gave disease-related causes for withdrawal. The participation rate among Greenlandic children was higher than among Danish children, and children resident in Sisimiut at the beginning of the study were more likely to participate than children born in or moving into Sisimiut after the study began (Table 1).

Illness Surveillance

The median age of the children at enrollment was 142 days; the median age at first day of illness information in the monitoring period was 174 days. Information on respiratory symptoms was obtained for a median of 256 days (Table 1).

In total, 11,081 interviews with the parents or guardians were attempted during the monitoring period; in 1,638 (14.8%) of these attempts, no contact was made. Of the 9,443 successful interviews, 34.2% were made in the children's homes, 34.8% in child-care centers, and 14.1% by telephone. In 16.9% of the interviews, the place of interview was not noted.

Table 1. Study population, Sisimiut, Greenland, 1996–1998

	Participants[a]		Non-participants[b]		
	n	(%)	n	(%)	p value
Sex					0.933[c]
Boys	145	(49.3)	22	(50.0)	
Girls	149	(50.7)	22	(50.0)	
Place of birth					0.05[d]
Sisimiut	267	(90.8)	35	(79.5)	
Other Greenlandic towns	20	(6.8)	6	(13.6)	
Denmark	7	(2.4)	3	(6.8)	
Ethnicity[e]					<0.001[d]
Inuit	237	(80.6)	26	(59.1)	
Danish	11	(3.8)	7	(15.9)	
Mixed	30	(10.2)	2	(4.5)	
Unknown	16	(5.4)	9	(20.5)	
Availability for enrollment					<0.001[c]
Available for enrollment before study period[f]	135	(45.9)	7	(15.9)	
Born in Sisimiut in study period	143	(48.6)	31	(70.5)	
Moved into Sisimiut in study period	16	(5.4)	6	(13.6)	
Age at first day in monitoring period (mon)[g]					
≤2	106	(36.1)			
3–5	42	(14.3)			
6–11	57	(19.4)			
12–17	47	(16.0)			
18–23	42	(14.3)			
Time of illness monitoring (days)					
25% quartile	133				
Median	256				
75% quartile	374				
Range	2–630				

[a] n = 294.
[b]Children whose parents refused to participate from the start. n = 44.
[c]chi-square test.
[d]Fisher's exact test.
[e]Inuit, both parents born in Greenland. Danish, both parents born in Denmark. Unknown, one or both parents place of birth unknown.
[f]April 1, 1996, – June 1, 1998.
[g]Monitoring period July 30, 1996, – August 13, 1998. Median age 5.8 months.

Respiratory Symptoms, Fever, and Diarrhea

The prevalence of respiratory symptoms, fever, and diarrhea was highest in the age group 6–11 months. Respiratory symptoms and diarrhea, but not fever, were reported more often for boys than for girls (Table 2). The corresponding incidence of respiratory symptoms was almost 3 times higher than the incidence of fever and 6 times higher than that of diarrhea. The incidence of respiratory symptoms and fever was similar for boys and girls, but boys had substantially more episodes of diarrhea than girls. For all three illnesses, a steep rise in incidence was seen from the age ≤5 months to 6–11 months, followed by decreasing incidence up to 2 years of age (Table 3). For respiratory symptoms, 5% of the children had no episodes, 37% had 1 to 4 episodes, 41% had 5 to 9 episodes, and 16% had ≥10 episodes. The median number was 5 (IQR 3 to 8 episodes). For fever and diarrhea, the numbers were much smaller, as 17% and 46%, respectively, of the children had no

Table 2. Prevalence of respiratory symptoms, reported fever, and diarrhea in 294 children, Sisimiut, Greenland, 1996–1998

	Days with symptoms	Days observed (with symptom information)	Percentage ill (prevalence)	p value[a]
Respiratory Symptoms				
Total	32,018	76,914	41.6	
Sex				<0.001
Boys	16,060	35,795	44.9	
Girls	15,958	41,119	38.8	
Age (mo)				<0.001
≤5	3,242	12,110	26.8	
6–11	9,331	20,926	44.6	
12–17	9,898	22,154	44.7	
18–23	9,547	21,724	43.9	
Fever, reported				
Total	3,763	76,524	4.9	
Sex				0.96
Boys	1,748	35,520	4.9	
Girls	2,015	41,004	4.9	
Age (mo)				<0.001
≤5	366	12,101	3.0	
6–11	1,306	20,778	6.2	
12–17	1,213	22,064	5.5	
18–23	878	21,581	4.1	
Diarrhea, reported				
Total	2,017	76,541	2.6	
Sex				<0.001
Boys	1,037	35,526	2.9	
Girls	980	41,015	2.4	
Age (mo)				<0.001
≤5	194	12,081	1.6	
6B11	708	20,792	3.4	
12–17	629	22,066	2.9	
18–23	486	21,602	2.2	

[a]Differences in prevalence with respect to sex and age were tested by assuming a binominal distribution.

episodes. The median number of episodes of fever and diarrhea was two and one, respectively (Figure 1). The median duration of respiratory symptom episodes was 14 days (IQR 7 to 33 days) and 3 days for both fever (IQR 2 to 6 days) and diarrhea episodes (IQR 2 to 7 days), respectively (Figure 2).

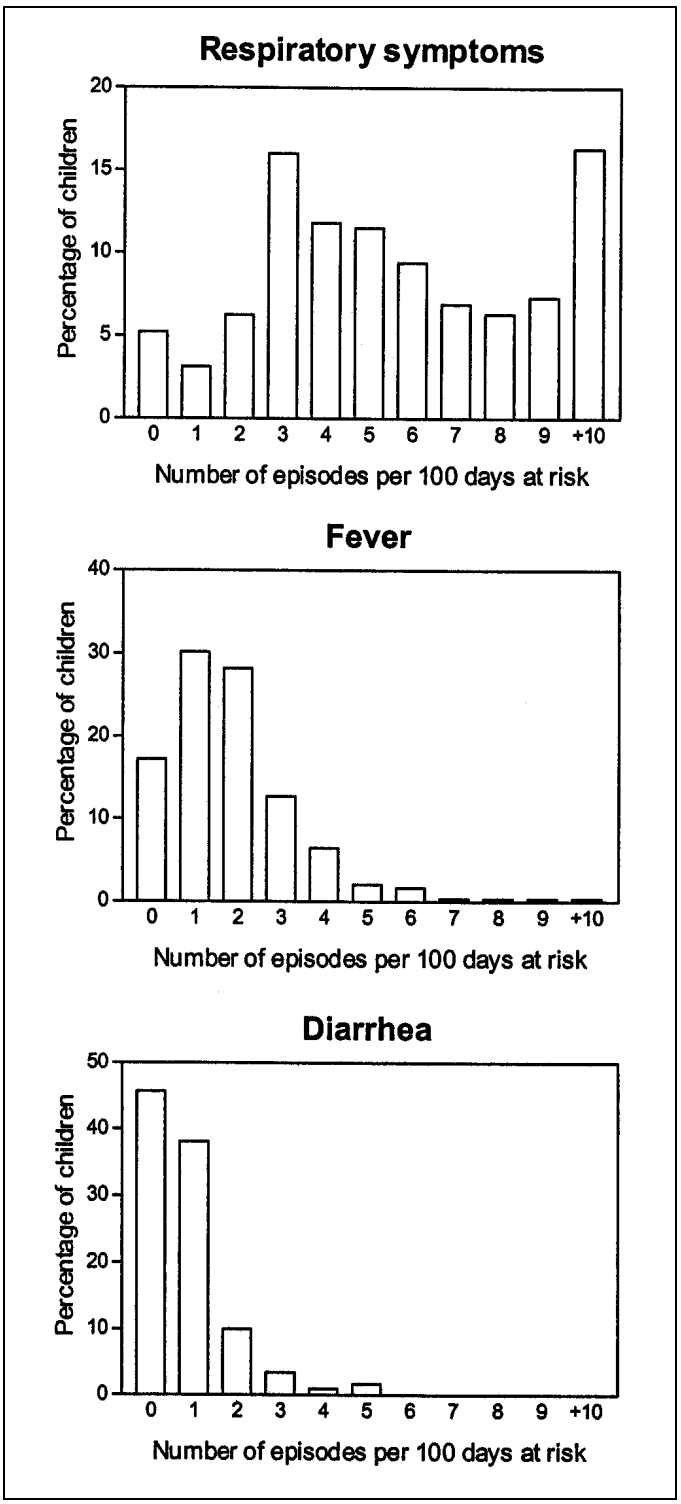

Figure 1. Distribution of number of episodes (respiratory symptoms, reported fever, and diarrhea) per 100 days at risk in 294 children, Sisimiut, Greenland, 1996–1998.

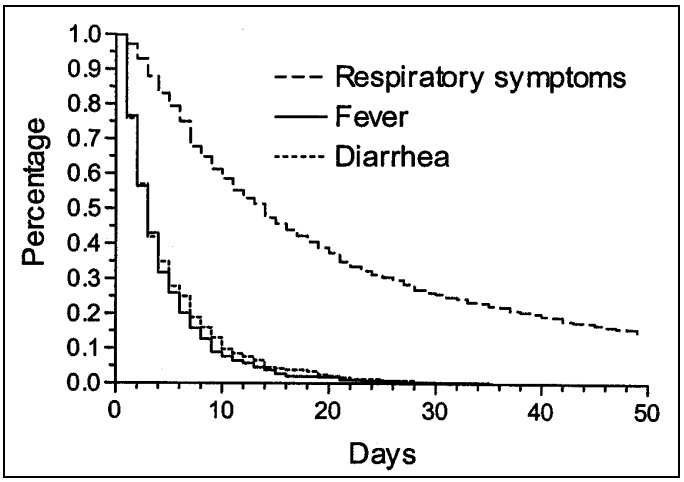

Figure 2. Duration of episodes (respiratory symptoms, reported fever, and diarrhea) in 294 children, Sisimiut, Greenland, 1996–1998.

The most frequently reported symptom was nasal secretion, reported in 26,254 (83%) of days with symptoms, either as the only symptom (37%) or in combination with symptoms of URI (46%). Symptoms of LRI (fast or difficult breathing and chest indrawing) were found in 1.8% of days with respiratory symptoms. Ear discharge indicative of acute or chronic otitis media was present in 4.9% of days of observation.

URI and LRI Episodes

Six of the 294 participating children did not have 7 consecutive days free of respiratory symptoms before any episode of ARI, leaving 288 children at risk of clinically characterized episodes of acute respiratory infections. Of the 1,547 episodes of respiratory symptoms, 918 were classified as episodes of URI (527), LRI (292), or clear nasal discharge (99) (Table 4). The incidence of LRI was higher in boys than in girls ($p<0.001$), but no difference in the sex of the child was observed for URI ($p=0.329$) (Figure 3).

The remaining 629 (41%) of the 1,547 episodes of respiratory symptoms could not be clinically characterized. This group included episodes ending before visit (129), episodes without abnormal clinical signs at examination (301), and episodes for which clinical examinations could not be conducted (199). These episodes were distributed similarly with respect to sex and age.

No seasonal pattern of the incidence of the overall ARI, URI, or LRI was observed (Figure 4). In addition, no seasonal variation was observed for the severe episodes of ARI (e.g., those that required medical attention).

Severity of Clinical Episodes

Median duration of URI episodes was 14 days (IQR 7–25 days) and of LRI episodes 19 days (IQR 9–39 days). Activity restriction characterized 65% of clinical episodes (58% URI, 75% LRI); 40% of episodes (32% URI, 56% LRI) resulted in outpatient hospital visits. Only one URI and eight LRI episodes caused hospital admittance. No children in the study died from ARI.

Table 3. Incidence of episodes of respiratory symptoms, reported fever, and diarrhea in 294 children, Sisimiut, Greenland, 1996–1998

	No. of new episodes	Days at risk	Incidence/ 100 days at risk	95% CI[a]	p value[b]
Respiratory symptoms					
Total	1,547	33,228	4.66	4.43, 4.89	
Sex					0.625
Boys	685	14,508	4.72	4.38, 5.09	
Girls	862	18,720	4.60	4.31, 4.92	
Age (mo)					<0.001
≤5	201	6,870	2.93	2.55, 3.36	
6–11	471	8,439	5.58	5.10, 6.11	
12–17	462	8,838	5.23	4.77, 5.73	
18–23	413	9,081	4.55	4.13, 5.01	
Calendar periods[c]					0.081
Jan–Mar	393	8,957	4.39	3.97, 4.84	
Apr–Jun	391	8,954	4.37	3.95, 4.82	
Jul–Sep	403	7,944	5.07	4.60, 5.59	
Oct–Dec	360	7,373	4.88	4.40, 5.41	
Fever, reported					
Total	1,106	63,584	1.74	1.64, 1.85	
Sex					0.538
Boys	503	29,505	1.70	1.56, 1.86	
Girls	603	34,079	1.77	1.63, 1.92	
Age (mo)					<0.001
≤5	112	10,059	1.11	0.93, 1.34	
6–11	371	16,689	2.22	2.01, 2.46	
12–17	358	18,217	1.97	1.77, 2.18	
18–23	265	18,619	1.42	1.26, 1.61	
Calendar periods[c]					<0.001
Jan–Mar	309	16,609	1.86	1.66, 2.08	
Apr–Jun	230	16,921	1.36	1.19, 1.55	
Jul–Sep	314	15,326	2.05	1.83, 2.29	
Oct–Dec	253	14,728	1.72	1.52, 1.94	
Diarrhea, reported					
Total	523	69,255	0.76	0.69, 0.82	
Sex					0.016
Boys	268	31,833	0.84	0.75, 0.95	
Girls	255	37,422	0.68	0.60, 0.77	
Age (mo)					<0.001
≤5	48	10,623	0.45	0.34, 0.60	
6–11	158	18,705	0.84	0.72, 0.99	
12–17	179	19,981	0.90	0.77, 1.04	
18–23	138	19,946	0.69	0.59, 0.82	
Calendar periods[c]					<0.001
Jan–Mar	215	17,423	1.23	1.08, 1.41	
Apr–Jun	50	18,516	0.27	0.20, 0.36	
Jul–Sep	101	17,419	0.58	0.48, 0.70	
Oct–Dec	157	15,897	0.99	0.84, 1.15	

[a]CI, confidence interval.
[b]Likelihood-ratio test.
[c]Months accumulated in monitoring period (July 30, 1996 – August 13, 1998).

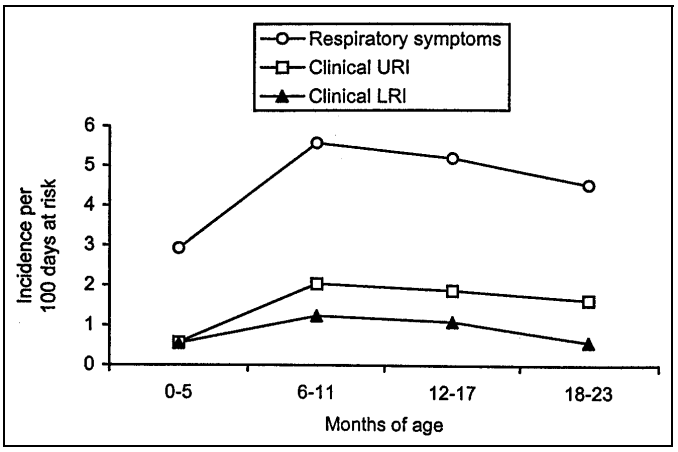

Figure 3. Age-specific incidence of episodes of respiratory symptoms and episodes clinically characterized as upper (URI) or lower respiratory tract infections (LRI) per 100 days at risk in 288 children, Sisimiut, Greenland, 1996–1998.

Discussion

The relatively small size of Sisimiut allowed us to invite all children in this community to participate, in contrast to other studies, in which subgroups of children were chosen as study populations (17–22). The participation rate of 87.6% in this study was high compared with rates of 74% and 78% reported in other community studies in the United States (19,20). We had a low dropout rate (17.7%) and the main cause of lack of follow-up (71.2%) was migration from Sisimiut. We do not believe that our results were biased by selective dropout.

Although living standards in Sisimiut are slightly higher than in Greenland as a whole (23,24), enough similarities exist within towns and settlements in Greenland that the estimates of incidence and prevalence can be considered representative of the population as a whole.

We found that respiratory symptoms were reported in 41.6% of days of observation and that the incidence of respiratory symptom episodes was 4.7 episodes per 100 days at risk (32.6 episodes/100 weeks at risk). For episodes that were clinically characterized, the incidence of URI and LRI combined was 2.5 episodes per 100 days at risk (17.3 episodes/100 weeks at risk). Because health service in Sisimiut is free and easily accessible, we believe that the episodes not diagnosed through the clinic were not severe.

The results we present are high, compared with those in both developing and industrialized countries. In the BOSTID community studies of children ≤5 years of age from Kenya, Nigeria, Papua New Guinea, the Philippines, Thailand, Colombia, Uruguay, and Guatemala, prevalence of ARI was reported within the range of 21.7% to 40.1%, and incidence of ARI in the range of 12.7 to 27.5 episodes per 100 child weeks at risk (13). In community studies from Tecumseh, Michigan, and Seattle, Washington, incidence of ARI ranged from 8.6 to 11.7 episodes per 100 child weeks (children ≤1 year of age)

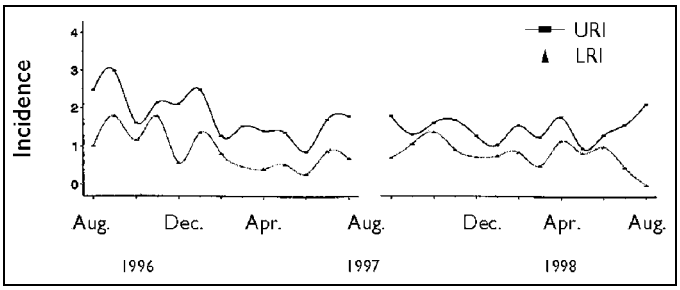

Figure 4. Incidence of clinical episodes of upper respiratory tract infections (URI) and lower respiratory tract infections (LRI) by calendar month in 288 children, Sisimiut, Greenland, 1996–1998.

(25,26). In addition, duration of episodes in our study (median 14 days) tended to be relatively long. In the BOSTID and Tecumseh studies, the median episodes lasted 1 to 2 weeks (13) (except for one study, which reported a median duration of 5 weeks [25]).

Some differences in design between our study and others may have affected our comparison. To determine an episode, we used the exact days on which parents reported symptoms. Other studies have used whole weeks as units of time, which may overestimate the duration of episodes and underestimate time at risk. We included nasal secretion as a respiratory symptom, while other studies omitted clear nasal secretion from case definitions. Because we found that nasal secretion was reported for 85% of days with symptoms, including this symptom increased the prevalence and incidence in our results. Similar increased prevalence and incidence have been found in studies that also included mild nasal discharge as part of the case definitions (27,28). Although we made a conservative estimate of ARI by excluding clear nasal discharge from clinical definitions and including only clinically verified episodes of URI and LRI, we still found a high incidence of 17.3 episodes per 100 child weeks at risk in Sisimiut. In addition, our study population consisted of children ≤2 years of age. As the incidence of ARI depends on age, the highest incidence seen in children in this age group, the focus on age group might distort the comparison with the BOSTID studies. Nevertheless, compared with two BOSTID studies that specifically studied children ≤3 years of age, our estimate of 17.3 episodes per 100 child weeks at risk for ARI was higher than the estimates found in these studies (13.2 and 15.4 episodes/100 child weeks at risk) (22,29).

Given that rhinitis symptoms were reported at a high rate, the episodes could be expected to be less severe than in other studies; accordingly, we observed no fatal episodes of ARI. We found that 65% of clinically verified episodes caused activity restriction, and 40% prompted contact with the health center (LRI episodes being more severe than URI episodes). While not completely comparable because of differences in definitions, the severity of episodes appeared to be within the same range as that observed in Tecumseh, where 33% of all respiratory illnesses caused activity restriction (in all age groups) and 47% caused physician visits (children ≤1 year) (19,25).

Table 4. Number and characteristics of clinically characterized episodes of acute respiratory infections in 288 children, Sisimiut, Greenland, 1996–1998[a]

	No. of new episodes	Days at risk	Incidence/ 100 days at risk	95% CI[b]	p value[c]
URI					
Total	527	33,228	1.59	1.46, 1.73	
Sex					0.329
Boys	219	14,508	1.51	1.32, 1.72	
Girls	308	18,720	1.65	1.47, 1.84	
Age (mo)					<0.001
≤5	39	6,870	0.57	0.41, 0.78	
6–11	172	8,439	2.04	1.76, 2.37	
12–17	167	8,838	1.89	1.62, 2.20	
18–23	149	9,081	1.64	0.41, 0.78	
LRI					
Total	292	33,228	0.88	0.78, 0.99	
Sex					<0.001
Boys	159	14,508	1.10	0.94, 1.28	
Girls	133	18,720	0.71	0.60, 0.84	
Age (mo)					<0.001
≤5	38	6,870	0.55	0.40, 0.76	
6–11	104	8,439	1.23	1.02, 1.49	
12–17	97	8,838	1.10	0.90, 1.34	
18–23	53	9,081	0.58	0.45, 0.76	
Clear nasal discharge[d]					
Total	99	33,228	0.30	0.24, 0.36	
Sex					0.021
Boys	32	14,508	0.22	0.16, 0.31	
Girls	67	18,720	0.36	0.28, 0.45	
Age (mo)					0.002
≤5	7	6,870	0.10	0.05, 0.21	
6–11	32	8,439	0.38	0.27, 0.54	
12–17	33	8,838	0.37	0.27, 0.53	
18–23	27	9,081	0.30	0.20, 0.43	

[a]Six of the 294 participating children did not have 7 consecutive days free of respiratory symptoms before any episode of ARI, leaving 288 children at risk of clinically characterized episodes of acute respiratory infections.
[b]Abbreviations used: CI, confidence interval; URI, upper respiratory tract infections; LRI, lower respiratory tract infections
[c]Likelihood-ratio.
[d]Based on the medical students' clinical examinations only, as doctors at the community health center did not discriminate between clear and purulent nasal secretions.

We found a significantly increased risk of LRI in boys compared with girls, which is similar to findings of other studies (13,30), although the increased risk for boys in our study was higher than that observed in the BOSTID community studies (13). In contrast, there was no difference in sex of the

child with respect to URI, which corresponds with results of Greenlandic studies showing no difference between boys and girls for otitis media and common cold (31–33). Similarly, our finding of the highest risk of both URI and LRI for the 6- to 11- month age group followed by a decline in older age groups is in agreement with results of other studies (13,25). Possible mechanisms for this may include cessation of breast-feeding, degradation of maternal antibodies passively transferred from birth, and attendance at child-care centers.

As some of the episodes were very long, these symptoms could reflect allergy rather than infection. However, episodes characterized by clear nasal secretion as a possible sign of allergic rhinitis were few (only 99 [6.4%] of 1,547 episodes reported), and these episodes were excluded from clinical definitions of ARI. We have recently shown that atopy, defined as elevated specific immunoglobulin E in serum, is half as prevalent in schoolchildren of Sisimiut as in Danish schoolchildren of the same age (34). We are confident that our findings represent infections rather than allergies.

Surprisingly, we found no clear seasonal variation in the incidence of respiratory symptom episodes, clinically verified episodes of URI or LRI, and episodes prompting contact with the health center. Based on hospital contacts and drug prescriptions, the highest incidence of ARI in Greenland has been described in July and in the winter (3,35,36), although other studies failed to demonstrate any seasonal pattern (37). In Greenland, no routine data are available from hospital admissions or routine surveillance for respiratory tract infections or respiratory pathogens to elucidate these findings further. In Alaska, seasonal trends in the incidence of LRI and invasive pneumococcal disease have been described, with highest incidence in the spring and lowest in the fall (4,38); another study found the highest rate of hospitalizations from respiratory syncytial virus in December and lowest in March (39). While seasonal variation may correlate with weather conditions such as low temperature, humidity, and precipitation, proving a causal role of these factors is difficult (30). Instead, causes may be related to crowding in the home correlated with weather conditions (30). While marked variation in monthly average temperature is seen in Sisimiut, ranging from 6.3ºC to -14ºC in July and March, respectively, relative outdoor humidity varies little (80%–87%). Greenlandic children spend much time outside all year round, even in winter. In child-care centers, the children sleep outside in baby carriages all year unless the temperature drops below -15ºC. The lack of seasonal variation in ARI could therefore reflect a pattern of little seasonal variation in indoor stay for children of this age but could also reflect different and opposing patterns of various infectious agents. Studies examining possible seasonality of specific pathogens (e.g., respiratory syncytial virus or *Haemophilus influenzae*) are warranted.

Although our study focused on acute respiratory tract infections, we also collected data on diarrheal diseases and episodes of fever without other prominent symptoms. These data show that the high illness rate in Sisimiut is specifically caused by ARI and not other infections in childhood, in contrast to data from many developing countries, where young children have high incidences of different kinds of infections. This observation corroborates the point that Sisimiut should be regarded as a modern Greenlandic society with a high incidence of respiratory tract infections and not a developing country setting with high rates of poverty-related diseases, such as diarrhea and malnutrition.

This first population-based community study of ARI in Inuit children $\leq$2 years of age based on active surveillance showed a high occurrence of the disease overall. A total of 41.6% of days were spent with symptoms of respiratory tract infections, and the incidence of new episodes of ARI was 2.5 per 100 days at risk. Of all episodes, 65% caused activity restriction, and 40% caused contact with the health center. The prevalence of this disease calls for intervention programs, and further studies are in progress to elucidate risk factors that may allow for specific interventions.

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Streptococcus pneumoniae, Brooklyn, New York: Fluoroquinolone Resistance at Our Doorstep

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To examine the resistance rates and epidemiology of *Streptococcus pneumoniae* in Brooklyn, New York, isolates were collected during two boroughwide surveillance periods in 1997 and 1999. Of 138 isolates, 67% were susceptible to penicillin and 34% to ciprofloxacin. Susceptibility rates to ciprofloxacin decreased dramatically from 1997 to 1999 (47% to 16%, p=0.0003). Five isolates (3.6%) were resistant to levofloxacin. Western Brooklyn had lower rates of susceptibility to penicillin compared with eastern neighborhoods. More isolates in the eastern neighborhoods belonged to the Spanish/French 9/14 clone, and isolates in the western neighborhoods tended to belong to the Spanish/USA 23F clone. Residents of the western neighborhoods were more likely to be white and elderly and less likely to be receiving Medicaid or public assistance, characteristics associated with increased health-care and antibiotic use. Brooklyn residents appear to be at high risk for fluoroquinolone-resistant *S. pneumoniae.* Our results underscore the need for vigilant regional surveillance.

Streptococcus pneumoniae with reduced susceptibility to penicillin has become common in many areas, including Europe (1–3) and North America (4–8). In 1995, 20% of bloodstream isolates in New York City were nonsusceptible to penicillin (9), as were 29% from the northeast United States from 1996 to 1997 (6). In a national survey from 1995 to 1998 involving >4,000 isolates, 24% were resistant to penicillin (10). The Southeast has recorded the highest resistance rates (approximately one third of isolates) (10). Pneumococcal isolates from nonsterile sites (1,3,9) and from children (3,5,8) tend to be more resistant. In addition, recent receipt of antibiotics has been consistently recognized as a risk factor for having resistant *S. pneumoniae* (3,11–15). Many isolates resistant to penicillin are also resistant to other antimicrobial drugs (1,8,16), and therapeutic options have become quite limited. In this report, we describe the resistance rates and epidemiology of *S. pneumoniae* in the borough of Brooklyn, New York.

Methods

Isolates of *S. pneumoniae* were collected during two boroughwide surveillance efforts conducted in 1997 and 1999. Consecutive single patient isolates were collected from the microbiology laboratories of 16 major hospitals in Brooklyn. Serotypes were determined by a quellung reaction. All susceptibility tests were performed in the research laboratory of the investigators. Isolates collected in 1997 underwent susceptibility testing by the broth microdilution technique with Mueller-Hinton broth containing 4% lysed horse blood, according to the National Committee for Clinical Laboratory Standards (NCCLS) methods (17). Fluoroquinolone MICs of the 1997 isolates were confirmed by E-test, as were all susceptibility tests performed with the 1999 isolates. Susceptibility breakpoints were defined according to NCCLS standards (17) or the manufacturer's recommendations. An isolate was considered resistant if it had either intermediate or high-level resistance to an antibiotic.

Iolates of *S. pneumoniae* were fingerprinted with contour-clamp homogeneous electric-field (CHEF) electrophoresis, according to established methods (18). Genomic DNA in agarose plugs was digested with *Sma*1 and placed into 1% agarose gel. Electrophoresis was performed on a CHEF DR II apparatus (Bio-Rad Laboratories, Hercules, CA) with a pulse time of 2 to 30 seconds for 22 hours at 14°C. The gel was then stained with ethidium bromide. Strains were considered identical if they shared every band, closely related if they differed by one to three bands, possibly related if they differed by four to six bands, and unrelated if they differed by seven or more bands (19). Isolates were compared with previously identified clones collected from North America (20).

1990 census data were used to determine boundaries and demographic information for the Brooklyn neighborhoods (21). Categorical data were compared by using chi-square analysis or Fisher's exact test. This study was approved by the Institutional Review Board at the State University of New York Downstate Medical Center.

Results

One hundred thirty-eight isolates of *S. pneumoniae* were collected, 81 from 1997 and 57 from 1999. For the isolates for which clinical data were available, 56% were isolated from blood cultures and 41% from respiratory tract cultures; 68%

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were from adults and 32% from children. Overall, the percentage of isolates susceptible to penicillin was 67% (Table) and was similar for the two collection periods. Four isolates (3%) had penicillin MICs of 4 μg/mL. Resistance to erythromycin was detected in 25%. All the isolates were susceptible to vancomycin, quinupristin-dalfopristin, and linezolid; 34% were susceptible to ciprofloxacin, and 23% had MICs of ≥4 μg/mL. The ciprofloxacin-susceptibility rate decreased sharply from 1997 to 1999 (47% to 16%, p=0.003). Five isolates (3.6%) were resistant to levofloxacin. Two of these five isolates were also highly resistant to gatifloxacin and moxifloxacin. All but one of the penicillin-resistant isolates belonged to serotypes present in the seven-valent conjugate vaccine (9V 11%; 14 11%; 19F 36%; 23F 21%; and 6B 18%).

Fifty-two isolates were characterized by pulsed-field gel electrophoresis (PFGE), including 35 isolates resistant to penicillin. Twenty-one clones were recognized, including the Spanish/USA 23F clone (8 isolates) and the Spanish/French 9/14 clone (11 isolates). All but one of the Spanish/USA 23F clones were resistant to erythromycin, compared with only two isolates of the Spanish/French 9/14 clone. Two other clones previously recognized in North America (20) were also recovered; they contained eight and four penicillin-resistant isolates, respectively. The remaining 21 isolates, largely penicillin-susceptible, belonged to 17 clones consisting of 1 or 2 isolates.

Isolates that were nonsusceptible to ciprofloxacin were distributed among the different PFGE types. Of 39 isolates examined, 4 belonged to the Spanish/USA 23F clone, 11 to the Spanish/French 9/14 clone, and 6 to another clone. The remaining 18 isolates belonged to smaller clones or were unique isolates; 11 of these isolates were susceptible to penicillin. Of the levofloxacin-resistant isolates, one belonged to the Spanish/USA 23F clone, one to the Spanish/French 9/14, and two to a third clone; one isolate was not studied. All these isolates were penicillin resistant.

The penicillin-susceptibility rates in Brooklyn neighborhoods were also examined. The four western neighborhoods had lower rates of susceptibility to penicillin than the eastern neighborhoods (57% versus 75%, p=0.046). Significantly more penicillin-resistant isolates in the eastern neighborhoods belonged to the Spanish/French 9/14 clone (42% versus 7%, p=0.047), and more isolates in the western neighborhoods belonged to the Spanish/USA 23F clone (27% versus 5%, p=0.14). Compared with Brooklyn's east side, the population in the four western neighborhoods was more likely to be white (70% versus 36.8%, p<0.001) and elderly (14.3% versus 11.7%, p<0.001) and less likely to be receiving Medicaid (20.8% versus 28.5%, p<0.001) or public assistance (11.8% versus 18.3%, p<0.001).

Conclusion

Our results underscore the need for regional (or even neighborhood) surveillance of community pathogens. Brooklyn's boroughwide rate of penicillin-susceptible *S. pneumoniae* (67%) is comparable with reports in the United States, including the Northeast (6,10,22). However, in Brooklyn's western neighborhoods, just over half of isolates were susceptible to penicillin. A disconcerting rate of reduced susceptibility to fluoroquinolones was also noted. We found a marked decrease in the susceptibility rates for ciprofloxacin over a relatively short time period (from 47% in 1997 to 16% in 1999); 3.6% of all isolates were resistant to levofloxacin. Although susceptibility rates of 50% to 75% have been reported for ciprofloxacin (22,23), isolates frankly resistant (MIC ≥4 μg/mL) to ciprofloxacin or resistant to newer fluoroquinolones have been uncommon. Less than 10% of Canadian and U. S. isolates are reportedly resistant to ciprofloxacin (22–24), compared with 23% in our study. Levofloxacin resistance was found in 7 of 4,013 isolates collected nationally (10); we recovered 5 levofloxacin-resistant strains from our collection of 138 isolates.

Brooklyn is apparently at particular risk for the emergence of highly fluoroquinolone-resistant *S. pneumoniae*. Because few of our isolates were highly resistant to penicillin (MICs ≥4 μg/mL), β-lactam antibiotics remain a preferred option (11)

Table. Antibiotic susceptibility rates of 138 *Streptococcus pneumoniae* isolates

Antibiotic	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Range (mg/mL)	Susceptible (%)	Intermediate (%)	Resistant (%)
Penicillin	0.03	2	0.008-4.0	67	16	17
Ceftriaxone	0.015	1	0.004-2.0	87	11	2
Cefepime	0.06	1	0.03-4.0	81	12	7
Meropenem	0.015	0.5	0.004-2.0	82	14	4
Erythromycin	0.125	8	≤0.015->256.0	75	2	23
Clindamycin	0.125	0.5	0.03->256.0	93	1	6
Ciprofloxacin	2	4	0.5->32.0	34	43	23
Levofloxacin	1	2	0.5->8.0	96.4	2.2	1.4
Chloramphenicol	4	4	≤ 0.5-64.0	90		10
Linezolid	0.5	1	≤ 0.125-2.0	100		
Quinupristindalfopristin	0.5	0.5	0.125-1.0	100		

for treating community-acquired pneumonia in the area. Clearly, local surveillance is essential to aid clinicians in making therapeutic decisions.

The epidemiology of resistant community pathogens can be complex and related to several factors, including prior antibiotic exposure (3,12–15) and access to health care. Our results are in agreement with others (5,16) in showing that certain demographic groups are at higher risk of acquiring resistant bacteria, possibly secondary to increased antibiotic use (13,15,25). The population of Brooklyn's west side, which has the demographic characteristics of a population that uses high amounts of antibiotics, had penicillin-resistance rates that reached 50%. Our molecular epidemiology studies showed that the Spanish/USA 23F clone, along with two other North American clones, predominated in the western half of the borough. The Spanish/USA 23F clone is to known to be more resistant to other classes of antibiotics, including macrolides (20). In contrast, the Spanish/French 9/14 clone predominated in the east side. Most of these strains remain susceptible to macrolides (20), as were the isolates in our study.

Strategies to limit the spread of resistant *S. pneumoniae* include improved surveillance, reduced antibiotic usage, and greater vaccination of persons at high risk (5,16,26–28). Educational efforts aimed at both health-care providers and those at higher risk are needed to reduce inappropriate antibiotic usage. Aggressive surveillance measures are especially needed in Brooklyn to monitor the emergence of highly fluoroquinolone-resistant *S. pneumoniae*.

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Mycobacterium tuberculosis: An Emerging Disease of Free-Ranging Wildlife

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Expansion of ecotourism-based industries, changes in land-use practices, and escalating competition for resources have increased contact between free-ranging wildlife and humans. Although human presence in wildlife areas may provide an important economic benefit through ecotourism, exposure to human pathogens may represent a health risk for wildlife. This report is the first to document introduction of a primary human pathogen into free-ranging wildlife. We describe outbreaks of *Mycobacterium tuberculosis*, a human pathogen, in free-ranging banded mongooses *(Mungos mungo)* in Botswana and suricates *(Suricata suricatta)* in South Africa. Wildlife managers and scientists must address the potential threat that humans pose to the health of free-ranging wildlife.

Tuberculosis (TB), considered an important emerging disease in humans, is now the leading cause of death in adults worldwide (1). Although *Mycobacterium tuberculosis* is the most common infection in humans, *M. bovis* is responsible for an increasing proportion of human TB cases (1). *M. bovis* is widespread in domestic animals and has been extensively documented in both captive and free-ranging wildlife populations (2). A number of wildlife populations are endemically infected, for example, the European badger (*Meles meles*) in the United Kingdom (3) and the African buffalo (*Syncerus caffer*) in South Africa (2). These permanent reservoirs of infection pose a serious threat to public health and TB eradication programs. In contrast, *M. tuberculosis* is considered primarily a human pathogen and has been reported only in domestic or wildlife species living in close, prolonged contact with humans (4,5). We describe the first documented outbreak of *M. tuberculosis* in free-ranging wildlife and discuss the implications for ecotourism and wildlife health.

Material and Methods

Twelve troops of free-living suricates were monitored daily by behavioral ecologists from the universities of Cambridge and Pretoria (6). The groups occupied ranges on uncultivated ranch land along the dry bed of the Kuruman River in the southern Kalahari Desert in Botswana (S 25° 8′, E 20° 49′). Complete information on outbreak features was not available. One animal was captured and euthanized for postmortem examination.

An epizootic affecting banded mongooses (*Mungos mungo*) was first identified at the northern extreme of Chobe National Park along the Chobe River in the dry season, from June 13 to September 15, 1999 (S 17° 49.33′, E 25° 07.58′). To monitor the progression of the outbreak, morning and evening patrols were conducted along the range occupied by the respective troops, and all observations, geographic features of importance, and affected animals were georeferenced**.** Surveillance was thought to be comprehensive and not biased in terms of road systems, as the patrolled roads were parallel to the river (Figure) and the animals had no other water source during the dry season. Thus, all known troops lived along the watercourse in the floodplain rather than inland in the woodland. All infected animals were euthanized whenever possible, and postmortem examinations were conducted. The rate of identification of new clinical cases from the point of outbreak, where the first case was identified, into Kasane Township and the National Park was calculated as the average time (days) between identification of each new case divided by the distance (kilometers) between cases.

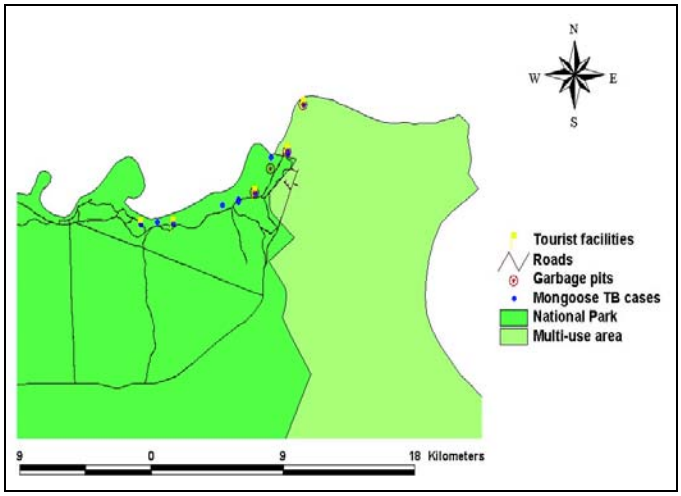

Figure**.** Locations of *Mycobacterium tuberculosis*-infected or suspected cases in banded mongooses, in relation to garbage pits, tourist facilities, and land use type, Chobe District, Botswana.

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Histopathologic and Bacteriologic Testing and Polymerase Chain Reaction Amplification

Various organs and tissues from seven banded mongooses and a suricate were fixed in 10% buffered Formol-saline, embedded in paraffin wax, cut into 4-µm sections, and stained with hematoxylin and eosin, as well as Kinyoun's method for acid-fast bacteria (AFIP modification) (7).

Pooled organ specimens from one banded mongoose (No. 2075) and one suricate (No. VLF005) and liver samples from another banded mongoose (No. 2344) were homogenized, and equal parts were decontaminated with either 2% HCl or 4% NaOH. After neutralization, sediments were placed on two slants of Löwenstein-Jensen (LJ) medium containing 0.5% pyruvate and one slant containing glycerine. The cultures were incubated at 37°C and checked weekly for growth. Acid-fast culture isolates were subjected to polymerase chain reaction (PCR) specific for *M. tuberculosis*-complex organisms (8) and subcultured onto the same type of medium for identification by growth characteristics and standard biochemical tests. PCR-positive *Mycobacterium* isolates were subjected to a second PCR test to differentiate *M. bovis* and *M. tuberculosis* (9).

Results

Epidemiologic Features

In the suricates, the epizootic occurred from October 1998 to December 1999, after an unknown infected male joined a study group consisting of 5 adult suricates and 15 pups. One human case of TB was known to have occurred in the study area in the human population living near the suricate burrows. Suricates were not observed feeding in garbage pits; however, they were seen foraging around roads and investigating human sputum.

In the banded mongooses, the epizootic spread quickly in six troops living along the Chobe River Front. New clinical cases were identified from the initial outbreak site at an average rate of 0.109 km/day into Chobe National Park and the township at an average rate of 0.282 km/day. Although the last case was observed on September 15, monitoring continued until January 21, 2001; no new cases were identified. Garbage pits and a known human TB case were in close proximity to initial outbreak points (Figure). Banded mongooses were observed feeding regularly at these garbage pits and would therefore be exposed to human excretions and any infectious material from TB-infected humans.

Clinical Signs and Gross Pathologic Findings

The first suricate noted to have clinical signs had enlarged cervical lymph nodes when it emigrated to the study site. The lymph nodes ruptured within a month and continued to discharge pus for 2 months (November–December 1998). By January 1999 the lesion had become a persistent nonhealing wound. The animal became progressively more debilitated and cachectic until he disappeared from the study site in April 1999. In June, clinical signs first appeared in other animals and then spread through the entire troop by December 1999. The signs included emaciation, weakness, and dyspnea with variable enlargement of the lymph nodes of the head, neck, and axilla. All troop members were euthanized, died, or disappeared and were presumed dead. Gross postmortem examination of one suricate showed abscesses filled with yellow fluid in the left parotid (3 cm in diameter), right axilla (1 cm), lung, liver, and mediastinal lymph nodes (5 mm), and pancreas (8 mm). The lungs were congested and edematous.

The mongoose troop size ranged from 8 to 35; in each troop, 2 to 4 animals had clinical signs. Affected animals were often found separated from the troop during periods of foraging. In some cases, although alert, they showed a pronounced lack of fear response to humans. Affected animals that were not euthanized disappeared within a few days after onset of illness and were presumed dead. Clinical signs included progressive cachexia, ataxia, and weakness. No other external abnormalities were noted. Necropsies of seven animals from five troops showed numerous miliary grayish white nodular masses (0.5 cm–2 cm in diameter) scattered over the liver and spleen surfaces, causing massive enlargement of the two organs; numerous grayish white infiltrative masses in the lung and kidney; enlargement of the mesenteric lymph nodes, which had necrotic gritty centers with chalky material; and occasional grayish white foci scattered throughout the length of the intestines.

Histopathologic Findings

Granulomas of varying size, predominantly consisting of aggregated epithelioid macrophages, were found in most of the organs and tissues examined and were consistently present in the liver, spleen, lymph nodes, and lungs. Such granulomas were occasionally noted in the adrenal gland, kidney, myocardium, pancreas, epididymus, pleura, intestine, peritoneum, and skin. Lesions were absent from the brain, skeletal muscle, urinary bladder, and testis. The smaller granulomas consisted purely of macrophages, while large ones showed central necrosis and sometimes contained small aggregates of lymphocytes and plasma cells. Giant cells were rare, and no calcification was seen. Acid-fast rods, typical of *Mycobacterium* species, were noted in the cytoplasm of macrophages in all eight mongooses but varied in numbers from scarce to abundant.

Bacteriologic and PCR Amplification Results

Acid-fast bacteria were detected on Ziehl-Neelsen–stained impression smears, and *Mycobacterium* species were isolated from both banded mongooses and the suricate specimens. For material from animal No. 2075, growth first appeared after 2 weeks on LJ slants with and without pyruvate. LJ-pyruvate cultures of the specimens from mongoose No. 2344 and suricate No. VLF005 yielded very few acid-fast colonies after a 5- to 6-week incubation period. Following PCR amplification, the *Mycobacterium* sp. isolated from mongoose No. 2344 and the suricate produced a 372-bp DNA product. Both isolates

showed a 336-bp DNA product, characteristic for *M. tuberculosis* when amplified by the protocol of de Wit et al. (9). This method had been found useful in differentiating isolates of *M. bovis* and *M. tuberculosis* (A. Michel, unpub. data). The isolates produced positive results in both the niacin production and nitrate reduction tests, confirming them as *M. tuberculosis*. A subculture of an isolate from banded mongoose No. 2075 was classified as a fast-growing *Mycobacterium* species after it had shown growth at 27°C and 37°C at 7 and 5 days, respectively. No growth was observed at 45°C.

Discussion

Because the lesions in the mongooses and the suricate were disseminated, the route of infection is not clear. However, an oral route of infection is suspected because the pulmonary lesions involved the interstitium and alveolar walls rather than the bronchioles, and mesenteric but not pulmonary lymph nodes were enlarged (banded mongoose). The behavioral pattern of both species would have facilitated exposure to human excretions in the environment and therefore to *M. tuberculosis* from any TB-infected humans.

Ecologic, environmental, and demographic factors influence the emergence of disease (10). TB incidence is increasing rapidly throughout the world with most cases in developing countries. In 1996, the Western Cape of South Africa had one of the highest incidences of human TB in the world (11). In Botswana, where most people are likely to have been infected with TB by adulthood (12), the TB infection rate in humans increased from 202 per 100,000 in 1989 (13) to 537 per 100,000 in 1999 (14). Concurrent HIV infection may shorten the time for TB infection to progress to overt disease, leading to increased severity of clinical signs and amount of Mycobacteria shed into the environment (15). In 1999, HIV sentinel surveillance in Botswana indicated that 36% of women receiving routine antenatal care were seropositive for HIV (16). In addition, concurrent helminthic infections may decrease the host immune response to TB, leading to reactivation of latent TB infections in humans and possibly increasing the level of TB in a community (17). Research is urgently required to better understand the epidemiology of *M. tuberculosis* in free-ranging wildlife and their potential to maintain infection in the absence of human reservoirs. Another factor influencing disease emergence is the dramatic increase in travel. In 1999, >89,000 visitors were recorded in Chobe National Park (18). Changes in the health, mobility, and number of humans in the vicinity of wildlife may have led to an increased level of *M. tuberculosis* being shed into the environment, resulting in this spillover of infection into a wildlife population.

Expansion of ecotourism, changes in land-use practices, and escalating competition for resources has increased contact between free-ranging wildlife and humans. Tremendous attention has been given to the zoonotic potential of emerging diseases in wildlife populations and the threat they present to human health. Little attention, however, has been given to the reverse: the disease threat humans present to wildlife. A number of reports have suggested links between pathogen occurrence in wildlife populations and human exposure, but the diagnoses were not confirmed and proof of transmission was lacking (19). In other cases involving macroparasites, the pathogens have been found in a number of domestic or free-ranging wildlife hosts, complicating transmission routes and links to human reservoirs (20,21). This report, however, represents the first clearly documented case of a primary human pathogen infecting free-ranging wildlife. The report underscores the need to heighten awareness of humans as a potential reservoir of disease for wildlife and the role humans may play in the emergence of infectious disease in wildlife populations. This understanding will be essential for developing effective programs for public and wildlife health.

Ecotourism brings large numbers of people to wildlife areas and provides both important economic benefits and an instrument for the conservation of biodiversity. However, susceptible wildlife populations may be negatively affected by the increased exposure to humans and their pathogens. A better understanding of the dynamics of disease transmission between humans and wildlife is critical, and mechanisms must be identified that limit wildlife exposure to human pathogens. Attention to this area of wildlife management is essential to the long-term conservation and sustainable use of wildlife resources.

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Community-Acquired Methicillin-Resistant *Staphylococcus aureus*, Finland

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is no longer only hospital acquired. MRSA is defined as community acquired if the MRSA-positive specimen was obtained outside hospital settings or within 2 days of hospital admission, and if it was from a person who had not been hospitalized within 2 years before the date of MRSA isolation. To estimate the proportion of community-acquired MRSA, we analyzed previous hospitalizations for all MRSA-positive persons in Finland from1997 to 1999 by using data from the National Hospital Discharge Register. Of 526 MRSA-positive persons, 21% had community-acquired MRSA. Three MRSA strains identified by phage typing, pulsed-field gel electrophoresis, and ribotyping were associated with community acquisition. None of the strains were multiresistant, and all showed an *mec* hypervariable region hybridization pattern A (HVR type A). None of the epidemic multiresistant hospital strains were prevalent in nonhospitalized persons. Our population-based data suggest that community-acquired MRSA may also arise de novo, through horizontal acquisition of the *mecA* gene.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of nosocomial infections worldwide. Recent studies suggest that the epidemiology of MRSA may be changing, as the isolation of MRSA is no longer limited to hospitalized patients or persons with predisposing risk factors (1–4). However, the prevalence of MRSA colonization in healthy persons in the community has been shown to be low, even when MRSA is highly endemic in hospital settings (5).

Nosocomial MRSA strains in the community, including nursing homes and other nonacute-care facilities, may be transmitted by discharged patients and health-care workers (6,7). Whether strains of MRSA in the community also arise de novo, as a consequence of horizontal acquisition of the *mecA* gene, is unclear. The transfer of *mecA* DNA to a susceptible *S. aureus* strain has occurred in vitro (8) and recently in a hospitalized patient during antibiotic treatment (9). The mechanism of transfer of *mec* DNA from a donor to a recipient is not completely understood. However, the excision and integration of the *mec* DNA from and to the chromosome are apparently catalyzed by cassette chromosome recombinases A and B (Ccr A and B) coded by *mec*-associated genes (*ccrA* and *B*), with homology to the invertase-resolvase family of DNA recombinases (10).

In Finland, the prevalence of MRSA has remained low, although several hospital epidemics have occurred in the last decade (11). We recently recognized two distinct groups of MRSA, one representing multiresistant epidemic strains and the other only β-lactam-resistant strains. These two groups also showed differences in ribotypes and *mec* determinant profiles (12). The aim of this study was to estimate the proportion of community-acquired MRSA by analyzing the hospital contacts of persons from whom MRSA was found from 1997 to 1999. We also compared the MRSA isolates in persons with and without hospital contact in terms of strain type (determined by phage typing, pulsed-field gel electrophoresis [PFGE], and ribotyping), antibiotic resistance, and *mec* determinant profile.

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Materials and Methods

Surveillance and Typing Scheme of MRSA

Finnish microbiology laboratories report (generally electronically) all MRSA isolations to the National Infectious Disease Register at the National Public Health Institute (KTL). The KTL records the date, source of specimen, and the patient's birth date, sex, and place of treatment. Using this information and a time interval of 36 months, multiple isolations from the same person are deleted from the database. The microbiology laboratories also send the MRSA isolates to the Laboratory of Hospital Bacteriology at KTL for further analysis. Phage typing, PFGE, and antimicrobial drug susceptibility testing were performed as described (11). In brief, phage typing was performed with the universal set of phages (13) at 1x and 100x routine test dilutions, both with and without heat treatment of bacteria (14). Antimicrobial drug susceptibilities were tested by the disk diffusion method according to guidelines recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The antibiotics tested were oxacillin, ampicillin, penicillin, cephalexin, cefuroxime, gentamicin, tobramycin, erythromycin, clindamycin, chloramphenicol, ciprofloxacin, rifampicin, fusidic acid, mupirocin, and vancomycin. MICs of oxacillin were determined by E-test (AB Biodisk, Solna, Sweden) according to manufacturer's instructions. If the oxacillin MIC was $\leq$64 μg/mL, methicillin resistance was verified with the MRSA screen test (Denka Seiken, Japan) or *mecA*-polymerase chain reaction (15). For PFGE, genomic DNA prepared in agarose blocks was digested with *Sma*I restriction endonuclease, and chromosomal

fragments were separated with a Chef DR III (Bio-Rad Laboratories, Hercules, CA) for 24 hours with initial and final switching times of 10 seconds and 60 seconds, respectively. PFGE profiles differing by fewer than four bands were interpreted as identical or closely related (16). Bionumerics software, with Dice coefficient and unweighted pair group matching average for clustering, and optimization and tolerance values of 1% and 1%–2%, respectively, were used to verify the relatedness of PFGE profiles. MRSA isolates sharing identical or closely related PFGE profiles and phage types were considered to be the same strain type. If the PFGE profiles were related, but the phage types were different, ribotyping with one to three restriction enzymes (*Hin*dIII, *Eco*RI, *Cla*I) was performed to verify the relatedness of isolates (11). Riboprofiles with fewer than four bands' difference were regarded as identical or closely related. The genomic variation within the *mec* determinant hypervariable region (HVR) was analyzed for a subset of MRSA isolates as described (12). In brief, the two probes which were prepared from plasmid pBBB30 (17) recognize the mec hypervariable region. The probes are hybridized with genomic bacterial DNA which is first digested with EcoRI and BglII.

Study Population, MRSA Isolates, and Definitions

All MRSA-positive persons in Finland from 1997 to 1999 and their MRSA isolates (one from each) were included in the study. A sporadic strain was defined as a strain type isolated from one person only. An MRSA isolate was defined as hospital acquired if the MRSA-positive specimen was obtained 2 days after hospital admission, and for discharged patients, if the patient had been hospitalized within 2 years before the date of MRSA isolation.

Previous Contacts with a Health-Care Facility

National identity codes for each person with an MRSA-positive culture were obtained either through the primary diagnostic laboratory or from the infection control nurse of the health-care facility. Based on the first isolation date of MRSA and the national identity code, data on previous hospitalizations within 2 years before the MRSA isolation date were retrieved from the National Hospital Discharge Register (HILMO). The HILMO is a civil register comprising comprehensive health-care records, provided by all hospitals and health-care centers in Finland, including outpatient surgery. Each report to the register includes patient identity information, admission and discharge dates, a code of the health-care provider, type of service, specialty, the place (home or institution) from which the patient came to the institution, and data on surgical procedures.

Additional background information was collected for persons for whom no HILMO reports could be found by sending questionnaires to infection-control nurses at relevant health-care facilities. The information collected included 1) whether the MRSA-positive person was a patient or a staff member, 2) whether the specimen was taken on clinical or screening basis, and 3) whether the screening sample was taken because of a hospital contact abroad or because of an epidemic situation.

Statistical Analysis and Ethical Aspects

For categorical variables, proportions were compared by the chi-square test with Yates correction or Fisher's exact test, as appropriate. The means and medians of the continuous variables were compared by the Student's *t* test or Mann-Whitney *U* test, depending on the sample distribution.

With approval from the Ministry of Social Affairs and Health and the Finnish data protection authority, the National Research and Development Centre for Welfare and Health gave permission to use the data from the HILMO register.

Results

From 1997 to 1999, 520 MRSA isolations were reported to the National Infectious Disease Register; the annual incidence ranged from 2.3 to 4.1/100,000 persons. The Laboratory of Hospital Bacteriology received MRSA isolates from 529 persons. Three persons did not have a Finnish national identity code and were excluded from the study. The median age of the 526 persons was 51 years (range <1–96), and 291 (55%) were male.

Contacts with Health-Care Facilities

Of the 526 MRSA-positive persons, 108 (21%) did not have any verified link to health-care facilities 2 years before the MRSA isolation date, including 17 persons whose MRSA was isolated within 2 days of hospital admission. Their MRSA isolates were classified as community acquired. BEcause of signs of infection or clinical findings, specimens were taken from 69 persons and, 37 persons were specifically screened for MRSA. Of those 37, 21 had a known MRSA contact in their family, 13 were otherwise exposed to a known MRSA carrier, and 3 were known to have carried MRSA previously.

The HILMO register and the questionnaire survey showed 418 (79%) persons who had at least one connection to a hospital, and their MRSA isolates were classified as hospital acquired. According to the HILMO register, 376 persons were hospitalized in Finnish hospitals within 2 years before the MRSA isolation date: 156 hospitalized patients with MRSA isolated 2 days after hospital admission and 220 discharged patients. The time frame between the MRSA isolation date and the previous hospitalization for the discharged patients was <6 months for 156 (71%) patients, 6 to 12 months for 36 (16%), and >12 months for 28 (13%) patients. The questionnaire survey identified 42 additional persons who had an obvious contact with a hospital, including 23 staff members (12 working in a Finnish health-care facility and 11 who had recently worked abroad) and 19 patients, 15 of whom had recently been hospitalized outside Finland.

The median age of the persons who did not have a contact with a health-care facility was lower than that of persons who had contact (34 vs. 58 years, $p<0.01$). The proportion of children <15 years of age was higher in community-acquired than

in hospital-acquired MRSA strains (27 [25%] of 108 vs. 31 [7%] of 418, p<0.01).

Strain Types

Among the 526 MRSA isolates, our typing scheme showed 84 strain types, 56 (67%) of which were sporadic and 28 (33%) shared by at least two persons. The distribution of sporadic (in total 56 [11%] of 526) and shared (in total 470 [89%] of 526) strain types was similar in persons with and without connections to health-care facilities (Table 1).

Fourteen strain types, each of which were isolated from ≥10 persons, represented 421 (80%) of 526 MRSA isolations (Table 2, Figure). Three of the 14 most common strain types were more likely to be found in persons who did not have a contact with a health-care facility than in those who had such a contact: Mikkeli clone (41 [38%] of 108 vs. 75 [18%] of 418, p<0.01), E31 (10 [9%] of 108 vs. 6 [1%] of 418, p<0.01), and E22 (8 [7%] of 108 vs. 7 [2%] of 418, p<0.01).

Of all strains isolated from persons who had no hospital contact, 94% were nonmultiresistant. In addition, of the 14 most common strain types, all 7 nonmultiresistant strains, but none of the multiresistant strains, showed HVR type A (Table 2). Of the 56 sporadic MRSA strains, 41% were nonmultiresistant, including all but one of the strains isolated from persons without connections to hospitals (Table 1).

Table 1. Distribution of sporadic strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and strains shared by at least two persons and contact with a health-care facility, Finland

	Persons with hospital contact (%)	Persons without hospital contact (%)
	n=418	n=108
Sporadic strain types	43 (10)	13 (12)
Strain types shared by at least two persons	375 (90)	95 (88)

Discussion

Our population-based study showed that from 1997 to 1999 one fifth of all Finnish MRSA isolates came from persons who had no connection to health-care facilities, suggesting that these MRSA isolates may be community acquired. Three strain types identified by phage typing, PFGE, and ribotyping were associated with community acquisition, and none of these strain types were multiresistant.

To our knowledge, this is the first report of community-acquired MRSA on the national level. Previous reports have focused on single health-care institutions or certain restricted areas (1,3,6,12,18-20). Both data sources used in our study, the surveillance and typing scheme of MRSA and the hospital discharge register, were nationwide (20). The availability of national identity codes allowed us to link the two data sources and to study one MRSA isolate per person. The number of isolates routinely typed and verified as MRSA was equal to that of MRSA isolations reported to the National Infectious Disease Register, suggesting that the isolates of MRSA available for typing were nationally representative.

Community-acquired MRSA can be classified into the following categories: discharged hospital patients with MRSA, nursing-home residents with MRSA, MRSA transmitted to nonhospitalized patients, and MRSA arising de novo in the community (7). The first three categories include MRSA isolates of health-care facility origin, which are thought to represent a limited number of different genotypes, disseminate clonally, and express resistance to multiple antibiotics (1,21–23). De novo MRSA strains, in contrast, are thought to arise through acquisition of *mec* DNA into a previously susceptible *S. aureus* genotype (7,9,24). Our study focused on the last two categories. We first identified nonhospitalized MRSA-positive persons and thereafter compared their strains with those found in hospitalized patients.

Based on our study, the proportion (21%) of community-acquired MRSA was relatively high in Finland. However, the definition of community-acquired MRSA is not straightforward. The definition classically includes MRSA isolated outside hospital settings or from a patient within 48 to 72 hours of hospital admission. Because of the long-term persistence of MRSA colonization (25), contacts with hospitals or nursing homes before MRSA isolation should also be taken into account (6,19,26). Therefore, our definition of community acquisition covered a 2-year time period without a health-care facility contact before the MRSA isolation. If the cut-off period had been 1 year, the proportion of community-acquired isolates would have been 26%. If the questionnaire survey on the additional background data had not been performed, the proportion would have been 29%. Although this questionnaire survey was not comprehensive, it allowed us to characterize the persons who had no reports in the discharge register and to identify persons with a foreign or ongoing hospital contact.

Most community-acquired MRSA strains were nonmultiresistant, and children were more likely to have a community- than a hospital-acquired MRSA. These findings agree with those of previous reports, suggesting that nonmultiresistant MRSA is emerging as an important pathogen in the community (1,18,22,23). The majority (64%) of community-acquired strains were isolated on a clinical basis. However, one third of all community-acquired strains were isolated because of screening of persons exposed to a known MRSA carrier, most of whom were family members. Most exposed persons had the same strain type as their contacts (data not shown).

Among the 14 most common strain types, which represent 80% of all MRSA isolates, we identified 7 nonmultiresistant strain types showing a hypervariable region hybridization pattern A. Three of these strain types were associated with community acquisition and represented more than half of all community-acquired strains. In addition, three other strain types were frequently found in persons without connections to hospitals. The only exception was the internationally recognized UK EMRSA-15 (27), which was isolated mainly from patients or health-care workers who had recently returned from hospitals abroad. In contrast, the multiresistant strain types, including the UK EMRSA-16 (28) and the Iberian clone

Table 2. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains found in ≥10 persons in relation to contact with a health-care facility, 1997–1999, Finland[a]

Strain type	Persons with hospital contact (%)	Persons without hospital contact (%)	Total	Multiresistance[b]	HVR type	Yr first identified in Finland
Mikkeli clone (O11, E12)	75 (65)	41 (35)	116	No	A	1993
E1	50 (98)	1 (2)	51	Yes	D	1992
E24	49 (98)	1 (2)	50	Yes	C	1998
E5 (UK EMRSA 16)	36 (100)	0 (0)	36	Yes	C	1995
Kemi clone	25 (71)	10 (29)	35	No	A	1996
E27	17 (65)	9 (35)	26	No	A	1997
E31	6 (38)	10 (63)	16	No	A	1997
E22	7 (47)	8 (53)	15	No	A	1997
UK EMRSA-15	13 (93)	1 (7)	14	No	A	1997
E19	13 (100)	0 (0)	13	Yes	C	1997
Pori clone (O15)	8 (62)	5 (38)	13	No	A	1993
E20	12 (100)	0 (0)	12	Yes	C	1998
Iberian clone (E6, E7, E10, O8)	11 (92)	1 (8)	12	Yes	B	1991
O25	12 (100)	0 (0)	12	Yes	B	1997

[a]HVR, hypervariable region; E, epidemic, strain isolated in more than one health-care facility; O, outbreak, strain isolated in one health-care institute.
[b]Resistance to more than three antibiotic groups in addition to β-lactams.

(29), were almost exclusively found in persons who had contacts with hospitals.

Despite the fact that the HVR type A seems to coincide with nonmultiresistance, the hypervariable region itself has not been shown to contain any antibiotic resistance markers (17,30,31). Some of the multiresistant strains actually have deletions in this area (31). The hypervariable region has been analyzed by polymerase chain reaction and sequencing to distinguish different *mec* DNA types (32,33). The method identified five different subclones in 50 isolates representing one epidemic strain in Germany (33).

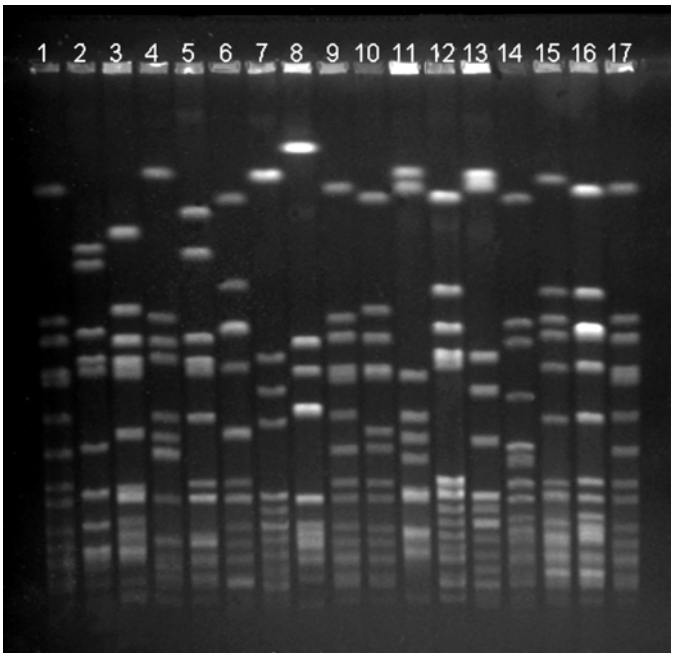

Figure. Pulsed-field gel electrophoresis (PFGE) profiles of the 14 most common methicillin-resistant *Staphylococcus aureus* (MRSA) strain types identified, Finland, 1997–1999. Lanes 1, 9, 17: *S. aureus* NCTC 8325 (molecular weight marker); lanes 2–4: strain types associated with community acquisition (Mikkeli clone, E22, E31); lane 5: E1; lane 6: E24; lane 7: E5; lane 8: Kemi clone; lane 10: E27; lane 11: UK EMRSA-15; lane 12: E19; lane 13: Pori clone; lane 14: E20; lane 15: Iberian clone; and lane 16: O25.

Our study had several limitations. First, some of the MRSA isolates classified as community acquired may have been isolated from nursing-home residents, since not all Finnish nursing homes report to the National Discharge Register. The possibility of misclassification concerns a small number of isolates, since only 10 of all persons with community-acquired MRSA were >64 years of age. Second, the differences in local sampling policies may affect the number and type of community-acquired MRSA identified. National guidelines for MRSA prevention in Finland are primarily directed to hospital use, and sampling and screening policies in community setting are not specified. Third, we did not gather clinical data and risk factors (6,34,35) other than previous hospital stays for MRSA acquisition. Further information should be collected from persons with community-acquired MRSA to develop a hypothesis on risk factors specific for community acquisition.

In conclusion, a large proportion of MRSA-positive persons may have acquired their strains outside the hospital setting, and their MRSA strains were nonmultiresistant, showed an HVR type A, and differed genotypically from epidemic strains found in hospitalized patients. None of the epidemic multiresistant hospital strains were prevalent in nonhospitalized persons. Our findings suggest that MRSA may also

emerge as a community-acquired pathogen as a consequence of horizontal acquisition of the *mecA* gene by a previously susceptible *S. aureus* strain type.

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Neurocysticercosis in Radiographically Imaged Seizure Patients in U.S. Emergency Departments[1]

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Neurocysticercosis appears to be on the rise in the United States, based on immigration patterns and published cases series, including reports of domestic acquisition. We used a collaborative network of U.S. emergency departments to characterize the epidemiology of neurocysticercosis in seizure patients. Data were collected prospectively at 11 university-affiliated, geographically diverse, urban U.S. emergency departments from July 1996 to September 1998. Patients with a seizure who underwent neuroimaging were included. Of the 1,801 patients enrolled in the study, 38 (2.1%) had seizures attributable to neurocysticercosis. The disease was detected in 9 of the 11 sites and was associated with Hispanic ethnicity, immigrant status, and exposure to areas where neurocysticercosis is endemic. This disease appears to be widely distributed and highly prevalent in certain populations (e.g., Hispanic patients) and areas (e.g., Southwest).

Neurocysticercosis is the most common parasitic disease of the central nervous system. It is endemic in many developing countries and has been cited as the primary reason that "epilepsy" is twice as common in these countries as in more industrialized nations such as the United States (1). The prevalence of neurocysticercosis in some of these developing countries exceeds 10% (2,3), where it accounts for up to 50% of cases of late-onset epilepsy (4).

International travel and immigration are bringing neurocysticercosis to areas where it is not endemic. Several case series have been published from a variety of institutions throughout the United States, especially in the Southwest (5–10), but none has directly assessed the prevalence of neurocysticercosis. Domestic acquisition of the disease has been documented not only in large, metropolitan centers that attract large numbers of immigrants but also in less urban areas of North and South Carolina (11). Local acquisition has even been demonstrated in such unlikely areas as an Orthodox Jewish community, where it was attributed to the employment of domestic workers from Central and South American countries (12).

Seizures are the most frequent, and often the only, clinical manifestation of neurocysticercosis; they occur in 70% to 90% of cases (10,13). Because seizure patients frequently go to emergency departments, we chose this setting to perform a prospective study to determine the prevalence and epidemiology of this disease.

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Materials and Methods

This study was a prospective case series of patients who visited any of a network of 11 geographically diverse, university-affiliated, urban emergency departments (*EMERGE*ncy ID NET) from July 1996 to September 1998. The approximate total annual visit census of these emergency departments is 900,000. Institutional review board approval for the study was obtained at all sites. A more detailed description of *EMERGE*ncy ID NET, including its administration and the processes of data transfer and compilation, has been published (14).

Emergency department patients >5 years of age were enrolled in the study if they had a known or suspected seizure and had undergone neuroimaging, either computed tomography scanning (CT) or magnetic resonance imaging (MRI). Patients <5 years of age were excluded to avoid enrolling a

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potentially large number of patients with febrile seizures. The treating physician recorded demographic and clinical data including age, sex, race, immigrant status, foreign travel, prior seizure history, seizure type, CT and MRI findings, presumptive diagnosis, and disposition.

When blood was drawn from a patient as part of the evaluation, an additional tube was obtained for this study. Serum specimens from 890 of the 1,801 patients enrolled were sent to the Centers for Disease Control and Prevention so that serologic testing for cysticercosis could be performed. Serum samples were tested by enzyme-linked immunoelectrotransfer blot for *Taenia solium*–specific antibodies, as described (15,16). Briefly, this assay uses seven purified glycoprotein antigens from larval cysts of *T. solium*, namely, GP50, GP42-39, GP24, GP21, GP18, GP14, and GP13, where the prefix GP stands for glycoprotein and the number indicates the molecular mass in kilodaltons. These antigens are used in an immunoblot format to detect infection-specific antibodies. Reactions to at least one antigen band are considered positive (15,16).

On the basis of a classification scheme proposed by Del Brutto (17), our case definition for neurocysticercosis required either 1) CT scan finding(s) characteristic of neurocysticercosis (i.e., multiple calcifications or multiple cystic lesions) with or without a positive serologic test, or 2) CT scan finding(s) consistent with neurocysticercosis (i.e., a single cystic, calcified, or hypodense lesion) and a positive serologic test. Radiologists at each site read CT scans without regard for or knowledge of the study. Study coordinators at each site then abstracted the absence or presence of findings relevant to the study from the radiology reports. Simple descriptive statistics were used to summarize the clinical features of patients with and without neurocysticercosis. Relative risk ratios (RRs) and their corresponding 95% confidence intervals (CIs) were determined by Fisher's exact test.

Results

A total of 1,801 eligible patients with 1,833 emergency department visits were enrolled during the 2-year study period. Twenty-eight patients had multiple visits; four patients underwent evaluation on three separate occasions (Table 1). A diverse group of seizure patients were enrolled in the study (Table 2).

From the entire study population, 2.1% (38) patients met the case definition for neurocysticercosis (Table 3). Of patients who underwent both CT scanning and serologic testing, 2.9% met the case definition. Thirty-four patients satisfied the case definition based on classic CT scan findings, and four satisfied the case definition based on a positive serologic test coupled with CT scan findings consistent with neurocysticercosis. Neurocysticercosis was identified at 9 of the 11 study sites; 6 sites enrolled more than one patient (Table 3). Patients with this disease tended to be younger than patients who did not meet the case definition (Table 4). Patients were also more likely to be Hispanic, have been born outside the United States, have visited or lived in an endemic region, be uninsured, and have a reported history of neurocysticercosis. Overall, approximately 9% of patients with Hispanic ethnicity who came to an emergency room with a seizure met the case definition for neurocysticercosis. The prevalence of the disease in the Hispanic patients with seizures ranged from 9% to 13.5% in the highest risk sites.

Table 1. Demographic and clinical characteristics of 1,833 neuroimaged emergency department seizure patients[a]

Characteristics	Patients (%)
Sex (male)	1,220 (67)
Race/ethnicity	
Black	753 (41)
White, non-Hispanic	643 (35)
Hispanic	320 (17)
Native American	43 (2.3)
Asian/Pacific Islander	33 (1.8)
Other or unknown	41 (2.2)
Insurance	
Medicare/private	462 (25.2)
Medicaid	391 (21.3)
Uninsured	762 (41.6)
Immigrant status[b]	
Born in USA	820 (61)
Not born in USA	178 (13)
Unknown	350 (26)
Exposure to disease-endemic region	
No travel outside USA	950 (51.8)
Exposure to disease-endemic region	342 (18.7)
Unknown travel history	541 (29.5)
Seizure type	
Generalized tonic/clonic	1,577 (86)
Focal motor	114 (6.2)
Partial complex	86 (4.7)
Unknown or undocumented	56 (3.1)
Seizure history	
Prior seizure history	810 (44)
No prior seizure history	896 (49)
Unknown seizure history	127 (7)

[a]The median age (interquartile range) in yrs for these patients was 40 (range 30–51 yrs).
[b]1,348 patients; immigrant status data was not collected on the first 485 patients.

Patients meeting the case definition for neurocysticercosis were not more likely to have a new-onset seizure (versus having an established history of seizures; RR 1.1; CI 0.56 to 2.01). Neurocysticercosis patients were, however, more likely to have focal motor or partial complex seizures than those without neurocysticercosis (RR 2.6; CI 1.3 to 5.5) (Table 4).

Serologic testing was performed on 49.4% (890) of the 1,801 patients enrolled, and results were positive in 2% (18)

Table 2. Emergency department physicians' diagnoses for 1,348[a] neuroimaged seizure patients

Diagnosis	Seizure patients (%)
Etiology uncertain	515 (38)
Alcohol or drug abuse/withdrawal	253 (19)
Head injury	105 (7.8)
Epilepsy	92 (6.8)
Other[b]	104 (7.7)
Brain tumor	42 (3.1)
Metabolic disorder (e.g., hypoglycemia)	39 (2.9)
Stroke	36 (2.7)
Neurocysticercosis	30 (2.2)
Nontraumatic cerebral hemorrhage	22 (1.6)
Syncope, possibly not seizure	25 (1.9)
Meningitis or brain abscess	18 (1.3)
Pseudoseizure	14 (1.0)
Toxoplasmosis	12 (0.9)
No diagnosis documented	42 (3.1)

[a]The method of categorizing discharge diagnoses was modified during the study. These data represent the last 1,348 of 1,833 patient encounters.
[b]Other category includes six patients with recent neurosurgery, three with toxic levels of anticonvulsant medications, three with reactions to non-anticonvulsant medication, three with systemic lupus erythematosus, and several other less frequently occurring causes.

cases. No significant differences were detected in age, seizure history, seizure type, prior history of neurocysticercosis, immigrant status, or exposure to an endemic region between the patients who underwent serologic testing and those who did not. Nine of the seropositive patients had CT scan findings consistent with neurocysticercosis; nine did not. Compared with the seronegative patients, seropositive patients were more likely to be Hispanic (RR 8.7; CI 3.1 to 24.1), have visited or lived in a neurocysticercosis-endemic region (RR 6.8; CI 2.2 to 20.5), and have an abnormal CT scan (RR 1.7; CI 1.2 to 2.3). Seropositivity was not significantly associated with either new-onset seizures or prior seizure history. Serology was positive in 27% of the patients who had neuroimaging findings characteristic of neurocysticercosis, 5.3% of those with consistent neuroimaging findings, and 1.1% of those with neuroimaging findings inconsistent with neurocysticercosis.

Seven patients reported a history of neurocysticercosis. Four (57%) of these patients had neuroimaging findings typical of or consistent with neurocysticercosis. Serologic testing was performed on two of the seven patients with one negative and one positive result.

The use of our case definition identified two patients who were not diagnosed with neurocysticercosis by the emergency department physician but who were later clinically diagnosed with neurocysticercosis. One was a child born in the United States to Laotian parents whose travel history was unknown; the other was a man of Hispanic ethnicity whose travel history and immigration status were not available. On the other hand, half of the patients diagnosed with neurocysticercosis by their treating physician did not satisfy our case definition. No confirmed cases of domestically acquired neurocysticercosis were identified during the study period.

Discussion

Immigrants continue to make up an increasing proportion of the U.S. population. By some reports, they will constitute up to 60% of the new immigrants and new births over the next few decades (18,19). By the year 2037, Latinos will outnumber whites as the dominant ethnic group in California, mirroring a population surge that is sweeping across the Southwest (20). Because neurocysticercosis is endemic in many of the countries from which these persons are emigrating, its presence in the United States reflects these immigration trends.

Table 3. Neurocysticercosis and selected demographic characteristics of seizure patients, U.S. sites

Site	Total seizure patients enrolled	Hispanic patients enrolled (%)	Immigrants enrolled[a] (%)	Neurocysticercosis patients identified (%)
Albuquerque, NM	107	58 (54)	9 (8)	6 (5.6)
Atlanta, GA	146	4 (3)	6 (4)	0 (0.0)
Charlotte, NC	300	11 (4)	17 (6)	4 (1.3)
Kansas City, MO	164	12 (7)	3 (2)	1 (0.6)
Los Angeles, CA	91	52 (57)	21 (23)	9 (9.9)
New Orleans, LA	174	9 (5)	8 (5)	2 (1.1)
New York, NY	184	50 (27)	54 (29)	1 (0.5)
Orlando, FL	68	6 (9)	9 (13)	0 (0.0)
Philadelphia, PA	185	20 (11)	19 (10)	1 (0.5)
Phoenix, AZ	243	90 (37)	27 (11)	10 (4.1)
Portland, OR	171	8 (5)	5 (3)	4 (2.3)
Total	1,833	320 (17)	179 (10)	38 (2.1)

[a]Immigration data were not obtained from the first 490 patients enrolled.

Table 4. Demographic and clinical characteristics of neurocysticercosis patients[a] and non-neurocysticercosis patients

Features	Neurocysticercosis patients n=37 (%)	Non-neurocysticercosis patients n=1,796 (%)	Relative risk 95% CI
Sex, male	27 (73.0)	1,189 (66.0)	
Racial/ethnic background[b]			
Black	4 (10.8)	746 (41.6)	
White, non-Hispanic	3 (8.1)	640 (35.7)	
Hispanic	29 (78.4)	291 (16.2)	17.1 (7.9 to 37.1)
Insurance status			
Medicare/private	7 (18.9)	455 (25.3)	
Medicaid	3 (8.1)	386 (21.5)	
Uninsured	22 (59.5)	738 (41.1)	2.5 (1.2 to 5.2)
Immigrant status[c]			
Born in US	5 (21.0)	815 (62.0)	
Not born in US	12 (50.0)	166 (13.0)	11.1 (3.9 to 31.0)
Unknown	7 (29.0)	343 (26.0)	
Exposure to endemic region			
No travel out of US	0 (0)	950 (52.9)	
Exposure to endemic region	28 (75.7)	314 (17.5)	158 (9.7 to 2,581)
Unknown travel history	9 (24.3)	532 (29.6)	
Prior history of neurocysticercosis[c]			
Positive prior history	3 (16.0)	5 (0.5)	21.6 (7.8 to 59.8)
No prior history	16 (84.0)	906 (99.5)	
Seizure type			
Generalized	26 (70.3)	1,551 (86.4)	0.38 (0.18 to 0.80)[d]
Tonic/clonic	4		
Focal motor	2 (5.4)	112 (6.2)	
Partial complex	7 (18.9)	79 (4.4)	
Unknown/ undocumented	2 (5.4)	54 (3.0)	
Seizure history			
New onset	19 (51.0)	877 (49.0)	1.1 (0.56 to 2.02)
Prior seizure history	17 (46.0)	793 (44.0)	
Serologic testing			
Seropositive	9 (36.0)	9 (1.0)	NA[e]
Seronegative	16 (64.0)	856 (99.0)	
Disposition			
Admission	16 (43.0)	865 (48.0)	1.0 (0.5 to 2.0)
Discharge	15 (41.0)	801 (45.0)	

[a]A patient was a person who met the case definition for neurocysticercosis. See text. The median age for neurocysticercosis patients was 32 yrs, with a range of 25–44 yrs; the median age of non-neurocysticercosis patients was 40 yrs (range 30–52 yrs).
[b]n = 36 for this category.
[c]n = 1,343; immigrant status was not collected from the first 490 patients enrolled.
[d]Generalized seizure versus focal motor or partial complex seizures.
[e]NA, not applicable. Comparison was not done since serology was part of the case definition for neurocysticercosis.
CI, confidence intervals.

Previous etiologic surveys of seizures in the United States and other industrialized countries have focused on brain tumors, strokes, and birth defects (21–23). Infections such as toxoplasmosis and meningitis or meningoencephalitis constitute a small minority of causes in seizure patients. More recently, HIV and its attendant complications have become prominent causes of adult-onset seizures (23). Neurocysticercosis, while a prominent cause of seizures in less developed nations, has not appeared in these studies.

An increasing number of neurocysticercosis cases (5–10) have been reported throughout the United States, which suggests that the prevalence of this disease may be on the rise. Because these previous case series were conducted retrospectively, primarily through chart reviews over periods as long as a decade, understanding the epidemiology and impact of neurocysticercosis is difficult. To our knowledge, this prospective study is the first to address the prevalence of neurocysticercosis in seizure patients in the United States.

Neurocysticercosis was identified at 9 of 11 sites and was responsible for 2.1% of seizures overall. In some sites, e.g., Los Angeles, California, and Albuquerque, New Mexico, the prevalence was nearly 10%. That neurocysticercosis has not appeared in previous U.S. studies on the epidemiology of seizures and now appears in our study as the cause of up to 10% of seizures in some areas suggests a substantial increase in frequency of this disease. Another study from a Los Angeles–area hospital corroborates this finding: 12% of the seizures seen in the authors' emergency department were attributable to neurocysticercosis (24).

Previous reports on neurocysticercosis in the United States, mostly retrospective case series, have focused on the clinical and epidemiologic aspects of the disease (5–10) Those studies are somewhat limited by the inadequacies and incompleteness inherent in retrospective data collection. It also seems problematic when epidemiologic information (e.g., exposure to a disease-endemic area) constitutes part of the case definition/inclusion criteria (e.g., exposure to cysticercosis-endemic area) but then epidemiologic information is subsequently reported as a result (e.g., percentage of patients who had visited an area where cysticercosis is endemic). In contrast, our data were collected at the time of evaluation, and our case definition was based solely on clinical criteria. As such, our study provides additional corroboration to the findings of previous studies reporting strong associations between neurocysticercosis and Hispanic ethnicity, immigrant status, and prior exposure to disease-endemic regions. Neurocysticercosis patients were also more likely to be uninsured; however, lack of insurance was also associated with being Hispanic and an immigrant. Consistent with previous studies, most neurocysticercosis patients did come to the emergency department with a generalized tonic clonic seizure, but such patients were more likely to have focal motor or partial complex seizures than were the seizure patients without neurocysticercosis. On the basis of our results, neurocysticercosis must be strongly

considered in emergency department seizure patients of Hispanic descent since nearly 1 in 10 were affected, a figure that was even higher in certain areas.

From 1988 through 1990, 7.2% of neurocysticercosis cases reported to the Los Angeles Department of Health Services were locally acquired (25). The rate of domestic acquisition has been even higher (17% to 26%) in some studies of pediatric neurocysticercosis (6,8). These rates of domestic acquisition appear to have increased from earlier studies in the late 1970s and early 1980s, when the rates were in the range of 2% to 3%. Because years can pass before symptoms develop, the incidence of domestically acquired cases will likely continue to rise.

The apparent increase in the prevalence of neurocysticercosis carries a substantial economic impact. Nearly half of the seizure patients in our study were admitted to the hospital. The average cost of hospitalization for seizures in one study was $1,615 per patient, not including physician charges (26). The economic toll extends beyond such direct costs. Compared with the general population, seizure patients are seen in the emergency departments 2½ times more frequently, admitted to the hospital 3 times more frequently, and treated by specialists 3 times more frequently; they also receive psychological counseling 7 times more frequently (27) These figures still underestimate the true economic impact of neurocysticercosis because up to 30% of patients who visit an emergency room do not have seizures but rather a variety of other neurologic symptoms such as headache, visual changes, ataxia, and confusion. Hydrocephalus may develop in a substantial number if patients, requiring neurosurgical intervention.

Any study of neurocysticercosis is limited by the difficulty in clearly establishing the diagnosis. The only true measure for the diagnosis of neurocysticercosis is brain biopsy, which is clearly impractical. This study therefore implemented a case definition that incorporates the classification scheme proposed by Del Brutto (17) but is therefore limited by the predictive value of CT scan and serology. Serology has previously been demonstrated to be sensitive in cases with multiple cysts (94%) but less sensitive with single cysts or calcified lesions (28%) (28). Specimen storage and periodic bulk mailing may have further affected intrinsic test performance.

We found a considerable discrepancy between patients who were diagnosed with neurocysticercosis by their physicians and patients who met our case definition. Because we did not specifically ask the treating physicians how they arrived at their diagnosis, the exact reasons for this discrepancy are unclear. However, emergency room physicians appear to rely considerably on epidemiologic information when diagnosing neurocysticercosis. Of patients diagnosed by their physicians, 98% were Hispanic (compared with our 76%) and 80% were immigrants (compared with our 50%). Ten percent of the patients with physician-diagnosed neurocysticercosis had normal CT scans.

Additional limitations to the study include the fact that the participating network sites are university-affiliated emergency departments. This fact may limit the generalizability of our results to other patient populations. Ideally, serologic testing would have been performed on all patients, but laboratory testing is not routinely performed for all seizure patients seen in emergency departments. Patients who did undergo serologic testing were, however, not statistically different from those who did not, on the basis of the demographic information collected.

In summary, while neurocysticercosis accounts for a small proportion (2.1%) of all seizures in university-affiliated, U.S. emergency department patients, its geographic distribution appears diverse; the highest concentration is in the Southwest and in Hispanics. Our observations are consistent with current immigration trends that suggest the growing importance of neurocysticercosis in the United States. Continued surveillance and further studies of screening and treatment strategies appear warranted.

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"Most of the fundamental ideas of science are essentially simple, and may, as a rule, be expressed in a language comprehensible to everyone."

Albert Einstein

Two New Rhabdoviruses (*Rhabdoviridae*) Isolated from Birds During Surveillance for Arboviral Encephalitis, Northeastern United States

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Two novel rhabdoviruses were isolated from birds during surveillance for arboviral encephalitis in the northeastern United States. The first, designated Farmington virus, is a tentative new member of the *Vesiculovirus* genus. The second, designated Rhode Island virus, is unclassified antigenically, but its ultrastructure and size are more similar to those of some of the plant rhabdoviruses. Both viruses infect birds and mice, as well as monkey kidney cells in culture, but their importance for human health is unknown.

Since the appearance of *West Nile virus* (WNV) in North America in 1999 (1), interest in surveillance of bird mortality has heightened among epidemiologists and other public health personnel (2). This interest is based on recent experience indicating that surveillance of bird deaths, especially of crows and other members of the family *Corvidae*, is a sensitive method for detecting WNV activity in a region (2–5). Consequently, many public health diagnostic laboratories in the United States are now actively testing dead birds for WNV. We describe two new rhabdoviruses that were isolated from birds during surveillance studies for WNV and *Eastern equine encephalitis virus* (EEEV) activity in the northeastern United States. This finding serves as a reminder that WNV and EEEV are not the only viruses that may be associated with bird deaths in this region.

Methods

Viruses Studied

We examined three virus isolates from birds. Virus strains RI-166 and RI-175 were both isolated from brain tissue of dead pigeons (*Columba livia*) collected at two localities in Rhode Island in summer 2000, as part of WNV surveillance activities. The two dead pigeons were collected on September 15 and 16 in Barrington, Bristol County (#175), and East Providence, Providence County (#166), respectively. No trauma or obvious gross pathology was noticed in the brain of either bird at necropsy. Brain tissue, including nearly equal portions of cerebrum and cerebellum, was collected and immediately frozen at –80°C until processed for culture. Frozen tissue was thawed, and a small portion was completely homogenized in 3.0 mL of medium 199 (Sigma, St. Louis, MO). Homogenized brain tissue was centrifuged at 3,500 × *g* for 20 minutes at 2°C in a refrigerated centrifuge; then 100 µL of the supernatants was immediately added to 25-mL flasks containing Vero cell monolayers. Tissue cultures were incubated at 37°C and 5% CO_2 and examined for cytopathic effect (CPE) on days 3–7 postinoculation.

The third virus, designated CT-114, was originally isolated from an unknown wild bird captured in central Connecticut in 1969 by the late Robert B. Wallis, during surveillance for EEEV (6). The original isolation of CT-114 virus was made by intracerebral injection of newborn mice; no other information is available about this isolate.

Antigens and Immune Reagents

Antigens for the three virus unknowns were prepared from infected newborn mouse brain by the sucrose-acetone extraction method (7). Hyperimmune mouse ascitic fluids (HMAF) to RI-166 and CT-114 viruses were prepared in adult mice as described (8). The adult mouse immunization schedule was four intraperitoneal injections per week of 10% crude suspensions of infected suckling mouse brain in phosphate-buffered saline mixed with Freund's adjuvant. To induce ascites formation, sarcoma 180 cells were given intraperitoneally with the final injection.

Of the other rhabdovirus antigens and immune reagents used to characterize the three virus unknowns, some antigens were sucrose-acetone–extracted infected mouse brain, while others were medium from infected Vero cell cultures. The latter viruses, antigens, and HMAF were from the Arbovirus Reference and Reagent Collection maintained at the University of Texas Medical Branch (UTMB).

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Serologic Tests

Complement fixation (CF) tests were performed by a microtechnique (7) with two full units of guinea pig complement. Titers were recorded as the highest dilutions giving 3+ or 4+ fixation of complement on a scale of 0 to 4+.

Indirect immunofluorescent antibody (IFA) tests were done on Vero and mosquito cells grown in eight-chamber Lab Tek tissue culture slides (Nunc, Inc., Naperville, IL). The mosquito cells tested were the C6/36 clone of *Aedes albopictus* cells (9) and a *Culex quinquefasciatus* cell line (10). After addition of virus, the Vero and mosquito cells were incubated with appropriate media at 37°C and 28°C, respectively. Culture slides with Vero cells were fixed in cold acetone when the cells showed 2+ to 3+ viral CPE; the mosquito cells were fixed after 6 days of incubation. The IFA tests were performed by using HMAF at dilutions of 1:10 and 1:20 and a commercial fluorescein isothiocyanate-conjugated goat antimouse immunoglobulin G (Sigma, St. Louis, MO) (11).

Transmission Electron Microscopy

Immediately after removal of the medium, Vero cell monolayers infected with RI-175 and CT-114 viruses were fixed in a mixture of 1.25% formaldehyde and 2.5% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.3, to which 0.03% trinitrophenol and 0.03% $CaCl_2$ were added, as described (12). After primary fixation, monolayers were washed in cacodylate buffer. Then the cells were scraped off the plastic, pelleted by light centrifugation in buffer, and postfixed in 1% OsO_4 in the same buffer. They were stained en bloc with 1% uranyl acetate in 0.1 M maleate buffer at pH 5.0, dehydrated in ethanol, and embedded in Poly/Bed 812 (Polysciences, Warrington, PA). Ultrathin sections were cut on a Reichert/Leica Ultracut S ultramicrotome (Leica Microsystems, Inc., Bannockburn, IL), stained with 2% aqueous uranyl acetate and 0.4% lead citrate, and examined with Philips 201 or Philips CM-100 electron microscopes at 60 kV (Philips Electron Optics, Eindhoven, the Netherlands).

Results

Biological Characteristics

Viruses RI-166 and RI-175 were initially isolated in cultures of Vero cells at the Center for Vector-Borne Disease (CVBD), University of Rhode Island. Media from the positive cultures were tested by immunoassay for WNV, EEEV, *Highlands J virus*, Jamestown Canyon virus, La Crosse virus, *Saint Louis encephalitis virus*, and Flanders virus antigens, with specific monoclonal and polyclonal antibodies; results were negative. Both viruses RI-166 and RI-175 were subsequently sent to UTMB for further study and characterization. When added to Vero cell cultures, both viruses produced extensive CPE within 48 hours. Newborn Institute for Cancer Research outbred mice that were injected intracerebrally with both RI-166 and RI-175 viruses became sick and moribund within 96 hours. RI-166 virus was also added to cultures of C6/36 and *Cx. quinquefasciatus* cells; it did not produce CPE, and no viral antigen could be detected in the mosquito cells when examined by IFA 6 days later.

Virus CT-114 was initially isolated by Robert Wallis at the Department of Epidemiology and Public Health, Yale University School of Medicine, following intracerebral injection of a homogenate of bird tissue into newborn mice. The virus was subsequently transferred to UTMB. Virus CT-114 produced illness and death in newborn mice 24–48 hours after intracerebral injection, as well as massive CPE in Vero cells within 48 hours; however, it did not produce CPE in the mosquito cells. Specific viral antigen was detected by IFA in *Cx. quinquefasciatus* cells injected with CT-114 virus, but not in C6/36 cells.

Ultrastructure of Isolates

Virions of isolate CT-114 were bullet shaped and were found budding mostly into the intracytoplasmic vacuoles, either as single virions into a small vacuole, or as several virions budding into the same large vacuole (Figure, A and B). Virions of CT-114 were 55 nm–60 nm in diameter and 145 nm–150 nm long, with a periodicity of striations of 10.5 nm (Figure, B).

Virions of the isolate RI-175 were seen budding predominantly into the extracellular space from the plasmalemma of the Vero cells (Figure, C and D). The virions were bacilliform, measuring 90 nm–100 nm in diameter, up to 500 nm long, and with a 20- to 25-nm periodicity of striations. In some cross-sections, the spiral packaging of the nucleocapsid could be seen and had the appearance of tubules 9 nm in diameter (Figure, C). Large groups of virions could be observed outside the cells.

Antigenic Characteristics

On the basis of their rhabdovirus-like morphology, RI-166, RI-175, and CT-114 antigens and HMAFs were examined by CF against 36 rhabdovirus antigens and HMAFs in our reference collection. The 36 agents included *Carajas virus; Chandipura virus*; *Cocal virus; Isfahan virus*; *Maraba virus*; *Piry virus;* vesicular stomatitis virus, types Alagoas, Indiana, and New Jersey; *Vesiculovirus* species Boteke, Jurona, Klamath, La Joya, Malpais Spring, Radi, and Yug Bogdanovac; *Iriri virus,* Flanders virus, Mosqueiro virus, Mossuril virus, Kern Canyon virus, Nkolbisson virus, Le Dantec virus, Connecticut virus, New Minto virus, sawgrass virus, Chaco virus, Timbo virus, Bangoran virus, Inhangapi virus, Joinjakaka virus, Kannamangalam virus, Kotonkan virus, Marco virus, Tibrogargan virus, and Yata virus (13,14).

In addition, RI-166 antigen was also tested against 26 other rhabdovirus HMAFs: Calchaqui, Gray Lodge, Kwatta, Mount Elgon bat, Perinet, Porton, Duvenhage, Lagos bat, Mokola, Rabies, Bahia Grande, Hart Park, Kamese, Keuraliba, Almpiwar, Aruac, Bimbo, Charleville, Coastal Plains, Gossas, Kolongo, Navarro, Obodhiang, Parry Creek, Rio Grande, and Sandjimba. In CF tests, RI-166 (selected as the prototype) and RI-175 viruses were indistinguishable (Table 1); but RI-166

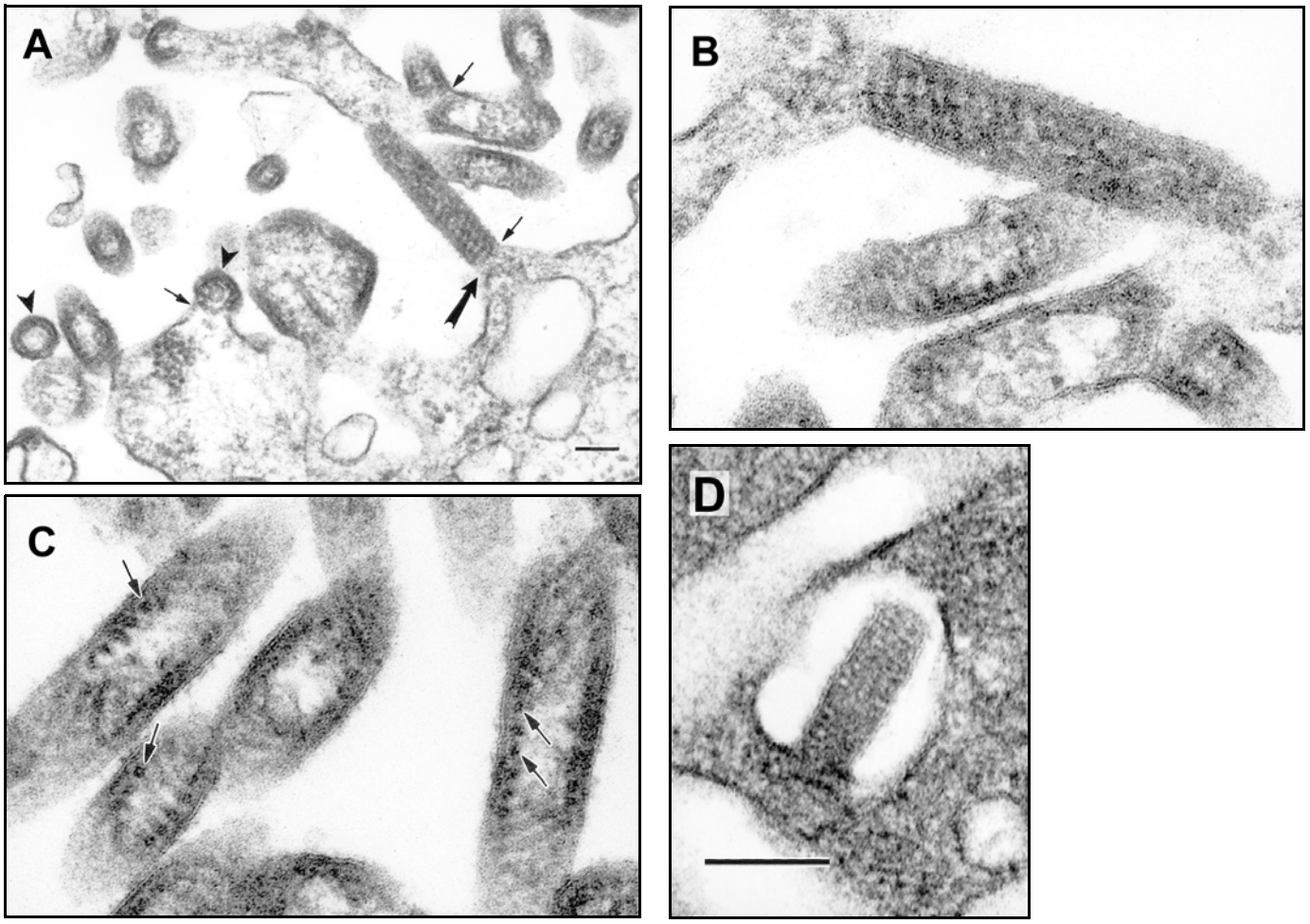

Figure. Ultrastructure of the new rhabdoviruses in infected Vero cells. A. Virions of isolate RI-175 budding from the surface of a Vero cell and from cell surface projections (arrows). Arrowheads mark cross-sections of virions. The virion indicated with a large arrow is enlarged in B. B. A virion of isolate RI-175 budding from host cell plasmalemma into an extracellular space. C. Details of the virion ultrastructure of isolate RI-175, showing spiral packaging of the nucleocapsid and its tubular structure in the cross-sections (arrows). D. A virion of the isolate CT-114 budding into an intracytoplasmic vacuole. Bar = 100 nm.

antigen and HMAF did not react with any of the other rhabdovirus antigens or HMAFs listed. Because of the geographic region where they were isolated, we initially suspected that RI-166 and CT-114 might be Connecticut or Flanders viruses. However, no antigenic relationship was shown by CF test (Table 1). The antigen RI 907-36 was prepared from a 1999 isolate of Flanders virus from Rhode Island. Likewise, no relationship could be demonstrated between RI-166, CT-114, Connecticut, or Flanders viruses by IFA test (data not shown). Based on these findings, we conclude that RI-166 is probably a new, unassigned vertebrate rhabdovirus. The name Rhode Island virus is proposed for this virus.

In CF tests, CT-114 HMAF reacted with five vesicular stomatitis serogroup antigens: Chandipura, Isfahan, Maraba, Jurona, and La Joya (Table 2). Antigenically, CT-114 was most closely related to Jurona and La Joya viruses. Both Jurona and La Joya viruses are tentative members of the genus *Vesiculovirus* (14–16). Based on the morphology and antigenic relationships of CT-114, we conclude that it is also a provisional member of the *Vesiculovirus* genus. The name Farmington is proposed for this new virus.

Conclusion

The isolation of these new rhabdoviruses from birds demonstrates the value of direct culture for detecting new and unexpected viral agents. Rhode Island virus was initially isolated in Vero cells; Farmington virus was detected by intracerebral inoculation of newborn mice. To save time and reduce costs, many arbovirus diagnostic laboratories in the United States have stopped culturing field specimens and instead are using techniques such as antigen-capture enzyme-linked immunosorbent assay (17) or polymerase chain reaction (18–20) to detect viral antigens or nucleic acids in insect pools, blood, and tissue samples. While these newer techniques are rapid and quite sensitive, they detect only those viruses for which one has a capture antibody or a specific primer set. Furthermore, these techniques do not detect novel or unexpected viral agents nor antigenic or virulence changes in known

Table 1. Cross-reaction of CT-114 and RI-166 viruses with other selected rhabdoviruses by complement fixation test

	Hyperimmune ascitic fluid					
Antigen	Connecticut	New Minto	Sawgrass	Flanders	CT-114	RI-166
Connecticut	256/≥64[a]	0	128/32	0	0	0
New Minto	0	256/≥64	0	0	0	0
Sawgrass	16/32	16/32	1,024/64	0	0	0
RI 907-36	0	0	0	≥256/≥32	0	0
CT-114	0	0	0	0	256/64	0
RI-166[b]	0	0	0	0	0	128/≥8
RI-175 [b]	0	0	0	0	0	128/≥8

[a]Reciprocal of ascitic fluid titer/reciprocal of antigen titer.
[b]RI-166 and RI 175 antigens were fluids from infected cell cultures.

viruses. A recent commentary (21) on the changing paradigm for arbovirus identification discussed these limitations of the more rapid molecular methods and stressed the importance of isolating viruses and obtaining phenotypic as well as genotypic information on them.

The isolation of Rhode Island virus from dead pigeons suggests that this virus may be an occasional avian pathogen. During the summer of 2000, a total of 335 birds, representing 31 avian species, were tested for virus at the CVBD. Rhode Island virus was isolated from 2 of 15 pigeons tested, suggesting that its host range may be restricted. Further experimental studies are needed to determine its pathogenesis and host range. In the northeastern United States, WNV, and to a lesser degree, EEEV, are the arboviruses usually associated with bird deaths (3,22). However, as surveillance for WNV continues and more dead birds are collected and cultured, other novel avian viral pathogens, such as Rhode Island virus, will probably be encountered.

At present, little is known about the ecology of Rhode Island or Farmington viruses. The ultrastructure and antigenic relationships of Farmington virus suggest that it is a novel vesiculovirus. The ability of Farmington virus to infect the *Cx. quinquefasciatus* cell line is also compatible with a vesiculovirus, since most of the rhabdoviruses in this genus are arthropod associated (23,24). Jurona and La Joya viruses, the vesiculoviruses most closely related antigenically to Farmington virus, were both isolated from New World mosquitoes. Jurona virus has been isolated from *Haemagogus* sp. and from a human in northern Brazil (25); La Joya was isolated from *Cx. dunni* in Panama (13).

Rhode Island virus is more intriguing. Its isolation from dead birds and its ability to infect mice (both newborn and adult) as well as Vero cells, are strong evidence that it is a vertebrate rhabdovirus. Yet its ultrastructure and relatively large size more closely resemble some of the plant rhabdoviruses (26). Further studies of this interesting new rhabdovirus and potential avian pathogen are warranted.

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Table 2. Cross-reaction of CT-114 and RI-166 viruses and selected vesicular stomatitis serogroup viruses by complement fixation test

	Hyperimmune ascitic fluid						
Antigen	Chandipura	Isfahan	Maraba	Jurona	La Joya	CT-114	RI-166
Chandipura	256/≥32[a]	0	0	0	0	8/8	0
Isfahan	32/≥16	64/≥32	8/8	0	0	8/16	0
Maraba	8/≥8	0	512/≥32	0	0	8/16	0
Jurona	0	0	0	1,024/≥32	0	16/≥32	0
La Joya	0	0	0	0	512/≥32	16/≥32	0
CT-114	0	0	0	0	0	256/≥16	0
RI-166[b]	0	0	0	0	0	0	128/≥8

[a]Reciprocal of ascitic fluid titer/reciprocal of antigen titer.
[b] RI-166 antigen was fluid from an infected Vero cell culture.

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Cryptosporidium Oocysts in a Water Supply Associated with a Cryptosporidiosis Outbreak

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An outbreak of cryptosporidiosis occurred in and around Clitheroe, Lancashire, in northwest England, during March 2000. Fifty-eight cases of diarrhea with *Cryptosporidium* identified in stool specimens were reported. *Cryptosporidium* oocysts were identified in samples from the water treatment works as well as domestic taps. Descriptive epidemiology suggested that drinking unboiled tap water in a single water zone was the common factor linking cases. Environmental investigation suggested that contamination with animal feces was the likely source of the outbreak. This outbreak was unusual in that hydrodynamic modeling was used to give a good estimate of the peak oocyst count at the time of the contamination incident. The oocysts' persistence in the water distribution system after switching to another water source was also unusual. This persistence may have been due to oocysts being entrapped within biofilm. Despite the continued presence of oocysts, epidemiologic evidence suggested that no one became ill after the water source was changed.

Outbreaks of cryptosporidiosis associated with drinking water have been an emerging problem for the past 20 years. In the 1990s, cryptosporidiosis became the most common cause of outbreaks associated with public drinking water supplies in the United Kingdom (1). This disease is also responsible for several of the largest outbreaks of waterborne disease seen in the United States (1). Yet substantial areas of uncertainty over many aspects of the epidemiology of this infection remain. One of the most pressing such areas is determining what concentration of oocysts in drinking water is considered safe.

In the United Kingdom, recent legislation was enacted that set a legal limit of 1 oocyst/10 L when water was sampled continuously over a 24-hour period (2). However, this level was set as a treatment standard and was not derived from known public health standards. With current knowledge, proposing standards for cryptosporidia based on public health criteria is not possible, primarily because published reports of outbreaks have not had accurate measures of the concentration of oocysts in the water at the time when infection was thought to have occurred. We report, to our knowledge, the first outbreak to have occurred when a fairly accurate estimate of the concentration of oocysts in the water could be made.

The Outbreak

In March 2000, an outbreak of cryptosporidiosis occurred in and around the town of Clitheroe in Lancashire County in northwest England. This small market town, nestled in the hills near the Ribble River, is a thriving community that attracts many tourists. The surrounding countryside supports arable and dairy farming. Before this outbreak, reported cases of cryptosporidiosis were low. In the years 1997–1999, the mean annual attack rate of laboratory-confirmed cryptosporidiosis was 4.83 per 10,000 residents per year, compared with 13.57 for the region as a whole.

During March 1–15, 2000, the Ribble Valley Environmental Health Department reported nine cases of cryptosporidiosis to the East Lancashire Health Authority. All the patients lived in or near Clitheroe. Provisional information provided by the water company indicated that six of these nine patients lived in a single water zone supplied by the same water treatment works. On the basis of this information, an outbreak was declared, and an outbreak control team was established. The team met for the first time on March 16.

Methods

Epidemiologic Investigation

Environmental health and public health department personnel interviewed patients with cryptosporidiosis in person or by telephone, using a structured questionnaire (3). Analysis was performed by using the computer program Epi-Info (version 6.02; Centers for Disease Control and Prevention, Atlanta, GA). Patients were defined as those with a positive stool sample who lived in or visited the implicated water zone and who had onset of diarrhea since March 1, 2000. Cases were defined as primary when no other member of the household had had diarrhea in the 2 weeks before the onset of symptoms; possible secondary cases were defined as those in which a member of the same household had had diarrhea in the previous 2 weeks. The case definitions included those who had traveled abroad for $\leq$7 days.

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Microbiologic Investigation

General practitioners in the area submitted stool samples to the local hospital microbiology laboratory. Stools were examined by microscopy with the modified auramine phenol stain (4). Positive samples were then sent to the Public Health Laboratory Service's Cryptosporidium Reference Unit for genotyping.

Environmental Investigations

The local water company provided information on the water supply, instituted a water-sampling schedule (from domestic properties, water treatment works, and fire hydrants during flushing operations), and analyzed the water samples to identify *Cryptosporidium* oocysts. Most of the samples were 10-L grab samples analyzed according to the U.K. standard method (5). The large-volume samples were analyzed by the method in the Water Supply (Water Quality) Amendment Regulations of 1999 (2). The source of water to the affected area (Grindleton Springs) was visited by members of the outbreak control team.

The local water company supplied rainfall statistics for the weeks preceding the outbreak. Local authority engineers were consulted for information on previous high water or flood warnings.

After the incident, the water company constructed a physical model of the affected reservoir, Lowcocks, with a geometric scaling ratio of 32:1. Flows were tracked by using salt injection with an array of conductivity probes suspended above the tank and injecting colored dyes for visualization. As the ratio of the two respective inlet flows can vary, the baseline performance of the tank was evaluated over a range of operational, but steady state, conditions. A series of transient tests was then conducted to mirror the operation of the reservoir in the time leading up to and covering the incident until the boil water notice was issued on March 21.

Result

Descriptive Epidemiology

Fifty-eight cases met the case definition. Of these, three were in patients who had traveled abroad for ≤7 days in the 2 weeks before illness. Fifty-one cases were identified as primary, and seven as possible secondary. The dates of onset of cases (Figure 1) showed peaks on March 10 and 17. Ages of patients ranged from 7 months to 95 years, but most patients were <5 years (52%). Thirty (52%) of the patients were male and 28 (48%) female. All 58 patients (100%) had diarrhea; 18 (31%) had fever, 48 (83%) abdominal pain, 19 (33%) vomiting, and three (5%) blood in the stool.

Fifty-one patients lived in the same water supply zone and drank unboiled main tap water in the zone. The crude attack rate for residents of this zone was 29.6 per 10,000 population (based on general practitioner registered population of 17,252 linked by postal code of residences in the water supply zone). The crude attack rate for people within the same local government area but not living in the same water supply zone was 1.8 per 10,000 population, giving a relative risk associated with residence in the implicated water supply zone of 16.2 (95% confidence interval 7.5 to 35.0). The age-specific attack rate varied from 275 per 10,000 in children <5 years of age to 5.6 per 10,000 in those >44 years (Table 1). Seven patients lived in properties not in the affected water zone. However, six of these had drunk unboiled main water in the affected zone in the 2 weeks before illness; the other patient had visited a swimming pool in the zone. Other potential risk factors, such as travel, visit to a swimming pool, and consumption of certain foods, were included in the questionnaire. None was common in patients.

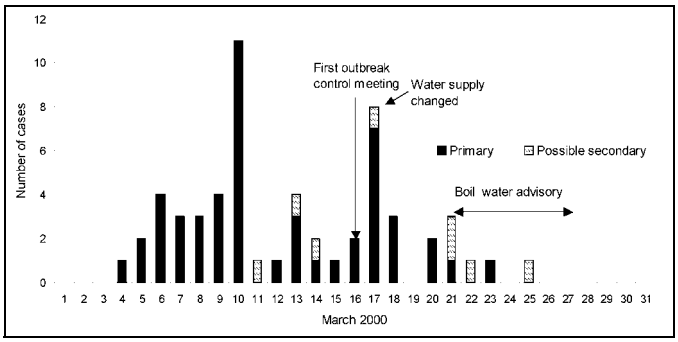

Figure 1. Date of diarrhea onset, 58 cryptosporidiosis cases, Clitheroe, 2000.

Microbiologic Testing

Of the 58 cases with a positive stool sample for *Cryptosporidium,* 47 specimens were typed. All were *C. parvum* genotype 2 (for nine cases there was insufficient material, and two specimens were untypable).

Environmental Results

Water Sample Analysis

Lowcocks Water Treatment Works (WTW), sourced from Grindleton Springs, supplied approximately 90% of the water to the affected zone. The supply was a spring source that fed a single service reservoir and from there moved into distribution. However, the reservoir could also be filled from a nearby larger water supply via an aqueduct. The supply was chlorinated but not filtered. As part of the risk assessment carried out under water quality amendment regulations (2), Lowcocks

Table 1. Age-specific attack rates for cryptosporidiosis in residents of water zone 97, Clitheroe, March 2000

Age group	Cases (n=51)	Population	Rate/10,000	95% confidence interval
≤4	26	945	275.1	170.8 to 379.4
5–14	9	2,283	39.4	13.7 to 65.1
15–44	12	6,822	17.6	7.6 to 27.5
≥45	4	7,202	5.6	0.1 to 11.0
Total	51	1,7252	29.6	21.5 to 37.7

WTW was classified as being at "significant risk" from *Cryptosporidium* oocysts in water supplied from the works. However, continuous monitoring had not yet begun before the outbreak.

The reservoir is rectangular with two inlets and a single outlet. The tank is 110 m long and 90 m wide with an operational depth between 3.5 m and 5.4 m. The spring has one inlet, which varies from 2 to 6 megaliters per day and another from the aqueduct, which varies from 1.5 to 5 megaliters per day. The calculated capacity of the reservoir is 53 megaliters. The ratio of aqueduct to spring water varies considerably during normal operation; full advantage is taken of the increase in availability of the spring's source after major rainfalls.

On March 17, a large-volume sample of water (1,627 L) from a pumping station fed from Lowcocks WTW yielded 76 oocysts of *Cryptosporidium* per 1,000 L. *Cryptosporidium* oocysts were also identified in a water sample taken from a domestic tap in the water zone on March 16 at a concentration of five oocysts per 10 L of water. From March 16 to April 6, a total of 192 samples (10-L grab samples) from domestic taps or fire hydrants in the affected zone were analyzed; 47 (24%) contained *Cryptosporidium* oocysts in concentrations ranging from 1 to 9/10 L. Six water samples from domestic taps in areas adjoining the affected water zone were negative (Table 2, Figure 2).

Table 2. Results of 10-L grab samples taken within distribution range of water works during investigation[a] of cryptosporidial outbreak, Clitheroe, March 16–April 6, 2001

Date	Samples taken	Samples positive	Oocyst counts of positive samples/L				
16 Mar	3	1	0.5				
17 Mar	6	5	0.1	0.2	0.1	0.2	0.1
18 Mar	8	4	0.2	0.2	0.3	0.3	
19 Mar	8	5	0.2	0.3	0.1	0.1	0.2
20 Mar	9	5	0.1	0.2	0.9	0.5	0.1
21 Mar	23	5	0.2	0.1	0.1	0.4	0.1
22 Mar	16	4	0.1	0.1	0.1	0.1	
23 Mar	15	2	0.1	0.2			
24 Mar	15	2	0.1	0.1			
25 Mar	12	2	0.1	0.1			
26 Mar	12	0					
27 Mar	9	0					
28 Mar	3	2	0.3	0.4			
29 Mar	3	0					
30 Mar	6	3	0.1	0.2	0.4		
31 Mar	9	3	0.1	0.1	0.6		
1 Apr	7	1	0.1				
2 Apr	7	1	0.1				
3 Apr	6	2	0.1	0.1			
4 Apr	6	0					
5 Apr	6	0					
6 Apr	3	0					

[a] Total volume examined each day (in L) = 10 X number of samples taken.

Site Visits

The concrete casings of two of the Grindleton Springs collection chambers showed signs of aging and were in a poor state of repair (one could look directly into one chamber through holes in the concrete). Evidence of recent livestock excreta (cattle) was present in the areas around, and in direct contact with, the covers to several of the spring collection chambers; manure was also spread in a field within 5 m of one wellhead.

Rainfall Statistics

Abnormally heavy rainfall (up to 58 mm per day) and flood alerts were reported for the area on February 27 and March 2–7.

Hydraulic Modeling

A number of detailed transient state tests were conducted in which the flows and levels were altered in line with the reservoir operation before and during the outbreak. Initially, the first "injection" of oocysts was assumed to have come into the reservoir on February 27, after the first associated heavy rainfall. However, results from these initial tests indicated that, because of the way the reservoir operated and its short nominal retention time (2 days) during part of this period, a large spike of oocysts entering the reservoir from the springs inlet on February 27 would have been effectively washed out by the time the sample was taken on March 17.

Two potential contamination events, one after each major rainfall event on February 27 and March 2, respectively, were then proposed. This hypothesis was modeled by injection of two discrete salt pulses into the model springs inlet at the appropriately scaled time in the modeling run. Results indicated three peaks of oocyst counts at the tank outlet. The first peak occurred when the tank was operating on only spring flow, corresponding to February 29. The second peak came on March 1, when aqueduct flow was introduced. The final peak occurred on March 2–3, after the second salt pulse (simulating the rainfall incident).

Based on the concentration found in the March 17 sample, the most probable peak concentration that the Clitheroe population would have been exposed to was 40 times greater, approximately 30 oocysts per 10 L. These values are based on tests in which the pulse was introduced instantaneously; in practice, contamination likely took place over several hours or days after each major rainfall event. While it is likely that the behavior of oocysts would not substantially differ in the water system and the salt and dye model, these numbers should not be considered exact; rather, they are a good indication of level of exposure over the period in question.

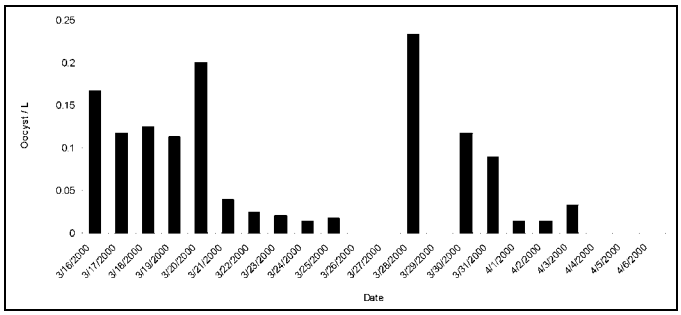

Figure 2. Mean daily cryptosporidia oocyst counts from 10-L grab samples taken during outbreak investigation, Clitheroe.

Control Measures

At the first outbreak control team meeting, 11 of 14 reported cryptosporidiosis cases were known to be in residents of the same water supply zone. As a result, the water supply to the affected area was changed to an alternate supply during the following night, and the system was flushed. The alternate supply was an approximately 50/50 blend of filtered surface water from two separate (protected) upland impounding reservoirs. The first source (Watchgate) provides up to 600 megaliters per day to a population of approximately 1.75×10^6; the second source (Hodder) provides up to 50 megaliters per day to a population of approximately 1.75×10^3. Both areas had had no observed increase in the rates of reported cryptosporidiosis.

At the third outbreak control team meeting, when results of sampling became available, it became evident that, although the water supply to the area had been changed by 9:30 a.m. on March 17 (and its distribution throughout the zone confirmed by chemical analysis of domestic water samples), substantial numbers of *Cryptosporidium* oocysts still existed in samples taken during the next 4 days (March 17–20). Initial samples from the source of the new water supply showed no evidence of contamination. Historic archived data available for both new sources showed only a low frequency of detected oocysts in the raw (untreated source) water for each site. During the incident, five samples of treated water were taken from the first site and 13 samples from the second source. A single oocyst was reported in one 10-L sample taken from the first site; no oocysts were detected in the other samples.

The outbreak control team agreed that there continued to be a risk to public health and issued a "Boil Water Advisory" on March 21. This advisory was rescinded on March 27 after extensive water system flushing operations and 2 days of domestic water samples being clear of *Cryptosporidium* oocysts. The peak in counts on March 28, although calculated from three samples, was associated with the sampling water from hydrants rather than from domestic taps.

Water sampling continued, but samples were taken from fire hydrants rather than domestic taps. While inspections of the water system showed no evidence of ongoing contamination, analysis of water continued to show cryptosporidia. When oocysts were detected in hydrant samples after the source of water had been changed, experienced operations staff inspected the route of the aqueduct, and boundary valves at the periphery of the affected distribution system were checked to ensure that water could not enter this system from an adjacent zone.

At this stage, no further new cases of cryptosporidiosis were being reported. The original source of water, Grindleton Springs, had been identified as having a plausible source of oocysts within the watershed (cattle excreta), a plausible pathway (through the damaged spring head structure to one of the chambers), and inadequate treatment for removing oocysts (microfiltration with a pore size >40 μ); this source of water had been isolated and discharged to waste. Thus, the change in sampling method, rather than ongoing contamination, might be causing the continuing positive oocyst results. For this reason, the boil water advisory was not reinstituted. Further flushing continued, no new cases of cryptosporidiosis were reported, and the last water sample positive for oocysts was on April 3.

Discussion

Use of U.K. Public Health Laboratory Service guidelines strongly associated this outbreak with the water supply because *Cryptosporidium* oocysts were detected in treated water and the descriptive epidemiology suggested that drinking tap water was the only common factor linking the cases (6). Environmental investigations suggested that contamination of Grindleton Springs with animal feces was the probable cause of the outbreak. Results of genotyping were consistent with an animal source.

This outbreak is unusual because of the very high attack rate of laboratory-confirmed cases. The crude attack rate for microbiologically confirmed cases of cryptosporidiosis was much higher than previously reported in the United Kingdom (7–9). We suggest that this high attack rate occurred because of low immunity in the population and the probable high concentration of oocysts at the time of the initial contamination. Although we have no direct measure of population immunity before this outbreak, the incidence of infection in previous years was low compared with that in the rest of the region. Furthermore, until the outbreak, the water supply was a groundwater source; various groups have suggested that such sources are associated with lower sporadic infections and lower population immunity (7,10).

The other major issue raised by this outbreak was the impact of changing the source of water. The outbreak control team had suggested that changing the water supply to the affected area at the beginning of the outbreak would remove the *Cryptosporidium* oocysts from the water. However, this measure did not result in the expected immediate clearance of contamination. Indeed, despite lack of evidence of a new contamination source and with ongoing extensive flushing operations, oocysts remained detectable at low levels for up to 19 days after the change. Counts did generally decline during the 10 days after the supply was changed; however, counts peaked on March 20 after a burst in the main supply pipe. Increased

counts on March 28–31 occurred when water samples started being taken from hydrants, rather than domestic taps. Hydrant water is discharged much more forcefully than that from domestic taps. The slow decline in oocyst counts after the change in supply may have been because of captured oocysts being released from the biofilm on the surface of the distribution pipes. Subsequent peaks associated with the burst and use of hydrants for sampling could have increased oocyst counts by stripping biofilm from the inner surface. *Cryptosporidium* oocysts do attach to biofilm in this manner (1,11,12)

Whatever the reasons for the continued detection of oocysts in water samples, few, if any, cases of infection were acquired after the source was changed. The epidemiologic analysis suggests that changing the water supply was the key public health measure. The boil-water advisory had little, if any, effect on reducing subsequent cases. The decision not to reintroduce the advisory when hydrant samples continued to show oocysts appears to have been justified.

Monitoring water samples, particularly with 10-L small-volume samples, highlighted the difficulties in interpreting the public health importance of oocysts in the water (13–15). Currently, the level of detectable *Cryptosporidium* oocysts in domestic water samples that poses no public health risk is unknown. The number of oocysts detected in the large-volume filtration of water from the WTW was below the limit currently defined as a national maximum permissible treatment standard (100 oocysts per 1,000 L) (2). However, this outbreak occurred 10 days after the most recent of three major rainfalls that could plausibly have given rise to contamination of the source water. Physical and computational fluid dynamics modeling suggested that the concentrations of oocysts in water leaving the WTW immediately after the heavy rainfall were 30 times the statutory treatment standard.

The introduction of continuous monitoring in the United Kingdom, together with existing surveillance for cryptosporidium infection in humans, will hopefully result in a better definition of an appropriate public health standard for this organism. However, recent human studies have shown a substantial intraspecies variability in the infectivity of *Cryptosporidium* oocysts (16). Furthermore, we have recently identified a novel strain of *C. parvum* that appears to be widespread in sheep but has never been described in humans (17). These observations suggest that identifying a standard in drinking water that would lead to a tolerable level of illness in the community may not be possible. Indeed, outbreaks of cryptosporidiosis associated with drinking water elsewhere in the United Kingdom have occurred despite the peak oocyst count's being well within the statutory standard (18,19). Several episodes have also been reported in which high oocyst counts (>10 oocysts in 100 L) have been detected in treated water with no episodes of illness subsequently being detected in the community (20).

Further research is required to define the public health importance of low levels of *Cryptosporidium* oocysts as well as the optimal water sampling strategy during an outbreak. Similarly, the effectiveness and utility of system flushing remain to be shown. The current treatment standard should be reviewed, as further evidence relating to the public health impact of levels of *Cryptosporidium* oocysts becomes available.

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Parachlamydiaceae: Potential Emerging Pathogens

Gilbert Greub* and Didier Raoult*

Parachlamydiaceae, which naturally infect amoebae, form a sister taxon to the *Chlamydiaceae* on the basis of the *Chlamydia*-like cycle of replication and 80% to 90% homology of ribosomal RNA genes. Because intra-amoebal growth could increase the virulence of some intracellular bacteria, *Parachlamydiaceae* may be pathogenic. Arguments supporting a pathogenic role are that *Chlamydia pneumoniae*, a well-recognized agent of pneumonia, was shown to infect free-living amoebae and that another member of the *Chlamydiales*, *Simkania negevensis*, which has 88% homology with *Parachlamydia acanthamoebae,* has caused pneumonia in adults and acute bronchiolitis in infants. The recent identification of a 16S rRNA gene sequence of a *Parachlamydiaceae* from bronchoalveolar lavage is additional evidence supporting potential for pathogenicity.

Nosocomial pneumonia, a frequent complication associated with considerable illness and death (1,2), is the leading cause of death from nosocomial infections (3). Community-acquired pneumonia, which is also common, is associated with a case-fatality rate of up to 8.8% (4). Despite use of standard diagnostic methods, no microbial cause could be identified in 47% to 55% of community-acquired pneumonia worldwide in adults (5–7) and 20% to 75% of nosocomial pneumonia (8,9). Emerging intracellular bacteria, which grow poorly or not at all on media used routinely for detecting human pathogens from clinical samples, could be the causative agents of these pneumonias of unknown etiology. During recent decades, several previously unrecognized intracellular bacteria have been discovered through the genotypic approach. In addition, use of amoebal coculture procedures (10) allows recovery of some fastidious gram-negative bacteria, such as the *Legionella*-like amoebal pathogens (11,12), *Candidatus Odyssella thessalonicensis* (13), *Sacrobium lyticum* (14), several *Afipia* species (15), and *Chlamydia*-like endosymbionts (16,17).

Amoebae: Microbial Trojan Horses

Although *Legionella* was the first pathogen demonstrated to multiply and persist in amoebae (18), several other fastidious intracellular bacterial pathogens, including *Chlamydia pneumoniae* (19), *Mycobacterium avium* (20), *Listeria monocytogenes* (21), and an *Ehrlichia*-like organism (22), may infect free-living amoebae. Extensive study of the ecology of *Legionella pneumophila* has confirmed empirical observations of its predilection for growth in hot water tanks and its localization in sediment (23). Rowbotham described the ability of *L. pneumophila* to multiply intracellularly within protozoa (18) and suggested that free-living amoebae could be a reservoir for *Legionella* species (24). As amoebae are common inhabitants of natural aquatic environments and water systems (25,26) and are resistant to extreme temperatures, pH, and osmolarity conditions while encysted (27), the *Legionella* reservoir is important. Growth of free-living amoebae at high temperatures (44°C to 53°C) was observed more frequently for strains isolated from hot-water tanks (mainly *Hartmanella vermiformis*) than for those isolated from moist sanitary areas (mainly *Acanthamoeba*, *Naegleria,* and *Valkhampfia* species) (26). This great tolerance of cysts and species-dependent thermotolerance of trophozoites could account for the difficulty in eliminating Legionellae from water systems (28). The resistance of *Acanthamoeba* spp. cysts to various disinfecting solutions (29–31) complicates the eradication of free-living amoebae. Moreover, a wide variety of *Enterobacteriaceae* have increased resistance to chlorination when ingested by *Tetrahymena pyriformis* (32). Thus, free-living amoebae could readily act as Trojan horses for bacterial endosymbionts (33,34).

The relationship between *Legionellaceae* and free-living amoebae, which serves as a model for other endosymbionts such as *Parachlamydiaceae*, is not restricted to the role of reservoir. Indeed, *Acanthamoeba* strains were found to produce *Legionella*-containing vesicles, which may be agents of transmission of legionellosis. The risk of transmission may be underestimated by plate count methods (35). In addition, Legionellae grown inside amoebae were more virulent (36,37), more motile (24), and more resistant to biocides (38) than are bacteria cultured in axenic media. The entry of Legionellae into monocytes was found to be enhanced by the intra-amoebal growth environment (39). In addition, intra-amoebal growth of *L. pneumophila* was shown to induce an antibiotic-resistant phenotype, while Legionellae cultured in broth did not (40). Similarly, *M. avium* living within *Acanthamoeba* had greater resistance to rifabutin, clarithromycin, and azithromycin than did strains living in macrophages (41). This finding could result from decreased uptake of antibiotics into the amoebae, an inactivation of the compound within amoebae, or a change in the bacterial phenotype. Replication of bacteria in amoebae was found not only to affect the bacterial host (through increased potential for spread, resistance to biocides and antibiotics, and acquisition of virulence traits) but also to enhance the pathogenicity of the free-living amoebae (42).

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The *Parachlamydiaceae*

These *Chlamydia*-like endosymbionts are small Gimenez-stained (43) coccoid bacteria (Figure 1) that naturally infect amoebae and are inconsistently stained with Gram stain. Electron micrographs of Acanthamoeba demonstrate the presence of bacteria at different developmental stages typical of the Chlamydiales, such as elementary and reticulate bodies (Figure 2). A new *Parachlamydiaceae* family was proposed (44) that forms a sister taxon to the *Chlamydiaceae*, as it has a Chlamydia-like cycle of replication and 80% to 90% homology of ribosomal RNA genes. This family comprises two genera, of which the type strains are *Parachlamydia acanthamoebae* (17) and *Neochlamydia hartmanellae* (45). Members of the Parachlamydia were proposed to have at least 95% homology of the 16S or 23S rRNA genes with *P. acanthamoebae* (44). However, comparison of the 16S rRNA gene sequences of four additional Parachlamydia with *P. acanthamoebae* showed substantial phylogenetic diversity within this genus (Figure 3), with 91.2% to 93.1% 16S rRNA gene sequence homology with *P. acanthamoebae* (46). The ecologic loci and prevalence of the *Parachlamydiaceae* are unknown, but the latter could be underestimated, as this fastidious gram-negative bacteria was recovered only by amoebal cultures, a procedure not performed routinely on clinical samples. Moreover, these *Chlamydia*-like organisms have potential for widespread dissemination, as they are mostly endosymbionts of *Acanthamoeba*, a free-living amoeba with worldwide distribution (27).

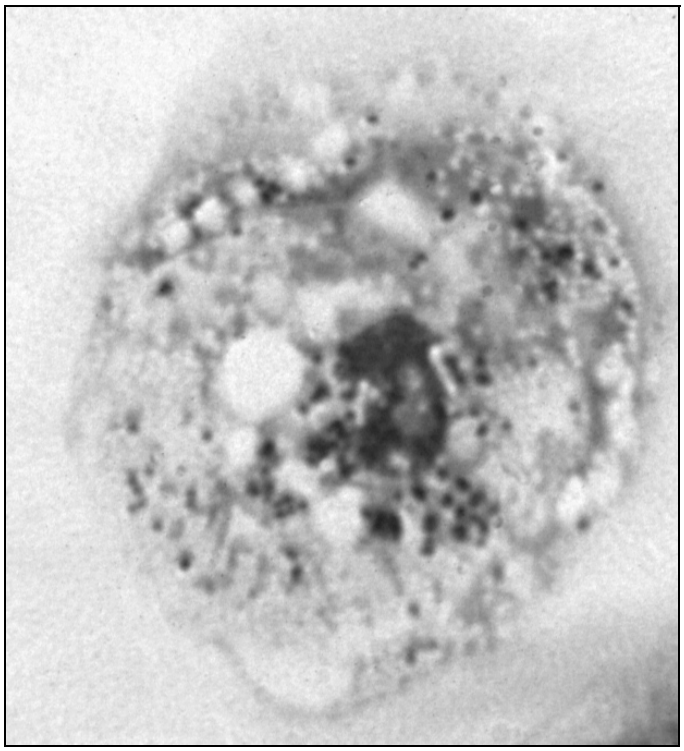

Figure 1. Hall's coccus within *Acanthamoeba polyphaga.* Diff Quick staining (Dade, Boehring, Paris, France). Magnification X 1,000.

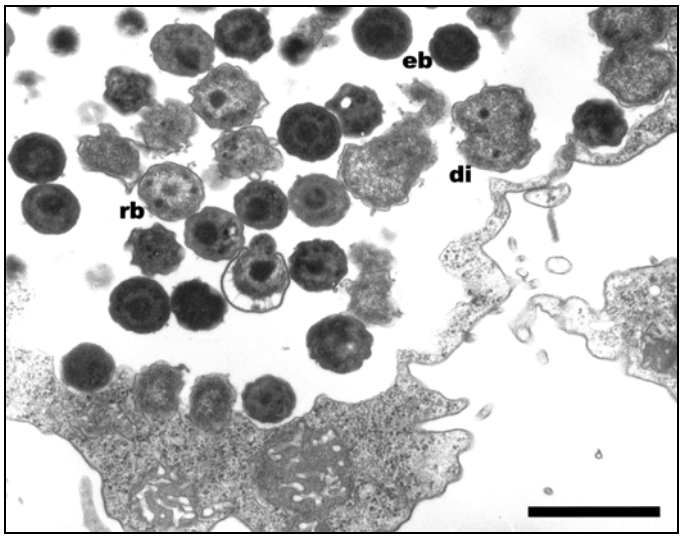

Figure 2. Hall's coccus within *Acanthamoeba polyphaga.* Electron microscopy, magnification X 12,000, bar = 1 µm.

Strains of *Parachlamydiaceae*

Nine strains of *Parachlamydia* have been described (Table). The first, *P. acanthamoebae,* was identified within *Acanthamoeba* BN9, an amoeba recovered from the nasal mucosa of a female volunteer (17). Its 16S rRNA sequence had 88.2% homology with *Simkania negevensis* and 87% homology with *Chlamydophila pneumoniae* (17). The second, Berg17 endosymbiont, also isolated from the nasal mucosa of a female volunteer, seems to have an rRNA signature similar to that of the Bn9 endosymbiont, as demonstrated by the binding of the $Bn9_{658}$ hybridization probe designed for in situ identification of *P. acanthamoebae* (17). The third, Hall's coccus, was found in an *Acanthamoeba* isolated from water taken from a humidifier in a case of humidifier-associated fever in Vermont (16). Its 16S rRNA gene sequence had >99% similarity with that of Bn9 endosymbiont and 86% to 87% with those of four *Chlamydia* species (16). Two additional *Parachlamydiaceae,* UWE1 and UWE25, were also found to infect *Acanthamoeba.* Both amoeba strains were recovered from soil samples from Washington State (46). A sixth strain, UWC22 endosymbiont, infected an *Acanthamoeba* recovered from infected corneal tissues (46). TUME1 endosymbiont was found in an amoeba recovered from municipal sewage sludge in Germany (46). The eighth strain, *Neochlamydia hartmannellae*, is the only strain of *Parachlamydiaceae* isolated from *Hartmanella vermiformis.* It did not grow on *Acanthamoeba* sp. or *Naegleria*, and its 16S rRNA gene sequence had only 92% homology with that of *P. acanthamoeba* and varied from 91.6% to 97.1% with the four latter endosymbionts of *Acanthamoeba* (45). The last one, CorvenA4, could not be isolated. Only its 16S rRNA sequence was retrieved from a respiratory sample (47).

Pathogenicity

Rationale for Potential Pathogenicity

Intra-amoebal growth may increase the virulence of some intracellular bacteria (39), prompting concern that other intracellular bacteria recovered from amoebae, such as the *Parachlamydiaceae,* could be pathogenic. Indeed, a bacterium able to survive exposure to the lytic enzymes of amoebal phagolysosomes would probably also survive the lytic activity of macrophages. This hypothesis is supported by the fact that mutants of *Legionella* that have similar cytotoxic defects and intracellular replication in mammalian macrophages and protozoa have been isolated (48), suggesting a common adaptive mechanism to the intracellular environment. Moreover, *Parachlamydia* can adapt to mammalian cells, as demonstrated by successful passage from an amoebal host to Vero cells (a monkey cell line) (17). Additional arguments in favor of a pathogenic role of the *Parachlamydiaceae* are that *Chlamydia pneumoniae*, a well-recognized agent of pneumonia, was shown to infect free-living amoebae (19) and that another member of the *Chlamydiales*, *Simkania negevensis* (49,50), which has 88% homology with *P. acanthamoebae* (46), has been shown to cause pneumonia in adults and acute bronchiolitis in infants (51,52).

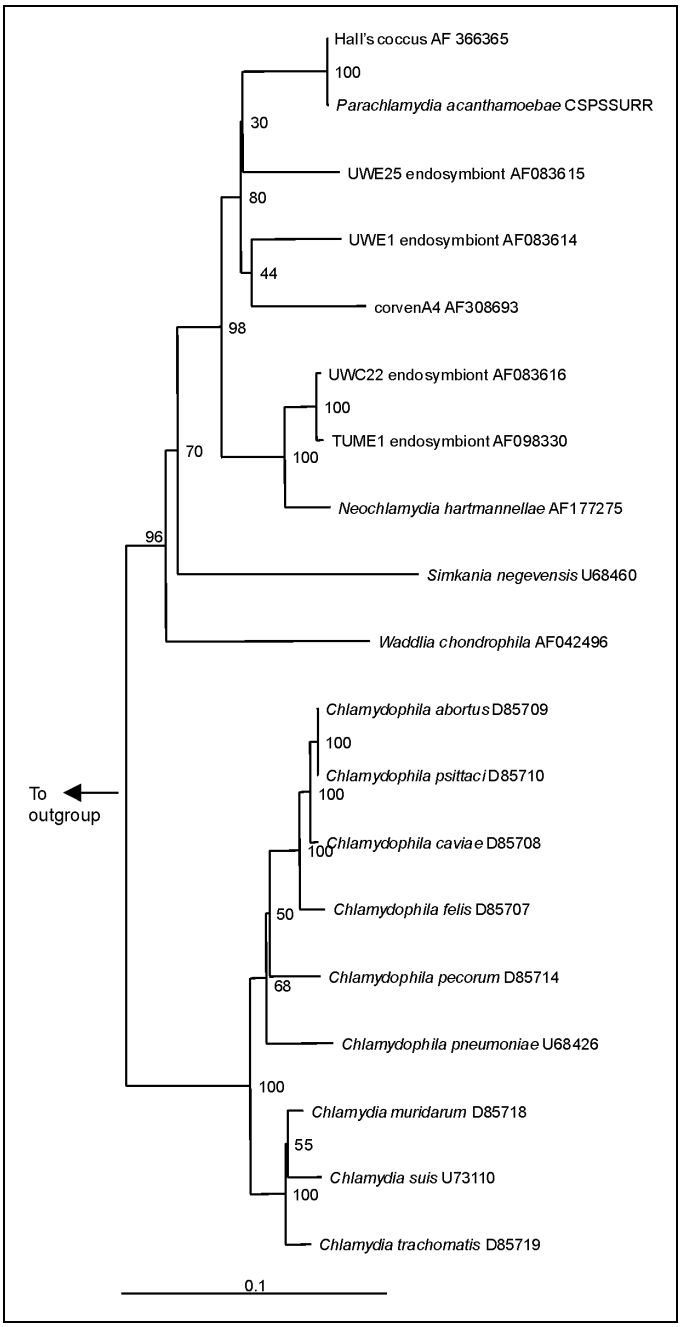

Figure 3. Neighbor-joining phylogenetic tree of the 16s rRNA gene sequence of *Chlamydiales*, including *Chlamydiaceae, Parachlamydiaceae,* and *Simkaniaceae,* compared with *Legionella pneumophila* (M 59157) as outgroup. Bar represents estimated evolutionary distance. The numbers at each node are the results of bootstrap analysis; each value is derived from 100 samples.

Strong evidence that some *Parachlamydiaceae* could be pathogenic came from the identification of Hall's coccus in an amoeba isolated from the source of an outbreak of humidifier-associated fever in the United States, as well as related serologic studies (16). In a study of 500 patients with pneumonia, fourfold rising titers against Hall's coccus were observed in two patients and convalescent-phase antibodies in three others (53). In a second study, two patients had convalescent-phase antibodies (16). These results were recently confirmed: 8 (2.2%) and 3 (0.8%) of 371 patients with community-acquired pneumonia were seropositive (titer >1/50) or had a fourfold rise in *Parachlamydia* antibody titers compared with none of 511 healthy study participants (54). The recent identification of a 16S rRNA gene sequence of *Parachlamydiaceae* from bronchoalveolar lavage provides additional evidence of potential pathogenicity (47). However, the contamination of this specimen by an amoeba harboring the *CorvenA4-Parachlamydia* could not totally be ruled out. These findings should be interpreted cautiously as water contamination probably led to the initial false attribution of *Afipia felis* as the causative organism of cat-scratch disease (55). The identification in respiratory tract specimens of three new *Chlamydia*-like strains, which had phylogeny closer to that of the *Parachlamydiaceae* and *Simkaniaceae* than the *Chlamydia* and *Chlamydophila* (56), is an additional argument in favor of a role of the *Parachlamydiaceae* in the pathogenesis of respiratory diseases.

In addition, a patient with adult Kawasaki syndrome was found to have a fourfold rise in antibody titer to *P. acanthamoebae* (54). A possible relationship between a previous respiratory infection and Kawasaki syndrome has already been reported (57,58). Thus, the role of *Parachlamydia* in the pathogenesis of Kawasaki syndrome should be explored further.

As *Parachlamydia* could potentially be resistant to lytic macrophages enzymes for years, it could enhance chronic inflammatory disease or chronic pathogenic mechanisms, such as the one leading to vascular damage. A role of *Parachlamydiaceae* in the pathogenesis of arteriosclerosis is suggested by

Table. Strains of *Parachlamydiaceae*

Strain	Sample, context and location	Host[a]	% 16S rRNA homology[b] to BN9	% 16S rRNA homology[b] to *C. pneumoniae*[c]	Ref
BN9 endosymbiont	Nasal swab of female volunteer, Germany	*Acanthamoeba* sp. strain BN9	100	87.6	17
Berg17 endosymbiont	Nasal swab of female volunteer, Germany	*Acanthamoeba mauritaniensis*	na[d]	na[d]	17
Hall's coccus	Water sample, humidifier fever, Vermont	*Acanthamoeba* sp.	99.6	87.4	16
UWE1 endosymbiont	Soil samples, Washington State	*Acanthamoeba* sp. strainUWE1	93.7	86.6	46
UWE25 endosymbiont	Soil samples, Washington State	*Acanthamoeba* sp. strain UWE25	93.2	86.8	46
UWC22 endosymbiont	Infected corneal tissues, Washington State	*Acanthamoeba* sp. strain UWC22	91.3	87.3	46
TUME1 endosymbiont	Municipal sewage sludge, Germany	*Acanthamoeba* sp. strain TUME1	91.0	87.2	46
Neochlamydia hartmannellae	Water system of a dental unit, Germany	*Hartmanella vermiformis*	91.5	86.8	45
CorvenA4	Bronchoalveolar washing, France	na[e]	91.4	85.0	47

[a]Bacterial strains were identified in free-living amoebae, isolated by culture on nonnutrient agar.
[b]Estimated with Clustal W [63] available on the website of Pôle Bio-Informatique Lyonnais, Lyon, France (http://pbil.ibcp.fr/).
[c]16S rRNA of *Chlamydophila pneumoniae* strain N16 (GenBank accession number U68426).
[d]Berg17 endosymbiont was shown to have a similar rRNA signature from Bn9 endosymbiont (binding of the $Bn9_{658}$ hybridization probe designed for in situ identification of *Parachlamydia acanthamoebae*); however, the 16S rRNA sequence of that strain is not available.
[e]Direct polymerase chain reaction amplification and sequencing from DNA extracted from the respiratory sample; no strain was isolated.

the presence in an abdominal aneurysm specimen of a *Chlamydia*-like strain that had a sequence closer to that of *P. acanthamoebae* than to *Chlamydia*, *Chlamydophila,* and *Simkaniaceae* (56). Some serologic studies have suggested that *Chlamydophila pneumoniae* could play a role in the pathogenesis of arteriosclerosis (59,60), although this observation was not confirmed in other studies (61,62). Such a discrepancy might result from serologic cross-reactions or confounding by a pathogen such as *Parachlamydia,* which in light of its homology could share epitopes, mode of transmission, or both with *C. pneumoniae*.

Based on this rationale, one may hypothesize that some *Parachlamydiaceae* could cause pneumonia. Thus, patients with nosocomial or community-acquired pneumonia of unknown etiology should ideally receive an extensive diagnostic work-up, including testing for *Parachlamydia*. In addition, patients with arteriosclerosis and Kawasaki disease or other infectious syndromes of unknown etiology should perhaps be tested for *Parachlamydia*. As *Parachlamydia* strains were all identified within free-living amoebae, recent history of swimming in ponds, rivers, or swimming pools might prompt a specific diagnostic approach.

Diagnostic Methods

No diagnostic tool is commercially available. Because of the fastidious nature of *Parachlamydiaceae*, molecular biology is probably the easiest and cheapest diagnostic approach. Serologic testing is also promising; however, it requires antigen and a laboratory capable of performing amoebal coculture. Serologic results may be useful for epidemiologic studies, as they may provide information on past or present contact with the antigen. Both molecular and serologic methods may yield results in <24 hours.

Although time-consuming, culture-based diagnostic methods have the advantage of enabling the recovery of strains. These methods encompass two main approaches. The first one directly targets the recovery of *Parachlamydiaceae,* with amoebae used as cell background. A convenient broth for amoebal coculture is Page's modified Neff's amoeba saline (PAS) (10), which is preferable to Nelson's and peptone-yeast extract-glucose medium because PAS is devoid of nutrients, thus reducing overgrowth of potential contaminants in clinical samples. Although incubation at 37°C may be ideal for bacterial recovery, lower temperatures (30°C–35°C) are generally used to prevent amoebal death or encystment (12,13,20). The coculture should be examined regularly for amoebal lysis or Gimenez-positive cocci. The second culture-based method is designed to recover free-living amoebae, which will then be examined for the presence of endocytobionts. Briefly, amoebal culture is performed by adding the clinical sample to nonnutrient agar (1.5 g agar in 100 mL PAS) supplemented with living *Enterobacter cloacae* or *Escherichia coli*, incubating at 25°C–30°C, and examining the plate daily for the presence of amoebae. To date, all *Parachlamydiaceae* strains have been recovered by the second approach.

Future Directions

The role of *Parachlamydia* sp. as an emerging pathogen needs to be confirmed. In view of the genetic diversity of the *Parachlamydiaceae* (46), their phylogeny needs to be elucidated, as the various species could be associated with species-specific pathogenicity. Search for additional *Parachlamydia* strains in hospital water systems could help define potential nosocomial exposures. Because the *Parachlamydiaceae* are difficult to culture, simpler approaches are being developed, including serologic and molecular tests. These methods could

be performed on a large number of samples from both healthy and ill persons. Patients with community-acquired pneumonia, nosocomial pneumonia, Kawasaki disease, and arteriosclerosis should be tested. Increased resistance to antimicrobial drugs, which may be associated with intra-amoebal growth, is another promising area for future study.

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Synopses. 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 of first author—both authors if only two.

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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

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Three Drinking-Water–Associated Cryptosporidiosis Outbreaks, Northern Ireland

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Three recent drinking-water–associated cryptosporidiosis outbreaks in Northern Ireland were investigated by using genotyping and subgenotyping tools. One *Cryptosporidium parvum* outbreak was caused by the bovine genotype, and two were caused by the human genotype. Subgenotyping analyses indicate that two predominant subgenotypes were associated with these outbreaks and had been circulating in the community.

Human cryptosporidiosis is predominantly caused by the human and bovine *Cryptosporidium parvum* genotypes, which differ in host range; the former infects mostly humans under natural conditions, and the latter infects both humans and some farm animals such as cattle, sheep, and goats (1). In many geographic areas, both *C. parvum* transmission cycles can occur in humans, but the importance of each genotype as a source of human infection probably varies (2–4). Both genotypes have been involved in waterborne outbreaks of human cryptosporidiosis in the United States, Canada, and the United Kingdom (2,5,6).

From April 2000 to April 2001, three drinking-water–associated outbreaks of cryptosporidiosis occurred in Northern Ireland. These outbreaks were epidemiologically unrelated and originated from geographically separate areas. Concerns have been raised about a possible relationship between *C. parvum* genotypes and subgenotypes associated with these outbreaks. In this study, for genotyping analysis, we investigated these outbreaks using a small subunit rRNA (SSU rRNA)-based polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotyping tool, as well as the *Cryptosporidium* oocyst wall protein (COWP) PCR assay. For subgenotyping analysis, sequence typing of the 60-kDa glycoprotein (GP60) was used.

The Study

The three drinking-water–associated outbreaks occurred in the greater Belfast area. Outbreak A occurred during April and May 2000; at least 129 cases were laboratory confirmed. Outbreak B occurred in August 2000, involving at least 117 cases. Outbreak C occurred in April 2001; at least 230 people were infected (7–9; unpub. data). An outbreak patient was defined as a person with microscopically confirmed *Cryptosporidium* infection who became ill during the outbreak period and who was a resident in the water supply areas. The attack rates for outbreaks A, B, and C were 34, 180, and 58 cases/100,000 persons, respectively. Outbreak B was thought to be caused by the ingress of human sewage from a septic tank into the drinking water-distribution system and C from the ingress of wastewater from a blocked drain.

For molecular analysis, 34, 42, and 44 microscopically positive stool samples from outbreaks A, B, and C, respectively, were used. One wastewater sample from a blocked drain implicated in outbreak C was also analyzed. Control isolates of the *C. parvum* genotypes were also included in the subgenotyping analysis. Fourteen control isolates were from sporadic *C. parvum* infections of the bovine genotype in a rural area in the west of Ireland about 100 miles from Belfast, where the water supply was entirely different. Ten control isolates were from sporadic *C. parvum* infections of the human genotype in northwest England during the same time as outbreak C.

C. parvum genotype in human fecal samples was first determined by a COWP gene-based PCR-RFLP tool (10). Oocyst suspensions were prepared from feces by using salt flotation (11). The oocysts were washed and resuspended in deionized water and stored at 4°C before use. To extract DNA, oocyst suspensions were incubated at 100°C for 60 minutes, digested with proteinase K (3 mg/mL) in lysis buffer at 56°C for 30 minutes, and extracted by spin-column filtration (QiAMP DNA kit, Qiagen, Crawley, UK). Extracted DNA was stored at -20°C before use. Genotypes were investigated by using the COWP gene primers cry15 and cry9 to amplify a 553-bp region, which was then subjected to endonuclease digestion by *Rsa*I (10).

Genotypes were confirmed by using an SSU rRNA-based PCR-RFLP tool (12). Subgenotyping was done by sequence analysis of the GP60 gene (13). Before molecular analysis, the wastewater sample was processed by both salt flotation (11) and immunomagnetic separation (Dynal, Lake Success, NY), following the manufacturer-recommended procedures (14). Both genotyping and subgenotyping tools used nested PCR amplification of targeted genes. The primers used for GP60 were 5′-ATA GTC TCC GCT GTA TTC-3′ and 5′-TCC GCT GTA TTC TCA GCC-3′ for primary PCR and 5′-GGA AGG AAC GAT GTA TCT-3′ and 5′-GCA GAG GAA CCA GCA TC-3′ for secondary PCR. The PCR reaction contained 1X Perkin-Elmer (Norwalk, CN) PCR buffer, 3 mM $MgCl_2$, 200 µM (each) deoxynucleoside triphosphate, 200 nM of the forward and reverse primers, 5 units of *Taq* polymerase, and 0.5–2 µL of DNA template (for primary PCR) or 2 µL of primary PCR product (for secondary PCR) in a total 100-µL reaction mixture. Each PCR reaction was then subjected to 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45

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seconds, and extension at 72°C for 60 seconds, with an initial denaturation at 95°C for 3 minutes and a final extension at 72°C for 10 minutes. PCR products were sequenced in both directions on an ABI3100 (Applied Biosystems, Foster City, CA) with forward and reverse primers. An additional sequencing primer (5′-GAG ATA TAT CTT GGT GCG-3′) was used in the sequencing of GP60 PCR products. We aligned the study's GP60 nucleotide sequences with each other and with sequences from the GenBank database with GCG software (Genetics Computing Group, Madison, WI). A neighbor-joining tree was constructed from the aligned sequences as described (15).

Thirty-three of the 34 stool samples from outbreak A were amplified by both the COWP and SSU rRNA-based nested PCRs. RFLP analysis of the PCR products showed that all 33 PCR-positive samples had the *C. parvum* bovine genotype. Thirty-two of the 42 stool samples from outbreak B were also positive by PCR, and all belonged to the *C. parvum* human genotype. Furthermore, in outbreak C, 36 of 44 samples had the *C. parvum* human genotype, and 8 had the bovine genotype. After further epidemiologic investigations, these eight bovine genotypes, although submitted to the primary diagnostic laboratory at the same time as the human genotypes, were considered contemporary sporadic cases and not part of outbreak C. These patients did not live in the distribution area of the water supply implicated in the outbreak. The patients lived in County Down (South Down), whereas the outbreaks occurred in south Antrimy and north Down. Results of the two genotyping methods were in complete agreement in both detection rates and genotyping result.

Subgenotype analyses of the GP60 gene showed that of the 30 stool isolates of the *C. parvum* bovine genotype examined for outbreak A, 25 isolates belonged to a single GP60 subgenotype and 5 isolates belonged to another subgenotype. In contrast, 14 samples of the *C. parvum* bovine genotype isolated from sporadic cases of human cryptosporidiosis from the west of Ireland, which were unrelated to any of the Northern Ireland outbreaks, belonged to nine subgenotypes. Subgenotype analysis of 31 stool samples from outbreak B showed the presence of only one subgenotype of the *C. parvum* human genotype. For outbreak C, all 36 *C. parvum* human genotype stool isolates were identical to the subgenotype involved in outbreak B. In addition, all eight *C. parvum* bovine genotype stool isolates, which were contemporary with, but not from, the area affected by the outbreak, were identical to the predominant subgenotype in outbreak A. The wastewater sample from the blocked drain implicated as the cause of outbreak C contained oocysts of the same subgenotype as the *C. parvum* human genotype. Of the nine sporadic isolates of the *C. parvum* human genotype from northwest England, eight belonged to the same subgenotype as the *C. parvum* human genotype involved in outbreaks B and C (Figure). Most infected persons each had only one genotype/subgenotype of *C. parvum*, judged by the RFLP profile, the absence of underlying signal in the chromatogram of the sequencing result, and at least five independent PCR analyses of each sample. The SSU rRNA technique can detect multiple *Cryptosporidium* parasites in individual samples (16).

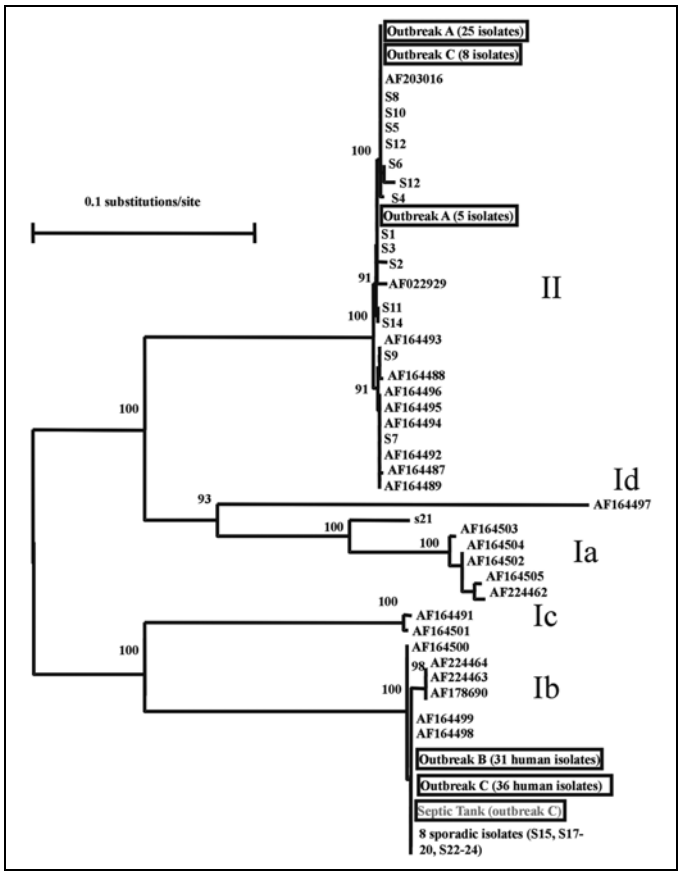

Figure. Genetic relationship among *Cryptosporidium* parasites found in three Northern Ireland outbreaks (outbreaks A, B, and C), sporadic cases in the west of Ireland (S1 to S14) and the northwest of England (S15 to S24), subgenotypes described by Strong et al. (11), and an unpublished sequence (AF203016) from the GenBank database. The isolates with accession numbers were mostly humans and cattle from the United States with the exception of AF164488, AF164492, and AF164493, which were isolated from humans in Zaire, Peru, and Brazil, respectively, but had been passaged in calves in the United States. Nomenclature for groups of subgenotypes is adapted from Strong et al.: Ia, Ib, Ic, and Id for subgenotypes of the *C. parvum* human genotype and II for subgenotypes of the *C. parvum* bovine genotype (11). Data presented are a neighbor-joining tree of GP60 sequences.

Discussion

Results of genotyping analysis support epidemiologic observations that these three drinking-water–associated outbreaks of cryptosporidiosis in Northern Ireland were unrelated, although they all occurred in the greater Belfast area over a 1-year period. Outbreak A was caused by the *C. parvum* bovine genotype, and outbreaks B and C were caused by the *C. parvum* human genotype. The occurrence of the *C. parvum* human genotype in outbreaks B and C suggests that these two outbreaks were, at least in part, caused by contamination of the drinking-water supply by seepage of raw sewage and through wastewater into the drinking water distribution systems, respectively. This finding illustrates the value of timely genotyping analysis during outbreak investigations. The source of contamination is further

supported by subgenotyping analysis of the wastewater sample from the blocked drain that was epidemiologically implicated in outbreak C. This sample contained one subgenotype of the *C. parvum* human genotype indistinguishable from the subgenotype found in most infected persons.

The failure to detect *Cryptosporidium* in 10 of the microscopically positive samples in outbreak B was most likely not because of rare *Cryptosporidium* genotypes; the SSU rRNA technique is *Cryptosporidium* genus specific and detects all known *Cryptosporidium* spp. (12,14–16). The presence of PCR inhibitors in the extracted DNA may have prevented the detection of *Cryptosporidium* by PCR.

Results of subgenotyping analysis nevertheless indicate that the three recent cryptosporidiosis outbreaks in Northern Ireland were caused by two predominant subgenotypes of *C. parvum* that probably had been circulating in the community before the outbreaks. These two subgenotypes of *C. parvum* are also the most common subgenotypes found in Northern Ireland and northwest England. The human subgenotype was found in 8 of 9 sporadic isolates from northwest England and the bovine subgenotype in 4 of 14 isolates in another part of Ireland.

The two subgenotypes of the *C. parvum* bovine genotype found in outbreak A and concurrent with outbreak C have not been found in most other areas (3,4). The only *C. parvum* isolate identical to one of the subgenotypes is an unpublished sequence (AF2030016) deposited in GenBank (Figure). The source of the other genotype, however, is unknown. In contrast, the subgenotype of the *C. parvum* human genotype involved in outbreaks B and C has a wide geographic distribution, with isolates from United States, Canada, United Kingdom, Portugal, and Peru (3,4). This subgenotype, the most common subgenotype of the *C. parvum* human genotype found in the United States, was responsible for several waterborne and foodborne outbreaks of human cryptosporidiosis (3). This subgenotype has a worldwide distribution and is the cause of many outbreaks. Whether the wide distribution of this subgenotype of the *C. parvum* human genotype and apparent association with multiple outbreaks in geographically distinct areas result from unusual biologic fitness of this parasite is unknown.

Acknowledgments

We thank Mike Mitchell for providing control oocysts. We also thank Anne Thomas, David Gomez, and Xu Jiru for providing technical support; and P. Donaghy, B. Morgan, and B. Smyth for information on outbreaks A, B, and C, respectively.

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Cluster of African Trypanosomiasis in Travelers to Tanzanian National Parks

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Game parks in Tanzania have long been considered to be at low risk for African trypanosomiasis; however, nine cases of the disease associated with these parks were recently reported. The outbreak was detected through TropNetEurop, a sentinel surveillance network of clinical sites throughout Europe.

African trypanosomiasis (sleeping sickness), a serious infection caused by a protozoan (*Trypanosoma brucei*), is usually spread to humans by the tsetse fly via infected animals and humans. Although the World Health Organization has reported a dramatic increase in incidence in Africa, the disease has remained a rare but well-documented cause of fever in travelers returning from endemic areas. In recent years, infection in returning travelers has been more likely to be due to the East African form (caused by *T. brucei rhodesiense*), rather than the West African form (which is due to *T. brucei gambiense*); the latter form causes a fulminant illness for which rapid diagnosis is necessary (1,2). We report details of nine recent cases caused by the West African form of this disease, one fatal; all of the cases occurred in travelers to Tanzanian national parks.

Case Reports

Game parks in Tanzania have long been considered to be low-risk areas for African trypanosomiasis (3). However, in February 2001, two index patients and seven additional European and South African patients were seen with trypanosomiasis acquired in the Tarangire and Serengeti National Parks, Tanzania (4). The patients were identified and reported in TropNetEurop, a sentinel surveillance network of clinical sites throughout Europe for monitoring imported infectious diseases.

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All of the South African patients but one were European nationals (Table). To our knowledge, all patients had traveled to the Tarangire and Serengeti National Parks, in addition to a number of other destinations. This area in East Africa has been implicated as being endemic for African trypanosomiasis. However, the case incidence in Tanzanian and foreign nationals has been very low in recent decades.

During their journey or briefly after their return, the patients, all febrile, were seen by general practitioners or emergency departments. Most patients were seen during the primary stage of disease (patients 1, 3, 4, 6, 7, 8; Table); however, several showed signs of the secondary stage, including cerebral manifestations. Most patients also showed a typical skin lesion, the trypanosome chancre. Diagnosis was established by thin and thick blood film. Although three patients had multiorgan failure, and specific medication was difficult to obtain, drug treatment proved successful in all but one patient, who died. Drugs for treatment were not chosen for the clinical stage the patients exhibited but rather for availability. Thus, patients with complications and a manifest secondary stage of disease received pentamidine only.

Conclusions

The temporal clustering of imported cases suggests a change in the local epidemiology of this disease and may herald further cases in tourists during the current travel season. For 1998, the World Tourism Organization recorded 450,000 visitors to Tanzania (5), for a potential annual incidence of trypanosomiasis in tourists to Tanzania of at least 9/450,000. This is an increase from near zero during recent years to 2/100,000. The risk for those visiting the Tarangire and Serengeti National Parks is obviously higher. Reaction of the Tanzanian authorities involved strengthening installation of insecticide-impregnated locations in Serengeti to include roads, lodges, staff quarters, and campsites. This initial program resulted in a dramatic decline of tsetse flies in Serengeti during the second half of 2001. This effort will have to be sustained by mandatory killing of flies at some keys areas including Serengeti, Tarangire, and Lake Manyara National Parks. The National Medical Research Program has been directed to screen more people for the disease around these foci.

For many of the patients, drugs for treatment were extremely difficult to obtain. For some European patients, treatment with suramin was possible only after informal help from member sites of the network. Drugs for treatment (suramin, melasoprol, and eflornithin) have now been obtained. Surveillance in cattle to establish their role in the epidemiology of the disease will also be conducted (Tanzania Chief Veterinary Officer, pers. comm.).

This report highlights the effectiveness and importance of sentinel surveillance methods for monitoring imported infectious diseases in Europe. TropNetEurop, the network that identified and reported the index cases, is known for its speed of reporting, often within days of diagnosis. The network's use of member sites as regional referral centers is based on an

Table. Patients with African trypanosomiasis, Tanzania

No.	Sex	Age	Nationality	Mo/yr of diagnosis	Clinical details and treatment	Travel history[a]
1	M	33	Italian	02/01	Skin lesion (back), fever, nausea/vomiting; no major complications; treatment with suramin	Tourist: Kenya; Lake Manyara, Serengeti, and Ngorongoro NPs
2	M	32	Italian	02/01	Skin lesion left leg; fever; multiorgan failure; anuria; treatment with pentamidine	Tourist: East Tsavo, Ngorongoro, and Serengeti NPs
3	F	44	British	02/01	Skin lesion left leg; fever; no major complications; treatment with suramin	Tourist: Nairobi, Amboseli, Lake Manyara, Ngorongoro, and Serengeti NPs
4	M	41	Swedish	03/01	Skin lesion right foot; fever; treatment with suramin	Tourist: Lake Manyara, Ngorongoro, Tarangire, and Serengeti NPs
5	M	68	South African	03/01	Fever; renal failure; acidosis; jaundice; DIC; treatment with melasoprol	Tourist: Serengeti NP
6	F	27	Norwegian	03/01	Skin lesion left side of face; fever; no complications; treatment with suramin	Research project on zebras: Ngorongoro and Serengeti NPs
7	M	60	Dutch	03/01	Fever; treatment with suramin	Tarangire NP
8	F	55	Dutch	04/01	Skin lesion left ankle; fever; headache; treatment with suramin	Tarangire NP
9	F	53	Dutch	06/01	Skin lesion right leg; fever; headache; intracerebral manifestation; coma; death; treatment with suramin and melasoprol	Lake Manyara, Ngorongo, and Serengeti NPs

[a]East Tsavo NP and Amboseli NP are in Kenya; all other NPs mentioned are in Tanzania.
NP, national park; DIC, disseminated intravascular coagulopathy.

anonymous reporting system at sentinel clinics. Discussion of the index patients by member sites triggered increased awareness within the network and led to the rapid recording of additional patients and a pattern that might have otherwise gone undetected.

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Dispatches 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.

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Excretion of Vancomycin-Resistant Enterococci by Wild Mammals

David J. P. Mallon,* John E. Corkill,* Sarah M. Hazel,* J. Sian Wilson,* Nigel P. French,* Malcolm Bennett,* and C. Anthony Hart*

A survey of fecal samples found enterococcal excretion in 82% of 388 bank voles (*Clethrionomys glareolus*), 92% of 131 woodmice (*Apodemus sylvaticus*), and 75% of 165 badgers (*Meles meles*). Vancomycin-resistant enterococci, all *Enterococcus faecium* of *van*A genotype, were excreted by 4.6% of the woodmice and 1.2% of the badgers, but by none of the bank voles.

Over the last decade, enterococci have emerged as a major cause of nosocomial infections, ranging from urinary tract and wound infections to life-threatening bacteremia (1–3). Enterococci are well suited as nosocomial pathogens because they readily colonize skin and mucous membranes, survive well in the environment, tolerate temperatures from 10°C to 45°C, survive in acid and alkaline conditions, and are intrinsically resistant to many antimicrobial drugs such as cephalosporins, fluoroquinolones, and aminoglycosides. The importance of this pathogen has been heightened by the emergence of multidrug-resistant enterococci, which has raised the specter of untreatable infections (2,4). Although a number of *Enterococcus* species exist, most human infections are caused by *E. faecalis* and *E. faecium*. *E. faecium* is isolated more frequently in Europe and *E. faecalis* in the United States (1,2,5,6). Among several phenotypes of glycopeptide resistance, VanA (teicoplanin and vancomycin resistance) and VanB (vancomycin resistance only) account for most resistant isolates (1). The genes encoding both VanA and VanB are transferable, inducible, and detectable in both *E. faecalis* and *E. faecium* (7).

We have recently shown that wild rodents can be a reservoir of antibiotic-resistant gram-negative bacteria (8). We now present data on carriage of vancomycin-resistant enterococci (VRE) by wild mammals.

The Study

From 1997 to 2000, fecal samples were obtained from woodmice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) in woodland and on small islands in a lake near farmland in the Wirral, northwest England. We collected 177 fecal samples from bank voles and 13 from woodmice captured on the islands and 211 from bank voles and 118 from woodmice captured in the woodland. Fecal samples from badgers (*Meles meles*) were obtained near their burrows in a 10X10-km area in Cheshire, England. The 165 samples from the badgers were collected in 2000. Fecal samples were stored frozen at -70°C until analyzed.

Isolation was attempted by using both enterococcosel agar and broth, neither incorporating vancomycin (BBL Microbiology Systems, Cockeysville, MD). On thawing, fecal samples were emulsified in an equal volume of sterile saline, and 10 µL was spread onto enterococcosel agar and incubated at 37°C for 48 hours. In addition, approximately 500 µL of each sample was added into enterococcosel broth and incubated at 37°C for 48 hours, after which samples were subcultured onto blood agar plates with a vancomycin disk (5 µg) on the main inoculum. Suspect colonies (esculin positive or from around the vancomycin disk) were further tested to confirm their identity as enterococci (Streptococcal Grouping Kit: Oxoid, Basingstoke, UK; API 20 Strep: Biomerieux, Basingstoke, UK). Provisionally identified VRE were tested for MICs of vancomycin and teicoplanin (E-test, AB BioDisk, Solna, Sweden).

Whole bacterial cell DNA was extracted by suspending 1 µL of pure culture in 250 µL of Chelex-100 (Bio-Rad Laboratories, Hercules, CA) in water slurry (5% wt/vol) and boiling for 10 minutes. Cell debris was removed by centrifugation (13,000 rpm for 15 minutes) and DNA extract stored at -70°C. Genetic identification of enterococci to species level and detection of *van*A, *van*B, *van*C1, or *van*C2/C3 and *van*D ligase genes were performed by using a multiplex polymerase chain reaction amplification (7). This amplification detects both structural genes encoding D-alanine—D-alanine ligases and glycopeptide resistance genes. As controls, strains of *E. faecalis* (NCTC 775) and *E. faecium* (NCTC 12202) and previously characterized strains of *E. faecium* encoding *van*A and *van*B were included. The primers used (7,9) were VANAA1: 5′-GGG AAA ACG ACA ATT GC-3′; VANAA2: 5′-GTA CAA TGC GGC CGT TA-3′; VANBB1: 5′-ATG GGA AGC CGA TAG TC-3′; VANBB2: 5′-GAT TTC GTT CCT CGA CC-3′; VANC1/C1: 5′-GGT ATC AAG GAA ACC TC-3′; VANC1/C2: 5′-CTT CCG CCA TCA TAG CT-3′; VANC23/C1: 5′-CTC CTA CGA TTC TCT TG-3′; VANC23/C2: 5′-CGA GCA AGA CCT TTA AG-3′; VANDD1: 5′-TAA GGC GCT TGC ATA TAC CG-3′; VANDD2: 5′-TGC AGC CAA GTA TCC GGT AA-3′; ddl FAECALIS-E1: 5′-ATC AAG TAC AGT TAG TCTT-3′; ddl FAECALIS-E2: 5′-ACG ATT CAA AGC TAA CTG-3′; Ddl FAECIUM-F1: 5′-GCA AGG CTT CTT AGA GA-3′; and Ddl FAECIUM-F2: 5′-CAT CGT GTA AGC TAA CTTC-3′.

Amplicons were separated by electrophoresis through agarose (1% wt/vol) gels (Sigma-Aldrich, Poole, UK). Gels were stained in ethidium bromide, viewed by UV transillumination, and photographed.

Pulsed-field gel electrophoresis (PFGE) of macrorestricted chromosomal DNA was performed to establish the genetic relatedness of the VRE isolates (10). Bacteria were harvested

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from solid media and embedded in lysozyme (25 mg/mL)–agarose blocks. Released nucleases were neutralized with 25 mg/mL proteinase K (Sigma-Aldrich) at 50°C overnight. Chromosomal DNA was digested with *Sma*I (40 U/block); gel slices were embedded in 1% (wt/vol) PFGE agarose gels (Bio-Rad Laboratories) and separated by electrophoresis on a CHEF 3D system (Bio-Rad Laboratories) in X 0.5 Tris-Borate-EDTA buffer. Electrophoresis conditions were an initial switch time of 1 second with a linear increase to 20 seconds after 20 hours at a buffer temperature of 14°C, 6 V/cm with a pulsing angle of 120°C (10).

Enterococcus spp. were detected in feces from 10 (76.9%) of 13 woodmice and 135 (76.3%) of 177 bank voles on the islands and 110 (93.2%) of 118 woodmice and 185 (87.7%) of 211 bank voles from the woodland site. A total of 123 (74.5%) of badger samples contained enterococci.

VRE were isolated from 6 (5.1%) of 118 woodmice samples from the woodland area only. Two (1.2%) of the badger samples yielded VRE. None of the bank voles at either site were excreting VRE. Seven of the eight VRE were obtained on direct culture on enterococcosel agar. The remaining VRE (from a woodmouse) was obtained from broth culture, as were each of the other VRE also isolated by direct culture. Each of the VREs had vancomycin MICs ≥64 mg/L and teicoplanin MICs 0.75–6.0 mg/L (Table). Biochemical identification with the API 20 Strep did not provide accurate species assignment. Each VRE had the *van*A gene but none of the other resistance genes, and each was confirmed as *E. faecium* by polymerase chain reaction (Figure). PFGE analysis showed that six of the isolates had band patterns differing by ≥10 bands, but two woodmice isolates were closely related, differing by only two bands.

Conclusions

This first study of enterococcal and specifically VRE excretion by wild mammals showed that enterococci appear to be part of the normal flora of most badgers (74.5%), woodmice (92%), and bank voles (82%), like many other animal species (1). We also found VRE in a small number of woodmice (5.1%) and badgers (1.2%). Each of our isolates was *E. faecium,* and each encoded the *van*A but not *van*B, *van*C, or *van*D genes. Although all had MICs for vancomycin ≥64 mg/L, only one had an MIC for teicoplanin of 6 mg/L, which does not correspond to the generally accepted VanA phenotype. However, an isolate of *E. raffinosus* has been described with a VanB phenotype yet *van*A genotype (11). On PFGE of macrorestricted chromosomal DNA, only two isolates (both from woodmice) were genotypically related. The remaining six appeared unrelated. The two related isolates were from different woodmice (tagged on capture with transponders), trapped 6 months apart.

How the mammals acquired VRE and whether they are long-term carriers are less clear. The woodmice and bank voles may have been exposed in the woodland to either avoparcin (a glycopeptide antibiotic related to vancomycin) or fecal material from farm animals; however, avoparcin had not been used for any livestock raised in proximity to the sites, and the samples (including the badger samples) were taken after the use of avoparcin as a growth promoter had been banned. None of the bank voles were excreting VRE, in spite of the fact that they can be reservoirs for enterococci. In general, bank voles tend to be herbivorous and have a limited territory, beyond which they rarely stray. Woodmice and badgers are omnivorous and will travel distances in search of food and territory (12).

In conclusion, we demonstrated that woodmice and badgers can excrete VRE in their feces and may be an unexpected reservoir for such bacteria. However, bank voles occupying the same habitat as the woodmice did not excrete VRE. How long such reservoirs may persist and if the bacteria can be transmitted to other animals, including humans, remain to be determined.

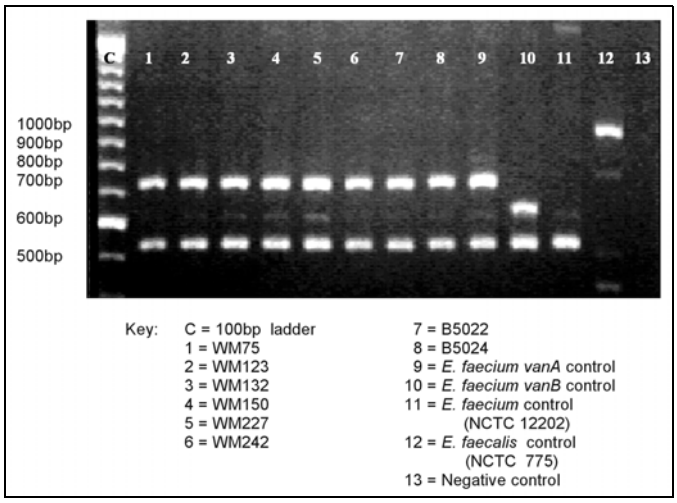

Figure. Polymerase chain reaction analysis of vancomycin-resistant enterococci isolates for glycopeptide resistance genotypes and species identification.

Table. Isolates of vancomycin-resistant enterococci from wild mammals[a]

Isolate identification no.	Vancomycin MIC (mg/L)	Teicoplanin MIC (mg/L)
WM[b] 75	64	2.0
WM 123[c]	>256	1.5–2.0
WM 132	>256	6.0
WM 150	>256	0.75
WM 227[c]	>256	1.5
WM 242	>256	2.0
B 5022	>256	3
B5024	96	3

[a]All isolates in this table are *Enterococcus faecium*, *van*A gene (polymerase chain reaction).
[b]Abbreviations used: WM, woodmouse; B, badger.
[c]Genetically similar by pulsed-field gel electrophoresis of macrorestricted chromosomal DNA (differing by two bands).

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Mr. Mallon is a biomedical scientist working towards a master's degree in medical microbiology. He is interested in the prevalence of vancomycin-resistant enterococci and their isolation from humans, wild and domestic animals, and the environment.

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Fatal Infection of a Pet Monkey with *Human herpesvirus 1*

Hartwig P. Huemer,* Clara Larcher,* Thomas Czedik-Eysenberg,† Norbert Nowotny,‡§ and Martin Reifinger‡

Concerns have been raised about pet monkeys as a potential threat to humans. We report the opposite situation, a danger to pets that arises from humans. Similar to herpesvirus B *(Cercopithecine herpesvirus 1),* which endangers humans but not its host species, *Human herpesvirus 1* can act as a "killer virus" when crossing the species barrier to New World monkeys.

A man was bitten by a marmoset (genus *Callithrix)* that had stomatitis. For exclusion of possible zoonotic pathogens, virus culture was performed on a specimen obtained from the marmoset's oral mucosa. Virus isolation and typing with antibodies revealed *Human herpesvirus 1* (HHV-1) infection, confirmed by type-specific polymerase chain reaction (PCR). Despite treatment, the monkey died 2 days after the sample was drawn. Standard veterinary practice is to consider whether diseases of primates that have been in close contact with humans might have been caused by human viruses. Acute stomatitis in pet monkeys can suggest HHV-1 infection, among other diseases, and systemic treatment with acyclovir may be appropriate.

Case Report

A 2-year-old male marmoset (*Callithrix jacchus)* was brought to a veterinary clinic with a 6-day history of severe necrotizing stomatitis, vomiting, and loss of appetite. The pet had been acquired by its owner 9 months earlier from an unknown source. Since then, it had usually been in close contact with its owner; she kept the pet on a leash and carried it directly on her body. A few days before being seen at the clinic, the animal had bitten a male visitor's hand. Treatment of the marmoset included removal of the necrotic mucosal surface under anesthesia, local administration of acyclovir, and systemic application of antiemetic, antiphlogistic, and antibiotic agents. For diagnosis and exclusion of a possible zoonotic infection, a few samples of the altered oral mucosa were taken. Two days after veterinary intervention, the marmoset died. The owner refused a necropsy. Since communication with the owner ceased before diagnosis, questions about possible herpetic lesions on her or her guest who had been bitten by the monkey could not be answered.

One specimen of the mucosal membrane was fixed in 10% buffered formalin, dehydrated in ethanol, cut into 4-mm sections, and stained with hematoxylin and eosin. Histologic examination showed severe necrotizing stomatitis with purulent inflammation and bacterial colonization of the debris. No epithelium remained nor any morphologically visible indication of a specific infection.

Another specimen of the oral mucosa was homogenized in sterile phosphate-buffered saline, and the supernatant was used for cell culture and PCR analysis. Virus culture was performed on Vero cells originating from African green monkey kidney tissue (ATCC # CCL-81). One to two days later, a typical cytopathic effect was visible, consisting of plaques and cell rounding (Figure), which led to total detachment of the cells within 3 to 4 days.

Cells were fixed with acetone/methanol, and immunofluorescence staining was carried out by using monoclonal and polyclonal antibodies against different species of *Alphaherpesvirinae,* including HHV-1 and -2, suid (SuHV-1), equid (EHV-1 to -4), bovine (BoHV-1), and nonhuman primate (CeHV-1) viruses.

Positive staining was obtained with several monoclonal anti-HHV-1 antibodies directed against major glycoproteins (gC, gD, gE) as well as nonstructural proteins (infectious cell protein 0). Because reaction was also found to type-specific monoclonal antibodies such as HC1, HC2, and HC3 (1), raised against gC of HHV-1, that virus was identified as the etiologic virus type. No cross-reactivity was observed with antisera against other species of herpesviruses, except a distinct reaction with a polyvalent anti-CeHV-1 antiserum due to the well-known cross-reactivity between HHV-1 and CeHV-1.

To discriminate between HHV-1 and HHV-2, type-specific PCR was performed according to the protocol of Piiparinen and Vaheri, using their published primers (2). Amplification products were detected in a 2% agarose gel stained with SYBR-Green. A 229-bp fragment was amplified, indicative of HHV-1 in the patient sample (lane S). Lanes 1 and 2 represent amplification products of HHV-1 strain Wal (229 bp) and HHV-2 strain D316 (241 bp), respectively (Figure).

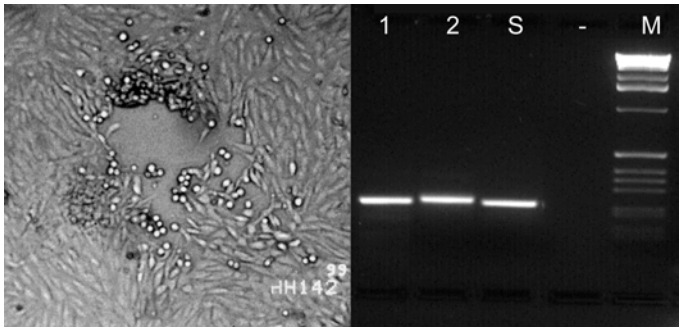

Figure. Left: cytopathic effect in Vero cells consisting of a plaque and rounding of the cells after homogenized altered mucosal membrane of the marmoset was added to the cell culture. Right: type-specific polymerase chain reaction (PCR). Lanes 1 and 2 show fragments of 229-bp DNA amplified from *Human herpesvirus 1* (HHV-1) and 241 bp from HHV-2 control strains, respectively. Lane S shows an HHV-1–specific PCR product amplified from an oral mucosa specimen of the marmoset; no product was obtained from supernatants of uninfected cell culture (lane -). Lane M, 1 kb DNA Ladder (GIBCO/BRL,Grand Island, NY).

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Additionally, a multiplex PCR reaction detecting HHV1-6 (including HHV-3, also called Varicella-zoster virus 1; HHV-4, commonly known as Epstein-Barr virus; and HHV-5, human cytomegalovirus) was performed by using the primer setup described by Tenorio et al. (3). When these authors' published set of primers was used, an HHV-1–specific fragment was also amplified (data not shown). Together with the reaction with different HHV-1–specific antibodies, including subtype-specific monoclonal antibodies, this was a clear indication of HHV-1 virus's being the causative agent, excluding other possible primate herpesviruses.

Discussion

The increasing number of pet monkeys kept in households in the United States has prompted concerns about the potential for transmission of the primate herpesvirus (formerly SHBV, now termed nonhuman primate virus or CeHV-1). Unlike the situation in the natural host, CeHV-1 can cause fatal encephalitis in humans. Persons working with certain macaque species may be at particular risk (4).

In this report, we describe the reverse problem—the growing evidence that human herpesviruses endanger other primates (5). Several nonhuman primate species have been reported to be susceptible to infection with human alphaherpesviruses. Experimental infections have been performed in owl monkeys, Cebus monkeys, Cotton-head Tamarins, and White-fronted Capuchins (6–8). Moreover, HHV-1 can naturally pass to primitive primates such as tree shrews (9).

In Old World primates, reports of human HHV-1 infections (10–12) indicate a virus-host relationship similar to that in humans, although sporadic fatal cases have been described, mainly in very young animals (13,14). In New World monkeys, however, HHV-1 seems to act more like a CeHV-1–type "killer virus" (5,15). The case presented here is the third confirmed case of naturally transmitted HHV-1 infection in marmosets (15–17), and several other cases have been suspected to be of similar origin (7,18–21).

An outbreak of a fatal HHV-1 infection was observed recently in a group of common marmosets *(C. jacchus*) housed as a family group at the German Primate Center, Göttingen, Germany (K. Mätz-Rensing et al., unpub. data). All marmoset family members died within 3 days, indicating that HHV-1 has the capability to spread from monkey to monkey (K.D. Jentsch, pers. comm.).

We were not able to determine an HHV-1 history for the people in our case's immediate surroundings, but a negative history would not be informative because asymptomatic shedding often contributes to HHV-1 transmission.

Because of the frequent but inapparent shedding of herpesviruses, direct contact of infected humans to animals should be limited. We suggest that keeping primates should be restricted to specialists, who are aware of the reciprocal hygienic risks. Use of gloves and eye protection, and even masks if aerosol transmission is suspected, is mandatory in standard biosafety precautions used in laboratory animal facilities. For more information, view the biosafety manual prepared by the Centers for Disease Control and Prevention and the National Institutes of Health at http://www.orcbs.msu.edu/biological/BMBL/BMBL-1.htm. Recommendations for precautions to be taken with any pet can be found at http://www.ahc.umn.edu/rar, http://www.cdc.gov/ncidod/op/pets.htm, and at http://www.cdc.gov/hiv/pubs/brochure/oi_pets.htm.

In contrast, increasing numbers of websites praise a vast variety of exotic pets and only rarely mention the potential health hazards (especially to children) from close contact or keeping pet monkeys in the household. Careful animal handling and certain hygienic restrictions should be strongly recommended for the sake of both owners and pet monkeys.

Dr. Huemer is associate professor of hygiene, microbiology, and preventive medicine at the University of Innsbruck. His research focus is mainly in the fields of virology and immunology.

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Serologic Evidence of Human Granulocytic Ehrlichiosis, Greece

To the Editor: Human granulocytic ehrlichiosis (HGE), a tickborne infectious disease, was first described in 1994 (1). Several cases have been reported in the United States; reports of acute cases in Europe have been rare, although European serosurveys of the prevalence of antibodies to the HGE agent have been conducted (2–4). No similar serosurvey has been conducted in Greece, although *Ixodes ricinus*, thought to be the principal tick vector in Europe (5), is present in northern Greece (6). Lyme disease, which is transmitted by the same tick, has never been reported, and the seroprevalence of Lyme borreliosis in Greece is very low (7).

We examined sera of 300 persons (100 men and 200 women) ages 15–78 years (mean age ± standard deviation 52.7±18.0 years), which were collected at six county hospitals in northern Greece and sent to our laboratory from April to October 2000. The participants were mostly farmers, all of whom lived in rural areas of northern Greece. All participants were healthy and had been hospitalized for routine blood tests. Each patient completed a questionnaire about medical history. The selected patients had no known history of rickettsiosis and reported no febrile or influenza-like illness during the past 6 months. Each participant provided oral consent for the serum to be used for detecting antibodies against several infectious agents related to zoonoses. The following information was recorded for each participant: age, sex, occupation, and area of residence.

Serum samples were tested by indirect immunofluorescence (IFA) with commercially available antigen (Focus Technologies, Cypress, California), which uses HGE-1–infected HL60 cells. Titers ≥64 were considered positive. All sera were also tested for *Rickettsia conorii*, *R. typhi*, *Coxiella burneti,* and *Ehrlichia chaffeensis* by IFA and for *Borrelia burgdorferi* by enzyme-linked immunosorbent assay and Western blot. Sera that reacted positively to more than one of these agents were excluded. Biostatistical analysis was performed by using the statistical package SPSS for Windows 10.0.1 (Standard version, SPSS Inc., Chicago, IL).

The overall prevalence of antibodies to the HGE agent was 7.3% (8.0% for men and 7.0% for women). No statistically significant differences were observed in the prevalence of antibodies in the six prefecture hospitals. Participants had no statistically significant differences in sex or age. Antibody titers to HGE were low (of 22 positive sera, 12 had titers ≥64 and 10 had titers ≥128).

Several serosurveys of the prevalence of antibodies to the HGE agent have been conducted across Europe in both healthy persons and patients with suspected or confirmed Lyme borreliosis (2,3,8). Since cases of *B. burgdorferi* infection are rare or nonexistent in Greece and the seroprevalence of Lyme borreliosis is very low, we selected as participants 300 healthy farmers who lived in rural areas. These persons compose a group at high risk for exposure to tick bites and therefore to *I. ricinus*. Our prevalence is higher than those observed in Bulgaria (2.9%) and Germany (1.9%) (2,3). This finding could be attributed to the fact that the prevalence in these countries was based on blood donors, unlike our survey. However, our prevalence is substantially lower than that in Slovenia, where 15.4% of the examined population had detectable antibodies to the HGE agent and several cases of HE have been confirmed (4). Our observation that no significant differences occurred in the prevalence of antibodies to the HGE agent in the six prefectures studied could be explained by the fact that these districts are small, with little variation in environmental and climatic conditions. Even though the antibody titers to the HGE agent were low in our survey, they suggest infection at an undetermined time (9). Seven of our sera were antibody positive to both the HGE agent and at least one other rickettsial agent or *B. burgdorferi*. This fact, which has been observed elsewhere (9), may result from coinfection or crossreaction. These sera were excluded. Our data suggest the possibility that HGE cases exist in Greece. Since such cases have been not been reported to date, they are likely underdiagnosed. Further research is needed to clarify the presence of the HGE agent in Greece.

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Hantavirus Infection with Marked Sinus Bradycardia, Taiwan

To the Editor: Hantaviruses are enveloped RNA viruses belonging to the family *Bunyaviridae* (1,2), for which a number of species have been identified, including the Hantaan, Seoul, Puumala, Dobrava-Belgrade, and Sin Nombre viruses (1,2). Each hantavirus is associated with a specific rodent reservoir (1,2). *Hantaan virus*, found throughout northeastern Asia, causes a life-threatening illness known as hemorrhagic fever with renal syndrome (HFRS). Main symptoms and signs of HFRS are fever, myalgia, severe vascular leakage with ascites and retroperitoneal edema and pain (abdominal, loin, or headache), shock, acute renal failure, proteinuria and hematuria, thrombocytopenia, and bleeding complications (3). *Seoul virus*, found worldwide, and *Puumala virus*, found in Scandinavia and Eastern Europe, cause mild forms of HFRS. *Sin Nombre virus*, found in the United States, causes hantavirus pulmonary syndrome, which is characterized by increased pulmonary capillary permeability and pulmonary edema and can progress to severe respiratory distress syndrome and shock as a result of low cardiac output (4,5).

Despite the fact that HFRS is frequently reported in People's Republic of China, no indigenous cases of HFRS have been reported in Taiwan. Previous serologic studies found that the Seoul strain is endemic in the areas of Taiwan and two isolated islands nearby, Kinmen and Matzu; in contrast, in the People's Republic of China, the Hantaan and Seoul strains concurrently predominate (6,7).

Our patient, a 38-year-old man, had onset of sore throat, headache, cough, myalgia, and intermittent fever (up to 38.3°C) on February 2, 2001. A resident of Matzu for more than 30 years, he had traveled to the People's Republic of China 3 months before the symptoms began. Laboratory tests at a local hospital showed thrombocytopenia (58,000/mL) and leukopenia (3,800/mL). Because his symptoms persisted, he was transferred to the National Taiwan University Hospital on February 7, 2001. Initial tests there showed a temperature of 36.4°C, heart rate 74 beats/min, and respiratory rate 18/min; there was no skin rash. The rest of the physical examination was normal. He had a platelet count 73,000/μL; leukocytes 5,670/μL with 59.1% segments, 19.8% lymphocytes, and 18.2% monocytes; urea nitrogen 7.4 mg/dL; and creatinine 0.94 mg/dL. Urinalysis showed proteinuria (300 mg/dL). His chest radiography was normal. Abdominal ultrasound showed a fatty liver.

After admission, the patient's laboratory values gradually improved and his proteinuria subsided. He had no fever. On February 10, 2001, he had marked sinus bradycardia (as low as 33 beats/min) and became fatigued. His blood pressure was 120–130/70–80 mmHg. No abnormal serum electrolytes, urea nitrogen, creatinine, creatine kinase, and troponin-I were noted. Echocardiogram showed normal atrium and ventricle size, good left ventricle contractility, and small amount of pericardial effusion. His heart rate gradually increased. He was discharged on February 15, 2001, without event.

A substantial increase of serum immunofluorescent immunoglobulin (Ig) G titers (1:640 on February 6; 1:5120 on February 19, 2001) and positive IgM titers of 1:80 against hantavirus antigen (Seoul type) confirmed that this virus was responsible for the illness.

A few reports of hantavirus infection with cardiac involvement have been published. A case report by Chun and Godfrey showed right atrium dilation with diffuse atrial hemorrhage, interstitial edema, and vascular congestion without surrounding myocardial fibers and conduction system involvement in a 19-year-old soldier who died from epidemic (Korean) hemorrhagic fever, sinus tachycardia, paroxysmal supraventricular tachycardia, and congestive heart failure (8). Marked sinus bradycardia (as low as 34 beats/min) in a patient with a severe form of hemorrhagic fever with renal syndrome (acute renal failure) has been reported (9). However, this finding was not observed in patients with mild cases of the disease.

The possibility that our patient acquired the infection during his travel to the People's Republic of China 3 months earlier is extremely low because of the length of the incubation period (typical incubation period 4–28 days) (10) and the different hantavirus strains prevalent in the People's Republic of China (6). Although viral genetic sequence data from the patient and rodents in Matzu were not available in this study, our patient was infected with the Seoul strain, which is highly seroprevalent in rodents in Matzu (6,7).

In summary, this case was probably the first indigenous case of hantavirus infection in Taiwan. Its characteristics suggest that marked sinus bradycardia should be included as a protean manifestation of hantavirus.

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Public Meeting: Annual Report on Antimicrobial Resistance Action Plan

Bethesda, Maryland
June 26, 2002

A public meeting will be held on June 26, 2002, from 10:00 a.m. to 5:00 p.m., to present the first annual report on implementation of *A Public Health Action Plan to Combat Antimicrobial Resistance (Part I: Domestic Issues)* and to receive comments from the public. This plan was prepared by the Interagency Task Force on Antimicrobial Resistance, which is co-chaired by CDC, FDA, and NIH and includes seven other Federal agencies and departments. The meeting will be at the Holiday Inn Select, Versailles Ballroom, 8120 Wisconsin Avenue, Bethesda, Maryland, 20814; Toll-Free:1-877-888-3001.

Time will be available for oral questions, comments, and suggestions from the public. In the interest of time, a limit of three minutes may be imposed and visual aids will not be permitted. However, written comments and suggestions for subsequent review by the Task Force are encouraged and can be submitted through July 31, 2002 to either of the addresses given below.

The Action Plan and meeting agenda are available at http://www.cdc.gov/drugresistance; the Annual Report will be posted when available, likely in early June. Anyone planning to attend the meeting should contact Ms. Vickie Garrett, Antimicrobial Resistance, CDC, 1600 Clifton Rd., MS C-12, Atlanta, GA 30333; telephone 404-639-2603; fax 404-639-4197 or should e-mail aractionplan@cdc.gov by June 22, 2002. Please include name, organization (if applicable), address, phone, fax, and e-mail address.

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A dictionary is a reference book containing an alphabetical list of words, with information about each word; it usually includes meaning, pronunciation, and etymology. The third edition of Brian Mahy's Dictionary of Virology provides definitions of words commonly and uncommonly used in the field of virology, words that so often divide us into subgroups. Given that new words are associated with new thought processes and the new procedures that accompany them, picking up the latest technical terms and buzzwords is difficult. In addition, these words are added to the astounding number of words we have all accumulated over 10, 20, 30, or 40 years, so that the sum can be overwhelming. Thus, having a book containing all these words is functional and valuable. One can run to a secluded place, check this book, and thereafter appear to know what a graduate student is talking about. Previous editions were available in the United States in hardcover only, but this latest edition is in a convenient paperback form.

This book is not a complete dictionary in the classical sense; it does not provide a pronunciation guide as a dictionary might. Mahy prefaces the book with the disclaimer: "Viruses which only infect bacteria, fungi, invertebrates or plants are outside the scope of this Dictionary." Perhaps the publisher drew the line at 422 pages, or perhaps this book should have been named "Dictionary of (for the most part) Animal Viruses". In any event, if you are a molecular biologist, clinician, or epidemiologist involved in work with animal viruses, a graduate student, a senior faculty member who is overly specialized, a journalist, or a layman without a life, this book is highly recommended.

A Dictionary of Virology includes useful, informative, and concise definitions and brief descriptions of a multitude of words and terms we use each day, and it also contains words used by others. The book's entries include not only the necessary dry descriptions but also miniexplanations of very complex matters. Key references will lead you in the right direction, should you want to know more.

As might be expected of a book containing this much information, there are some minor shortcomings. For example, names of virus strains are presented for some viruses without explanation as to why names of virus strains are not presented for all viruses. Virus (= species) names are promised as conforming to the latest taxonomic vogue (i.e., italics), but not all species names are italicized. The definition for *Highlands J virus* indicates that this is not the etiologic agent of disease (disease in horses and turkeys illustrates otherwise). The dictionary alleges that lyssa virus is to be a synonym for *Rabies virus*, but whereas *Rabies virus* is a lyssavirus (a member of the genus *Lyssavirus*), there are many lyssaviruses, only one of which is *Rabies virus*. *Trivittatus virus* is defined as a member of the "California encephalitis virus serogroup," but at least this week, it is considered a serotype of the species *California encephalitis virus* (California serogroup). A few spelling errors occur, which, I am certain, will be corrected in the fourth edition.

Never mind the minor and few errors of commission, there are few, if any, (I looked) gross errors of omission. A Dictionary of Virology, therefore, is not only unique, it is useful—quite remarkable for a dictionary—pleasant and informative reading. Do not keep this book on your nightstand; by the time you get to it, it will be too late in the day. Instead, carry it in your attaché case or in the glove compartment of your pickup truck. This book reflects the remarkable diversity of viruses and of the terms that have been coined to discuss them so that we may have the requisite common language. I recommend it highly.

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EMERGING INFECTIOUS DISEASES www.cdc.gov/eid

JOURNAL BACKGROUND AND GOALS

What are "emerging" infectious diseases?

Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include

- New infections resulting from changes or evolution of existing organisms.
- Known infections spreading to new geographic areas or populations.
- Previously unrecognized infections appearing in areas undergoing ecologic transformation.
- Old infections reemerging as a result of antimicrobial resistance in known agents or breakdowns in public health measures.

Why an "Emerging" Infectious Diseases journal?

The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and *West Nile virus* infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

Emerging Infectious Diseases represents the scientific communications component of CDC's efforts against the threat of emerging infections. However, even as it addresses CDC's interest in the elusive, continuous, evolving, and global nature of these infections, the journal relies on a broad international authorship base and is rigorously peer-reviewed by independent reviewers from all over the world.

What are the goals of Emerging Infectious Diseases?

1) Recognition of new and reemerging infections and understanding of factors involved in disease emergence, prevention, and elimination. Toward this end, the journal

   - Investigates factors known to influence emergence: microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.
   - Reports laboratory and epidemiologic findings within a broader public health perspective.
   - Provides swift updates of infectious disease trends and research: new methods of detecting, characterizing, or subtyping pathogens; developments in antimicrobial drugs, vaccines, and prevention or elimination programs; case reports.

2) Fast and broad dissemination of reliable information on emerging infectious diseases. Toward this end, the journal

   - Publishes reports of interest to researchers in infectious diseases and related sciences, as well as to public health generalists learning the scientific basis for prevention programs.
   - Encourages insightful analysis and commentary, stimulating global interest in and discussion of emerging infectious disease issues.
   - Harnesses electronic technology to expedite and enhance global dissemination of emerging infectious disease information.

About the Cover

Samuel Johnson (circa 1769)

Reduced copy, after Sir Joshua Reynolds
Courtesy of the National Portrait Gallery, London.

Samuel Johnson was a poet, critic, lexicographer, and the author of the famous Dictionary, which he began in 1747. In 1755, Johnson's Dictionary appeared in two large folio volumes. It represented about 9 years of work and was written almost single handedly. Johnson had only the assistance of a few amanuenses to copy out the quotations he marked. Johnson originally approached Lord Chesterfield as a potential patron, but Chesterfield gave Johnson only a token sum (10 pounds). Thus, Johnson worked more or less unsupported, except by advances from booksellers.

Although Johnson's financial situation was weak, his work remained without rival for almost 150 years, when the Oxford English Dictionary was created (1884–1928). For his dictionary, Johnson wrote the definitions of over 40,000 words, illustrating them with approximately 114,000 quotations drawn from every field of learning.

Samuel Johnson was also a sitter in 16 portraits. His circle of friends included Sir Joshua Reynolds, who painted Johnson in 1769. This portrait is known as the "Johnson Arguing" portrait. It illustrates Johnson's mental concentration. Reynolds wrote that, when Johnson was excluded from conversation, "He remained but a few moments without speaking or listening. His mind appeared to be preying on itself; he fell into a reverie accompanied with strange antic gesticulations."

Abstracted from: URL: http://newark.rutgers.edu/~jlynch/Johnson/Guide/dict.html, http://www.npg.org.uk/, and http://www.kirjasto.sci.fi/samuelj.htm

EMERGING INFECTIOUS DISEASES
A Peer-Reviewed Journal Tracking and Analyzing Disease Trends Vol.8, No.7 July 2002

In the next issue

Anthrax of the Gastrointestinal Tract

Emergence of Usutu Virus, an African Mosquito-Borne Flavivirus of the Japanese Encephalitis Virus Group, in Central Europe

Persistent High Incidence of Tuberculosis in Immigrants in a Low Incidence Country: Screening on Arrival Is Not Enough

Impact of Monitoring Antimicrobial Use and Resistance with Comparisons to a National Benchmark on Reducing Vancomycin Use and Vancomycin-Resistant Enterococci

Rickettsialpox in North Carolina: A Case Report

The First Outbreak of Dengue Hemorrhagic Fever in Bangladesh: Evidence for Continued Spread of Dengue in South Asia

For a complete list of articles included in the July issue, and for articles published online ahead of print publication, see http://www.cdc.gov/ncidod/eid/upcoming.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 404-371-5329 (tel), 404-371-5449 (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 and Historical Reviews, Dispatches, Commentaries, Another Dimension, Letters, Book Reviews, and News and Notes. 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.

Chinese, French, and Spanish translations of some articles can be accessed through the journal's home page at http://www.cdc.gov/eid.

Instructions to Authors

Manuscript Preparation. For word processing, use MS Word. Begin each of the following sections on a new page and in this order: title page, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, figure legends, appendixes, and figures. Each figure should be in a separate file.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and e-mail address). Include separate word counts for abstract and text.

Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Printed manuscript should be single-sided, beginning with the title page. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables and Figures. Create tables within MS Word's table tool. Do not format tables as columns or tabs. Send graphics in native, high-resolution (200 dpi minimum) .TIF (Tagged Image File), or .EPS (Encapsulated Postscript) format. Graphics should be in a separate electronic file from the text file. For graphic files, use Arial font. Convert Macintosh files into the suggested PC format. Figures, symbols, letters, and numbers should be large enough to remain legible when reduced. Place figure keys within the figure. For more information see EID Style Guide (http://www.cdc.gov/ncidod/EID/style_guide.htm).

Manuscript Submission. Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch) and 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 Road, NE, MS D61, Atlanta, GA 30333, USA; e-mail eideditor@cdc.gov.

Types of Articles

Perspectives. Articles should be under 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 of first author. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and 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. Articles should be under 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 of first author—both authors if only two. 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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research Studies. Articles should be under 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 of first author—both authors if only two. 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 (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should be under 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. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be 1,000–1,500 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed two); and a brief biographical sketch of first author—both authors if only two. Dispatches 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.

Commentary. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but should not include figures or tables.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

Letters. This section includes letters that present preliminary data or comment on published articles. Letters (500–1,000 words) should not be divided into sections, nor should they contain figures or tables. References (not more than 10) may be included.

Book Reviews. Short reviews (250–500 words) of recently published books on emerging disease issues are welcome. The name of the book, publisher, and number of pages should be included.

News and Notes. We welcome brief announcements (50–150 words) of timely events of interest to our readers. (Announcements may be posted on the journal Web page only, depending on the event date.) In this section, we also include summaries (500–1,000 words) of emerging infectious disease conferences. Summaries may provide references to a full report of conference activities and should focus on the meeting's content.