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Maintaining Fluoroquinolone Class Efficacy: Review of Influencing Factors

W. Michael Scheld*

Previous experience with antimicrobial resistance has emphasized the importance of appropriate stewardship of these pharmacotherapeutic agents. The introduction of fluoroquinolones provided potent new drugs directed primarily against gram-negative pathogens, while the newer members of this class demonstrate more activity against gram-positive species, including *Streptococcus pneumoniae.* Although these agents are clinically effective against a broad range of infectious agents, emergence of resistance and associated clinical failures have prompted reexamination of their use. Appropriate use revolves around two key objectives: 1) only prescribing antimicrobial therapy when it is beneficial and 2) using the agents with optimal activity against the expected pathogens. Pharmacodynamic principles and properties can be applied to achieve the latter objective when prescribing agents belonging to the fluoroquinolone class. A focused approach emphasizing "correct-spectrum" coverage may reduce development of antimicrobial resistance and maintain class efficacy.

Development of resistance to antimicrobial agents and the emergence of multiresistant pathogens have generated worldwide concern in the medical community. Infections caused by resistant bacteria are associated with higher rates of hospitalization, greater length of hospital stay, and higher rates of illness and death (1,2). The estimated annual cost of treating infections caused by resistant bacteria in the United States is several billion dollars (3).

Antimicrobial resistance develops when bacteria are exposed to an antimicrobial agent, and selective pressure favors the growth of the resistant pathogen. To decrease selective pressure, antibacterial therapy should only be prescribed in patients with known or suspected bacterial infections. The risk for resistance can be further reduced by using an antimicrobial agent that has potent activity against the suspected pathogens at the dose and dosing frequency that maximizes its effectiveness.

Historically, several approaches to antibiotic prescribing have been employed to address antimicrobial resistance. One approach is to use a newer more potent antimicrobial in settings where resistance has emerged to an older agent. However, if newer agents are overused or used inappropriately, resistance will invariably develop to the newer drug. For example, since the late 1980s and early 1990s, ceftazidime, a third-generation cephalosporin, has been widely used against gram-negative pathogens, including *Pseudomonas*. However, indiscriminate use led to decreased activity against gram-negative infections and may have contributed to emergence of potent broad-spectrum β-lactamases among *Enterobacter*, *Citrobacter*, *Klebsiella*, and other gram-negative species (4–7). Another approach to combating resistance is to continue using older agents as first-line choices, in preference to newer, more potent drugs, in an effort to preserve the activity of the new drugs. The newer agents are reserved for infections caused by mutated multiresistant strains. However, as resistance continues to increase to the first-line agents, poor outcomes and secondary costs associated with clinical failures increase. By withholding the more potent agents for selected cases, these agents are increasingly compromised by the emergence of mutants selected by the less potent compounds.

An approach designed to reduce the rate at which antibiotic resistance develops is antibiotic cycling or rotation. It has been used with some success in intensive-care units (ICUs), where one class of agent has been predominantly used for a predefined period, usually 3 months, followed by use of another class for 3 months. Although not widely used, rotation has been used succesfully by Kollef et al. (8). A second approach is the use of combination therapy, whereby the additive or synergistic action of two or more drugs is exploited. Overall, resistance potential is theoretically minimized by this technique since these agents are typically from different antimicrobial classes, and different sites in the bacterial cell are targeted. Finally, a more focused approach of using the agents that demonstrate the best pharmacokinetic and pharmacodynamic profile against suspected pathogens might also reduce antimicrobial resistance. The objective of this approach is to predictably eradicate bacteria so that resistant clones are not selected.

The fluoroquinolone class of antimicrobial agents is being used empirically in an increasing number of patients because resistance has developed to the more traditional empiric agents. Fluoroquinolones are active against a wide range of multiresistant pathogens since their mode of action is against different molecular targets than other antimicrobial classes (9). Moreover, mechanisms of resistance to fluoroquinolones, apart from two unusual exceptions (10,11), are unlike almost all other class resistance mechanisms, being neither plasmid nor integron mediated.

We propose a strategy to preserve susceptibilities to this important antimicrobial class. Appropriate and targeted use of the fluoroquinolone class is discussed and analyzed within the

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context of in vitro, pharmacokinetic, and pharmacodynamic activity. The epidemiologic and clinical aspects of fluoroquinolone usage are outlined in an attempt to identify outcome-optimizing drug selection strategies. Finally, once fluoroquinolone therapy has been chosen, evidence-based strategies for how this antimicrobial class can be used to minimize development of drug resistance are discussed.

Fluoroquinolone Differentiation: in vitro Perspectives

Individual members of the fluoroquinolone class demonstrate different spectra of activity and pharmacokinetic profiles. The first-generation fluoroquinolones (e.g., ciprofloxacin, ofloxacin, norfloxacin) are primarily active against gram-negative and some gram-positive organisms. The second-generation fluoroquinolone, levofloxacin, is the L-isomer of ofloxacin and demonstrates somewhat improved gram-positive activity. However, susceptibility data show levofloxacin to be less potent than ciprofloxacin against such gram-negative pathogens as *Pseudomonas aeruginosa* and certain enterobacteriaceae (12). The third-generation fluoroquinolones include moxifloxacin and gatifloxacin and have improved gram-positive, atypical, and anaerobic coverage compared with first- and second-generation fluoroquinolones. In particular, these newer representatives of the fluoroquinolone class manifest greater activity against *Streptococcus pneumoniae*, an important respiratory pathogen (12).

The relative activities of these fluoroquinolones, expressed as 90% MICs (MIC_{90}s), are shown in Table 1. Ciprofloxacin is the most active fluoroquinolone against *P. aeruginosa;* typical MICs of ciprofloxacin are two- to eightfold lower than those of levofloxacin or newer quinolones such as moxifloxacin and gatifloxacin (12–16)**.** Species of enterobacteriaceae also differ in their susceptibility to the quinolones (12). Ciprofloxacin is generally twofold more active against *Escherichia coli* and *Klebsiella pneumoniae* than levofloxacin and moxifloxacin (Table 1).

Conversely, ciprofloxacin (1.0–4.0 mg/L) and levofloxacin (1.0–2.0 mg/L) are not as active against *S. pneumoniae* as moxifloxacin (0.06–0.25 mg/L) or gatifloxacin (0.5–1.0 mg/L) (12–16). A recent survey conducted in the United States and Canada showed ciprofloxacin MIC_{90}s of 2 mg/L against *S. pneumoniae* to be identical to those of levofloxacin but higher than those of the third-generation fluoroquinolone gatifloxacin (0.5 mg/L) (17). In addition to improved gram-positive activity, third-generation fluoroquinolones have improved activity against some anaerobic species compared to first- and second-generation fluoroquinolones. MIC_{90}s for *Prevotella/Porphyromonas*, *Fusobacterium* species, and *Peptostreptococcus* species for levofloxacin are 1.0–8.0, 1.0–8.0, and 4.0 mg/L, as compared with moxifloxacin (0.5–4.0,0.125–4.0, and 0.5 mg/L), respectively (18). Activity of newer fluoroquinolones against a variety of atypical organisms is also improved. For

Table 1. Comparison of in vitro activity[a] of four fluoroquinolones against a range of pathogens[b]

Fluoroquinolone	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*S. pneumoniae*[c]	*S. aureus*[d]	Ref.
Ciprofloxacin	0.03	8	NR	4	0.5	12
	0.125–0.5	0.25–4	0.25	1-2	0.5–1	13
	0.016	8	0.06	4	0.5	14
	0.016	2	0.25	2	0.5	15
	0.25	4	0.06	2	1	16
Levofloxacin	NR	32	NR	2	0.25	12
	0.06–<0.5	0.5–>4	0.12–0.25	1–2	0.25	13
	0.03	32	0.13	2	0.25	14
	0.06	4	0.25	2	0.25	15
	0.12	16	0.12	1	0.5	16
Moxifloxacin	0.06	8	NR	0.25	0.06	12
	0.06–1	8	0.12–0.25	0.06–0.25	0.12	13
	0.008	32	0.13	0.25	0.06	14
	0.06	8	0.5	0.25	0.06	15
	0.5	8	0.5	0.25	0.12	16
Gatifloxacin	NR	32	NR	1.0	0.125	12
	0.06	>4	0.06–0.25	0.5	0.12	13
	0.016	32	0.13	1	0.13	14
	0.1	8	0.12	0.5	0.12	15

[a]MIC_{90} reported.
[b]*E. coli, Escherichia coli; P. aeruginosa*, *Pseudomonas aeruginosa; K. pneumoniae, Klebsiella pneumoniae; S. pneumoniae, Streptococcus pneumoniae*; *S. aureus, Staphylococcus aureus;* NR, not reported.
[c]Penicillin-susceptible *S. pneumoniae,* except in the case of Reference 12, which did not specify.
[d]Methicillin-susceptible *S. aureus,* except in the case of Reference 12, which did not specify.

example, for *Mycoplasma pneumoniae,* MICs are 1.0 mg/Land 0.5 mg/L for ciprofloxacin and levofloxacin, respectively, and 0.125 mg/L for both moxifloxacin and gatifloxacin (19).

Fluoroquinolone MIC_{90}s will increase as resistant mutants invariably emerge, although the rate at which resistance develops largely depends on appropriate use. Patient-, institution-, and infection-specific therapeutic decisions require that antimicrobial susceptibilities be routinely tested and reported. Accurately assessing these MIC changes depends on the precision of the test used. The standard doubling dilution techniques used in most hospital microbiology laboratories may not be precise enough to identify minor susceptibility changes within a bacterial population (20). Utilizing the E-test method, which is sensitive enough to detect these subtle MIC changes (21), as follow-up for monitoring and controlling resistant strains isolated with increasing frequency in the clinical laboratory might be a practical solution, even though this approach requires greater resource and acquisition costs. Detecting and reporting these susceptibility changes are important since they can predict changes in the resistance potential of a pathogen (22). These data may be used to develop appropriate prescribing patterns to preserve antimicrobial activity.

Moreover, susceptibility data may not be accurate because surrogate methods, such as class-representative disk testing, are used in many institutions (23). Fuchs et al. (24) found that an accurate prediction of levofloxacin resistance could not be derived from use of ciprofloxacin or ofloxacin disk testing. This study showed that levofloxacin MICs were underestimated. Accordingly, the drug whose clinical use is being considered must be tested directly.

After observing three failures in patients treated with levofloxacin for pneumococcal infections, Davidson et al. conducted a survey in 2000 (25) and found that 86% of Canadian laboratories tested only nonfluoroquinolone antimicrobial agents for *S. pneumoniae* susceptibility. Given the growing resistance to traditional first-line agents and the increasing number of guidelines promoting quinolones as an alternative first-line choice in some patients (26–28), relevant testing should be routinely performed. Highlighting the need for fluoroquinolone susceptibility testing, Sahm et al. (29) noted a significant ($p<0.005$) increase in pneumococcal levofloxacin resistance in1997–1998 and 1998–1999 from 0.1% to 0.6%, although the incidence of *S. pneumoniae* resistance to fluoroquinolones remains low (<1%) in the United States (30,31).

Resistance Selection in vitro: Mechanisms and Implications

Pathogenic bacteria employ a variety of strategies to persist and replicate under adverse conditions such as exposure to an antimicrobial agent. The efflux pump system is a mechanism that allows immediate survival of bacteria in the presence of an antimicrobial agent by actively expelling that agent across the cell membrane, thereby reducing the intracellular concentrations to sublethal levels. The pump's action is dependent on the antimicrobial's ability to bind to the bacterial efflux protein and be exported. Some fluoroquinolones, such as moxifloxacin and trovafloxacin, are not as affected by bacterial efflux mechanisms because of their bulky side-chain moiety at position 7, which hinders export (32).

Another resistance mechanism involves specific point mutations that reduce the binding of the antimicrobial agent to specific enzymatic sites by altering the target site. In this regard, fluoroquinolones bind to enzymes involved in DNA replication, including DNA gyrase and DNA topoisomerase IV. Specific mutations in the genes that code for these enzymes can result in decreased binding and activity of the fluoroquinolones (33). Different fluoroquinolones demonstrate stronger or weaker affinity to these enzyme-binding sites. First- and second-generation fluoroquinolones bind primarily to DNA gyrase or DNA topoisomerase IV, depending on the bacteria and the drug, whereas the third-generation fluoroquinolones generally bind strongly to both DNA gyrase and DNA topoisomerase IV. Thus, a single point mutation in DNA gyrase or DNA topoisomerase IV generally affects first- and second-generation fluoroquinolones to a greater extent than third-generation fluoroquinolones. Furthermore, the third-generation C-8 methoxyfluoroquinolones, moxifloxacin and gatifloxacin, appear to bind different molecular sites within these enzymes, thereby decreasing the cross-resistance between these agents and the older fluoroquinolones (34,35).

The rate at which resistance develops to an antimicrobial agent is a measure of the resistance potential of the agent and can be assessed in vitro. M'Zali et al. (36) compared the mutant selecting potential of ciprofloxacin and levofloxacin in *Pseudomonas aeruginosa*. In this study, clinical isolates of *P. aeruginosa* were repeatedly exposed to concentrations below the MICs for ciprofloxacin and levofloxacin. The fluoroquinolone-resistant strains emerged at a significantly increased rate with levofloxacin compared to ciprofloxacin ($p<0.001$). These findings were consistent with those of Gilbert et al. (37).

Likewise, Dalhoff et al. (38) compared the resistance selection potential of various fluoroquinolones in vitro after repeated overnight exposures to suboptimal concentrations of *S. pneumoniae*. In this study, the C-8-methoxyquinolones (moxifloxacin and gatifloxacin) showed a lower propensity to select resistant mutants compared with levofloxacin and ofloxacin.

Appropriate Fluoroquinolone Selection: Pharmacokinetic and Pharmacodynamic Considerations

Pharmacokinetic properties, including the concentration of drug in the serum over time (area under the curve [AUC]) and the peak serum concentration of the drug (Cmax), can be measured, and when considered in combination with in vitro activity, may be useful for predicting microbiologic success and clinical outcomes. In particular, the ratio of the Cmax to MIC or AUC to MIC (AUIC) can be predictive of drug efficacy, although which parameter is most predictive of clinical outcome is the subject of some disagreement. Generally, the higher the ratio, the better the outcome (39,40).

While fluoroquinolones are generally concentration-dependent bactericidal agents, differences in antibacterial activities exist among class members. Fluoroquinolones also differ in pharmacokinetic parameters, such as Cmax and AUC (39). These efficacy parameters, as they relate to *S. pneumoniae* and *P. aeruginosa,* for ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin are shown in Table 2. Cmax/MIC and AUIC are highest for ciprofloxacin against *P. aeruginosa;* against *S. pneumoniae,* these values are highest with moxifloxacin.

Although AUC/MIC and Cmax/MIC ratios are useful for predicting antimicrobial efficacy, they may not be as useful for predicting the potential for drug resistance to develop. In this regard, Thomas et al. (45) suggest that AUC/MIC should exceed 100 for gram-positive and gram-negative species to prevent resistance selection.

Alternatively, Zhao et al. (46) have hypothesized that the rate at which resistance develops to a fluoroquinolone is related to its MICs and mutant prevention concentrations. Studies involving a range of bacterial species suggest that the concentration to prevent mutant emergence in the clinical setting can be derived in vitro and is 2 to 4 times higher than the MIC for most fluoroquinolones (46); however, the clinical significance of these findings has not been clearly established. Derivation of the mutant prevention concentrations is a process involving spreading a high bacterial load onto a series of agar plates in which various concentrations of antimicrobial have been incorporated. The density of 10^{10} CFU/mL was selected to pinpoint frequency of mutation at levels of 10^{-7}, 10^{-8}, and 10^{-9}, as well as to model the bacterial load at the site of infection. The inoculated plates are incubated overnight and the MIC of surviving colonies determined. This method has been applied to two species, *S. pneumoniae* and *P. aeruginosa,* for several fluoroquinolones (Table 3) (47,48).

Moxifloxacin exceeds the mutant prevention concentrations for *S. pneumoniae,* and ciprofloxacin exceeds the mutant prevention concentration for *P. aeruginosa* (both, 2 mg/L) by achieving maximum serum concentrations of 4.5 mg/L and 3.0 mg/L, respectively. These serum concentrations significantly exceed the mutant prevention concentrations; therefore, these agents are postulated to prevent mutant selection of *S. pneumoniae* and *P. aeruginosa,* respectively. Levofloxacin does not achieve mutant prevention concentrations of 8 mg/L in serum and thus may not inhibit mutant selection (47,48).

Clinical Consequences of Inappropriate Use

Approval of ciprofloxacin in the United States in 1987 was accompanied by its rapid inclusion onto most hospital formularies. Initial use was predominantly for *P. aeruginosa* and other problematic gram-negative infections. However, after ofloxacin was introduced in 1992, some formularies substituted this drug for ciprofloxacin on the basis of cost alone. Similarly, levofloxacin was approved by the U.S. Food and Drug Administration in 1997–1998 for a broad range of infections and was added to formularies in an effort to reduce costs. The clinical consequences of these substitutions was not apparent at the time; however, the epidemiologic data soon emerged that reflected how varying levels of antimicrobial activity could make an impact on pathogen susceptibility and clinical outcomes.

Peterson and colleagues (49) noted decreases in *P. aeruginosa* susceptibilities to ciprofloxacin and ofloxacin of 21% and 23%, respectively, from 1992 to 1994. This decrease occurred after their medical center switched from ciprofloxacin to ofloxacin as the primary quinolone. In 1994, ciprofloxacin was reintroduced as the primary quinolone, and a 7% recovery in ciprofloxacin activity to *P. aeruginosa* was reported within 6 months.

Similarly, Rifenburg et al. (50) assessed the effect of fluoroquinolone usage on *P. aeruginosa* susceptibilities and collated data from 109 hospitals during 1993 to 1996. Greater use of ofloxacin was associated with lower *P. aeruginosa* susceptibilities. Bhavnani et al. (51) collected data from 145 hospitals and found a significant correlation between use of ofloxacin, but not ciprofloxacin, and decreasing *P. aeruginosa* susceptibilities. Additionally, the study suggested a deleterious effect of levofloxacin use on *P. aeruginosa* susceptibilities (51).

Introduction of levofloxacin in 1998 to replace ciprofloxacin in a tertiary-care university medical center resulted in a significant decrease in *P. aeruginosa* susceptibilities (from 74% to 57% in a 3-year period) and *E. coli* susceptibility to ciprofloxacin (from 99% to 89% in a 3-year period). Levofloxacin use rose from 91.2 to 272.8 defined daily dose (DDD)/1,000 patient days (199%) in the 3-year period (52). This vol-

Table 2. Comparison of pharmacokinetic and pharmacodynamic parameters for four fluoroquinolones and selected bacterial species[a]

				Streptococcus pneumoniae			*Pseudomonas aeruginosa*		
Fluoroquinolone	Dose (mg)	Cmax[a,b] (mg/L)	AUC_{24}[b] (mg x h/L)	MIC[c]	Cmax:MIC	AUIC	MIC[c]	Cmax:MIC	AUIC
Ciprofloxacin	500	3.0	28	2	1.5	14	4	0.75	7
	750	3.6	32	2	1.8	16	4	0.9	8
Levofloxacin	500	5.7	48	1	5.7	48	16	0.36	3
Moxifloxacin	400	4.5	48	0.25	18	192	8	0.56	6
Gatifloxacin	400	4.2	34	0.5	8.4	68	8	0.52	4.25

[a]Cmax, peak serum concentration of the drug; AUC, area under the curve; MIC, AUIC, ratio of the AUC to MIC.
[b]References 41–44.
[c]Reference 16.

Table 3. Mutant prevention concentrations (MPC)[a] for various fluoroquinolones to *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*[b]

Fluoroquinolone	Daily dose (mg)	Cmax[c] (mg/L)	*P. aeruginosa* MPC (mg/L)	*S. pneumoniae* MPC (mg/L)
Ciprofloxacin	500 b.i.d.	3.0	2	NR[d]
	750 b.i.d.	3.6	2	NR
Levofloxacin	500 q.d.	5.7	8	8
Moxifloxacin	400 q.d.	4.5	NR	2
Gatifloxacin	400 q.d.	4.2	NR	4

[a]MPC values are derived from a study of approximately 100 isolates and are considered provisional; b.i.d., twice a day; q.d., once a day.
[b]See references 47 and 48 for *S. pneumoniae* and *P. aeruginosa,* respectively.
[c]Cmax, peak serum concentration of the drug (41–44).
[d]NR, not reported.

ume of usage exceeds that of 50 DDD/1,000 patients, a threshold suggested by Austin et al. (53) as a predictive driver in selecting for antimicrobial resistance during a 2-year period. Zambrano et al. (54), at the same institution, recently reported a significant correlation between increased levofloxacin use and declining fluoroquinolone susceptibilities among ICU isolates of *K. pneumoniae* (96% to 79% [$p<0.008$]) and *P. aeruginosa* (82% to 67% [$p<0.01$]).

Similarly, another group reported (55) that after levofloxacin was added to the formulary, levofloxacin use as a proportion of total fluoroquinolone use increased from <2% to >22% over a 6-month period (from 3rd quarter 1999 to 1st quarter 2000). During the period of 1st quarter 1998 to 2nd quarter 2000, the susceptibility of *P. aeruginosa* to ciprofloxacin decreased by 11% (82% to 71%). The use of parenteral antipseudomonal agents such as gentamicin, imipenem, ceftazidime, and piperacillin/tazobactam increased concurrently, suggesting that physicians began using non-fluoroquinolone combination therapy when treating serious gram-negative infections. Furthermore, the antimicrobial cost reductions anticipated from switching to a less expensive fluoroquinolone on formulary were not realized. In 3rd quarter 2000, levofloxacin was replaced with ciprofloxacin as the main gram-negative fluoroquinolone, a substitution associated with a subsequent 6% increase in ciprofloxacin activity against *P. aeruginosa* during the next year.

Because the ICU has been a focal point of antimicrobial resistance, the Centers for Disease Control and Prevention initiated Project ICARE in 1996 (56). Specific data regarding fluoroquinolone use and fluoroquinolone susceptibility among *P. aeruginosa* isolates were presented for the period 1996–1999 by Hill et al. (57). No correlation was found between prevalence of quinolone resistance and total use of ciprofloxacin/ofloxacin. However, significant associations were found between fluoroquinolone resistance and combined use of ciprofloxacin, ofloxacin, and levofloxacin ($p<0.019$) and by use of levofloxacin alone ($p<0.006$) (57).

Likewise, recent studies suggest that using a less potent fluoroquinolone against *S. pneumoniae* for treating community and hospital respiratory tract infections may be affecting the activity of all fluoroquinolones against this respiratory pathogen (58–60). Fluoroquinolone resistance in *S. pneumoniae* has been reported, most notably from Hong Kong (58). A 1998 study of 181 *S. pneumoniae* isolates showed resistance to ciprofloxacin in 12.1%, to levofloxacin in 5.5%, and to trovafloxacin in 2.2%. By early 2000, levofloxacin resistance had increased to include 13.3% of all *S. pneumoniae* and 27.3% among penicillin-resistant strains (59). These strains also demonstrated elevated MICs to newer class members such as gatifloxacin (12.8%) and moxifloxacin (12.2%) (the latter occurring exclusively in highly penicillin-resistant strains). Additionally, fluoroquinolone resistance appears to be emerging in other countries such as Canada, where resistance rates have increased from 0% in 1993 to 1.7% in 1997/1998 combined (60).

Clinical Consequences of Inappropriate Fluoroquinolone Use

Inappropriate use of antimicrobial agents has been associated with adverse consequences, including therapeutic failure, development of resistance, and increased health-care costs. One example of a mismatch between pharmacodynamics and clinical infection was in the use of ciprofloxacin for community-acquired pneumonia. The pharmacodynamics of the dose typically prescribed in these cases (ciprofloxacin 250 mg b.i.d.) are inappropriate for treating pneumococcal pneumonia, especially in seriously ill patients (41). By 1994, approximately 15 cases of *S. pneumoniae* infections that did not respond to ciprofloxacin had been reported, primarily in seriously ill patients and associated with contraindicated medications and other important medical issues (61). These events prompted the U.S. Food and Drug Administration to modify the package insert to warn against empiric use of ciprofloxacin for respiratory infections in which *S. pneumoniae* would be a primary pathogen. Consequently, ciprofloxacin has been used less frequently in these types of infections.

By contrast, >50% of levofloxacin use has been for the treatment of respiratory infections. Since 1999, at least 20 case reports of pulmonary infections that did not respond to levofloxacin therapy have been published (25,62–69). Three of the patients died due to fulminant pneumococcal infections that were unresponsive to levofloxacin therapy at approved dosage (25,62,69). Very few of these cases were in immunosuppressed patients. Reports of pneumococcal failures on the standard dosage of levofloxacin, 500 mg every 24 h, have also been described in two clinical trials, one in a patient with acute exacerbation of chronic bronchitis and the other in a patient with community-acquired pneumonia (70,71) (Table 4). In some of the 21 case reports, the treatment failed, and the pathogen developed levofloxacin resistance during therapy.

Davidson et al. (25) recently published details of four cases of pneumococcal pneumonia in which levofloxacin therapy failed. Two of the patients had no history of prior fluoroquinolone use and were levofloxacin susceptible beginning therapy, but their *S. pneumoniae* isolates were levofloxacin-

Table 4. Clinical failures of *Streptococcus pneumoniae* infection with levofloxacin[a]

No. of cases	Age	Indication	Risk factors		Yr	Ref.	Country
			Coexisting conditions	Prior FQ use			
1[b]	58	Meningitis	HIV, splenectomy	NR	1999	62	USA
3	NR	RTI		Yes	1999	63	USA
1	63	CAP	COPD	No	1999	64	USA
1	50	CAP	COPD	No	2000	65	USA
1	84	CAP	COPD	Yes-Lev	2000	65	USA
1	53	HAP	none	No	2001	66	USA
7	39–83(avg. 63)	4 CAP/3 AECB	COPD (5)	5/7 (4-Lev, 1-Mox)	2001	67	USA
1	37	CAP	none	No	1999	25	Canada
1[b]	66	CAP	COPD	Yes-Cip + Lev	1999	25	Canada
1	80	AECB/CAP	COPD,	Yes-Cip	2001	25	Canada
1	64	CAP	none	No	2000	25	Canada
1	50	CAP	COPD	Yes-Lev	2001	68	USA
1[b]	79	CAP	none	N	1999	69	USA
21							
Clinical trials							
13 (7 on 500 mg)	NR	AECB	COPD	No		70	Neth.
4	NR	CAP	NR	No		71	USA
24 (11)							
Epidemiologic studies							
16[c]	-	LRTI	COPD	Yes-Cip	1995-96	72	Canada
27[d]	-	LRTI	COPD (17)	Yes-Lev	1998-99	73	HK
43							
Total			88 (74 on 500 mg) clinical/bacteriologic failures				

[a]FQ, fluoroquinolone; NR, not reported; RTI, respiratory tract infection; CAP, community-acquired pneumonia; COPD, chronic obstructive pulmonary disease; Lev, levofloxacin; Mox, moxifloxacin; HAP, hospital-acquired pneumonia; AECB, acute exacerbation of chronic bronchitis;
LRTI, lower respiratory tract infection; Cip, ciprofloxacin; Neth, the Netherlands; HK, Hong Kong.
[b]Death.
[c]3 deaths.
[d]4 deaths.

resistant after therapy. These resultant mutants exhibited increased MICs to the newer fluoroquinolones moxifloxacin and gatifloxacin as well, thus decreasing those agents' potential effectiveness.

Both Weiss et al. (72) and Ho et al. (73) demonstrated clear risk factors (Table 5) associated with the development of fluoroquinolone resistance, including prior exposure of the patient to first- or second-generation fluoroquinolones (i.e., ciprofloxacin, levofloxacin, and ofloxacin) and history of chronic obstructive pulmonary disease.

Conclusions

The fluoroquinolone class of antimicrobial agents is being increasingly used empirically as resistance has developed to the more traditional antimicrobial agents. Guidelines now recommend fluoroquinolones as first-line empiric therapy for urinary tract infections in regions were trimethoprim/sulfamethoxazole resistance is >10% to 20% (28), and fluoroquinolones are recommended as alternative empiric regimens in some patients with community-acquired pneumonia (26,27). Though increased use of these agents would be expected to lead to increased resistance, a targeted approach to fluoroquinolone prescribing, emphasizing their appropriate use, may reduce development of antimicrobial resistance and maintain class efficacy.

Evidence is mounting that suggests a link between inappropriate fluoroquinolone use, development of antimicrobial resistance against the entire fluoroquinolone class, and clinical failure. To maintain the activity of the fluoroquinolone class, clinicians need to implement an evidence-based approach to antimicrobial selection, particularly a strategy in which the most pharmacodynamically potent fluoroquinolone is matched, on an empiric basis when required, to anticipated bacterial pathogens.

Three major factors are associated with increasing resistance to fluoroquinolones (74): 1) underdosing, i.e., use of a marginally potent agent whose MIC is barely reached in serum or infected tissues; 2) overuse of agents known to encourage

Table 5. Risk factors for infection or colonization with fluoroquinolone-resistant *Streptococcus pneumoniae*[a]

Factor	Case patients (n=27)	Control patients (n=54)	Odds ratio (95% CI)	p value
Age (yr)[b]	72.5 (62.3–78.3)	75 (70–85)	–	0.01
Nursing home residence	14 (52%)	7 (13%)	7.2 (2.4 to 21.6)	<0.001
COPD	17 (63%)[c]	12 (22%)	5.9 (2.2 to 16.3)	0.001
Nosocomial origin	18 (66%)	14 (26%)	5.7 (2.1 to 15.6)	0.001
Interval from day of admission to isolation of LRSP (days)[b]	7 (1–20)	1 (1–3)	-	<0.001
No. of prior admissions[b]	4 (2–7)	1 (0–3)	-	<0.001
Recent hospitalization	16 (59%)	13 (24%)	4.6 (1.7 to 12.3)	0.003
Multiple hospitalization	15 (56%)	12 (22%)	4.4 (1.6 to 11.8)	0.004
Previous exposure to antimicrobial agents[d]				
Fluoroquinolones	8 (30%)/14 (52%)	0 (0%)/5 (9%)	–/10.6 (3.2 to 34.7)	<0.001/<0.001
β-lactam antibiotics	24 (89%)/25 (93%)	20 (37%)/32 (59%):	14.7 (3.9 to 55.4)/8.6 (1.8 to 40)	<0.001/0.006

[a]CI, confidence interval; COPD, chronic obstructive pulmonary disease; LRSP, levofloxacin-resistant *S. pneumoniae* (73).
[b]Median (interquartile range).
[c]Colonization in 3 patients.
[d]Exposure to antimicrobial therapy during the 6 weeks prior to hospitalization/12 months before hospitalization.

resistant mutants; and 3) the inability to readily detect and respond to changes in antimicrobial susceptibilities. Traditional reporting of susceptibility data may be misleading and may not readily identify initial changes in resistance patterns or differences between agents of the same class.

To preserve fluoroquinolone activity, the activity of these agents must be continually assessed, and these agents must be used appropriately. The individual attributes of a given drug should be matched with the likely pathogen at specific infective sites. Expecting a single fluoroquinolone to be suitable for all infections is unreasonable, and excessive use of any single fluoroquinolone for all indications will lead to resistance that will adversely affect the entire class.

Given the defined strategy of selecting the agent with the best pharmacokinetic and pharmacodynamic profile against the known or suspected pathogen, an appropriate therapeutic choice for most serious infections, such as nosocomial pneumonia in which *P. aeruginosa* is a known or suspected pathogen, would currently include ciprofloxacin in combination with an antipseudomonal β-lactam or an aminoglycoside antibiotic. This recommendation is based on the lower MIC_{90} and mutant prevention concentrations for this fluoroquinolone against *P. aeruginosa* and higher Cmax/MIC and AUC/MIC ratios compared to other members of the class. Likewise, for most other gram-negative infections of the skin and urinary tract, including *P. aeruginosa* infections, ciprofloxacin monotherapy is appropriate.

Ciprofloxacin, levofloxacin, and gatifloxain all achieve high concentrations in urine; thus, they would all be appropriate choices for treating urinary tract infections in the community. Ciprofloxacin would be the most appropriate therapy in cases where *P. aeruginosa* is a known or suspected pathogen. For other gram-negative infections, levofloxacin or gatifloxacin should be prescribed in appropriate doses to surpass the mutant prevention concentrations at the infection site.

For infections in which *S. pneumoniae* is anticipated to be the most likely pathogen (e.g., community-acquired pneumonia), moxifloxacin, which currently has the best antipneumococcal pharmacodynamic activity and the lowest mutant prevention concentrations against this organism, would represent a prudent therapeutic choice. By contrast, levofloxacin MIC_{90}s against *S. pneumoniae* are significantly higher than those of moxifloxacin and gatifloxacin. The AUC/MICs and Cmax/MICs are also lower for levofloxacin against *S. pneumoniae,* and serum concentrations of a standard dose of levofloxacin for community-acquired pneumonia do not reach the mutant prevention concentrations for *S. pneumoniae*. For these reasons, levofloxacin may not be the best choice for infections caused by *S. pneumoniae*. Furthermore, recent reports of levofloxacin failures in cases of community-acquired pneumonia caused by *S. pneumoniae* evoke concern.

The targeted strategy proposed in this review is being implemented in a variety of institutions since the introduction of the third-generation fluoroquinolones. Documenting the effect of this approach on hospital susceptibilities over time will be important. Additionally, susceptiblities in these hospitals need to be compared to those in hospitals that use a single fluoroquinolone more broadly.

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Dead Bugs Don't Mutate: Susceptibility Issues in the Emergence of Bacterial Resistance

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The global emergence of antibacterial resistance among common and atypical respiratory pathogens in the last decade necessitates the strategic application of antibacterial agents. The use of bactericidal rather than bacteriostatic agents as first-line therapy is recommended because the eradication of microorganisms serves to curtail, although not avoid, the development of bacterial resistance. Bactericidal activity is achieved with specific classes of antimicrobial agents as well as by combination therapy. Newer classes of antibacterial agents, such as the fluoroquinolones and certain members of the macrolide/lincosamine/streptogramin class have increased bactericidal activity compared with traditional agents. More recently, the ketolides (novel, semisynthetic, erythromycin-A derivatives) have demonstrated potent bactericidal activity against key respiratory pathogens, including *Streptococcus pneumoniae, Haemophilus influenzae, Chlamydia pneumoniae*, and *Moraxella catarrhalis*. Moreover, the ketolides are associated with a low potential for inducing resistance, making them promising first-line agents for respiratory tract infections.

As the 19th century drew to a close, the work of Joseph Lister ushered in the antimicrobial era. Lister was among the first scientists to appreciate the implications of Pasteur's theory that microorganisms are involved in human disease (1). Accordingly, he examined the inhibitory effect of various chemicals on the growth and viability of bacteria and directly applied the results to the practice of medicine by using phenol (as well as heat) to sterilize surgical instruments. After this early example of infection control through antisepsis, the next step was inevitable: when chemicals with antibacterial activity were discovered, they were soon used in the treatment of infected patients. The ensuing clinical success was so dramatic that these agents were hailed as miracle drugs. By the second half of the 20th century, the practice of medicine enjoyed almost complete dominance over infectious bacteria (2).

Ironically, these same miraculous drugs now jeopardize the miracle, as evidenced by the widespread emergence of antibacterial resistance in the last decade (3–7). For example, methicillin-resistant *Staphylococcus aureus* strains have recently appeared in community-acquired infections (8), and *Streptococcus pneumoniae* strains resistant to both penicillins and macrolides (the antibacterial agents used most frequently for pneumococcal infections) have become prevalent throughout the world. Indeed, rates of *S. pneumoniae* resistance to penicillin now exceed 40% in many regions, and a high proportion of these strains are also resistant to macrolides. Moreover, the trend is growing rapidly. Whereas 10.4% of all *S. pneumoniae* isolates were resistant to penicillin and 16.5% resistant to macrolides in 1996, these proportions rose to 14.1% and 21.9%, respectively, in 1997 (9). A more recent susceptibility study conducted in 2000–2001 showed that 51.5% of all *S. pneumoniae* isolates were resistant to penicillin and 30.0% to macrolides (10).

The urgent need to curtail proliferation of antibacterial-resistant bacteria has refocused attention on the proper use of antibacterial agents. That the use of any antibacterial agent or class of agents over time will result either in the development of resistance to these agents or in the emergence of new pathogenic strains that are intrinsically resistant is now widely accepted. An example of the development of resistance is the mutation of *S. pneumoniae* to produce a multidrug-resistant strain (11). An example of a new resistant pathogenic strain is exemplified by the emergence of *Enterococcus gallinarum* as a nosocomial pathogen due to its intrinsic resistance to vancomycin (12). Keeping these phenomena in check requires a comprehensive strategy that includes, whenever possible, the selection of antibacterial agents in dosages sufficient to be bactericidal (13). A bactericidal effect is desired because, to put it succinctly, dead bugs don't mutate. In other words, if microbial pathogens causing infection are killed by antimicrobial therapy, rather than inhibited, mutations that might already exist or occur under the selective pressure of the antimicrobial agent are less likely to be promulgated. This principle will be briefly reviewed in relation to respiratory tract infections.

Clinical Relevance of Bacteriostatic versus Bactericidal Activity

All of the effects of antimicrobial agents against microbes, including the delineation of microbial resistance, are based upon the results of in vitro susceptibility testing. Most of these

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susceptibility tests only measure bacteriostatic activity even though the agent being tested may have bactericidal activity. Thus, the clinical relevance of susceptibility testing itself could be questioned. Numerous authors have extensively reviewed this issue over the years (14–16). These authors point out the paucity of studies that have critically evaluated the effectiveness of antimicrobial therapy with results of in vitro susceptibility tests. Such critical evaluations are not easily done, as susceptibility tests do not take into account the normal host defense mechanisms. However, the detection of resistance is somewhat predictive of poor outcome, although in the normal host this may be less clinically important due to the interaction of host defenses (17,18).

The ability of bactericidal activity to influence therapeutic efficacy and clinical outcome has been evaluated in infections that typically are refractory to antimicrobial therapy. These infections include endocarditis, meningitis, osteomyelitis, and infections in the neutropenic host. All are similar in that antimicrobial penetration and host defense mechanisms at the site of infection are limited. Both experimental models of infection (19–22) and clinical studies (23–27) have shown that bactericidal activity predicts therapeutic efficacy and results in improved clinical outcome. Bactericidal activity has been considered less important in respiratory tract infections, with the exception being acute infectious exacerbations in cystic fibrosis (28). However, the relevance of pharmacokinetic and pharmacodynamics in the selection of antibiotics for respiratory tract infection has become increasingly recognized (29). Issues such as drug concentration at the site of infection, bactericidal activity, postantibiotic effect, and duration of therapy needed to achieve these effects are now being considered when antimicrobial agents are selected for the therapy of respiratory tract infections (29,30).

Although host factors may allow a bacteriostatic agent to be used successfully in an infected patient, these factors appear to be less able to curtail the emergence of resistance. Resistance, as a rule, occurs more rapidly with bacteriostatic agents such as tetracyclines, sulfonamides, and macrolides than it does with bactericidal agents such as beta-lactams and aminoglycosides (31–33). An example of this can be seen with *S. pneumoniae*. Beta-lactam agents have been the antimicrobial agents of choice for the therapy of pneumococcal pneumonia since penicillin was first clinically introduced in the 1940s (17). Penicillin resistant strains of *S. pneumoniae* have taken more than 4 decades to emerge. The emergence of macrolide resistance in *S. pneumoniae* has been rapid in comparison and has even been described during treatment of pneumococcal pneumonia (34). Bactericidal activity thus may be useful in the therapy of respiratory tract infections as a means to curtail, but not avoid, the emergence of resistance.

Antibacterial Resistance Mechanisms: Bactericidal versus Bacteriostatic Activity

The key to resolving the problem of antibacterial resistance lies in identifying the mechanisms that engender it (31–33). Among the most important mechanisms are decreased ability of antibacterials to penetrate the bacterial cell wall, active efflux of antibacterial agents, inactivation of antibacterial agents, destruction of antibacterial agents, alteration of antibacterial target sites, development of bypass pathways around antibacterial targets, and constitutive phenotypic variation in bacterial physiology.

Fundamental to many of these mechanisms is mutation of bacterial DNA. Subsequent exposure of the microorganism to a specific agent may then select the mutant, leading to the emergence of resistance. Some resistance mechanisms, such as bacterial production of beta-lactamase, are inducible or can be derepressed (35), requiring either upregulation or mutation of genetic material. Thus, if resistance is to be suppressed, the opportunity for bacterial upregulation or mutation of genetic material must be minimized.

One way to minimize upregulation, mutation, or both is by using bactericidal rather than merely bacteriostatic agents. Microorganisms inhibited by a bacteriostatic agent or exposed to an insufficient concentration of a bactericidal agent remain alive and, ipso facto, retain the potential to become resistant or promulgate any resistance selected by the exposure to the antimicrobial agent. An example of this principle can be seen with the upper respiratory tract pathogen, *Streptococcus pyogenes,* which to date has not developed resistance to penicillin, a bactericidal agent, but has developed resistance to erythromycin, a bacteriostatic agent (36–38). Erythromycin resistance in *S. pyogenes* largely is due to upregulation of efflux (36) or to ribosomal mutation (37). Antimicrobial agents that kill this pathogen should be less likely to promulgate any strains having such resistance. Another example is seen with the *omp* genes of gram-negative microorganisms. These *omp* genes encode porins that are sometimes flanked by insertion sequences. In the presence of the bacteriostatic agent, the mobility of insertion sequence–flanking *omp* genes can be attenuated and will result in disruption of the *omp* genes. The reduced expression of these porins may lead to reduced uptake of the inducer, the antibacterial agent. Specifically, insertion sequence interruption of the *ompK36* porin gene in respiratory tract pathogen *Klebsiella pneumoniae* has been shown to interfere in the expression of this porin gene and has resulted in clinical failure (39). If a bactericidal agent kills a pathogen such as *Klebsiella* before mutation of the porin gene, resistance is less likely to develop. These two examples illustrate the desirability of achieving bactericidal activity to curtail the emergence of resistance.

Bactericidal Activity Achieved by Combination Therapy

Bactericidal activity can be achieved through the mechanism of action for a single antimicrobial agent or by the use of combination therapy, or both. Sulfamethoxazole/trimethoprim (SMX-TMP) is an example of a combination of two agents, each of which alone is bacteriostatic, that achieves bactericidal activity. Sulfamethoxazole inhibits dihydropteroate synthase,

the bacterial enzyme that catalyzes the incorporation of *p*-aminobenzoid acid into dihydropteroic acid, the immediate precursor of folic acid, while trimethoprim was specifically synthesized as an inhibitor of dihydrofolate reductase (40). SMX-TMP has long been used for the therapy of respiratory tract infection (41) and has proven particularly useful in the treatment of acute exacerbations of chronic bronchitis. In fact, the World Health Organization continues to be recommend SMX-TMP as the first-line treatment for pneumonia in children because of its low cost and ease of dosing. Resistance to SMX-TMP has emerged more slowly than for either agent used alone (42). However, emergence of resistance to *S. pneumoniae* has occurred (43) and now may limit the use of SMX-TMP in respiratory tract infections.

Combinations of antimicrobial agents are also used in the therapy of bacterial endocarditis to achieve synergism leading to increased bactericidal activity and improved sterilization of infected valves. Bactericidal synergy for *S. epidermidis* can be demonstrated in vitro for the combination of vancomycin, rifampin, and gentamicin, which correlates well with the therapeutic results in an experimental animal model (44). In a comparable clinical study of patients with prosthetic valve endocarditis caused by *Staphylococcus epidermidis*, 90% were cured with a combination of vancomycin, rifampin, and/or gentamicin, compared with only 50% cured among those receiving vancomycin alone (45). Combination therapy for respiratory tract infections is less well studied except for acute respiratory tract infections occurring in cystic fibrosis patients. For example, combination therapy for treatment of *Pseudomonas aeruginosa* pulmonary infections in cystic fibrosis patients achieved a cure rate of 89% if peak serum bactericidal titers were $\geq$1:128 (28). In contrast, 100% of patients failed therapy if their peak serum bactericidal titers were <1:16.

Bactericidal Activity Achieved with Novel Bactericidal Agents

Fluoroquinolones

Other examples of the importance of bactericidal activity are the fluoroquinolones. Studies of the bactericidal action of the quinolones against *Escherichia coli* demonstrate at least two independent and important mechanisms of action. First, all quinolones exert bactericidal action by inhibiting topoisomerases. These bactericidal agents are only effective if the bacteria are actively dividing or synthesizing proteins and mRNA. The bactericidal activity of the quinolone nalidixic acid, for example, is minimized by chloramphenicol, which prevents protein synthesis, and by rifampin, which prevents RNA synthesis. However, ciprofloxacin and ofloxacin/levofloxacin respond differently. Although the bactericidal action of these fluoroquinolones against *E. coli* is reduced, bactericidal action is not entirely eliminated by chloramphenicol or rifampin. This lack of elimination of bactericidal action suggests that these agents possess a secondary bactericidal mechanism of action that does not depend on the synthesis of protein and RNA, and that may be active when the bacteria are in a nonreplicating state (46).

To understand this secondary bactericidal effect, consider the bacterial inducible SOS system (47). Consisting of approximately 20 genes, this system repairs structural damage to DNA caused by antibacterial agents, mainly through bypass repair (46–50). This mechanism tends to be error prone and often leads to mutants. Another effect of the SOS response, activated by fluoroquinolone-induced damage to the bacterial DNA, is the discontinuation of cell replication. The organism can refrain from replication for only so long before it dies.

In addition to high concentrations of fluoroquinolone, which trigger the secondary bactericidal mechanism, higher concentrations at DNA targets also play a role in the emergence of resistance because the postantibiotic effect of the fluoroquinolones is dependent upon concentration, time, and the microorganism. If the concentration of fluoroquinolone attained at the bacterial DNA targets is high enough to activate the SOS system for a duration that exceeds the capability of the particular microorganism to repair its DNA damage and replicate, the microorganism dies. No postantibiotic effect occurs, per se, since no microorganisms survive. If the fluoroquinolone concentration is not adequate, however, a race occurs between cumulative damage over time and the selection of a resistant mutant.

The concentration of fluoroquinolone required for SOS-mediated discontinuation of cell replication is expressed as a peak concentration/minimum inhibitory concentration (MIC) ratio and appears to require a ratio of approximately 10:1 (50,51). A rat model for *Pseudomonas* sepsis demonstrated that peak concentration/MIC ratios >20:1 once per day produced significantly ($p<0.5$) better survival—which may result in the selection of a mutant with altered topoisomerase—than did regimens using the same dosage on a more fractionated schedule (52). Dosages that led to peak concentration/MIC ratios <10 times the MIC did not result in as high a survival rate. Indeed, when the peak concentration/MIC ratio was <10 times the MIC, the best survival was predicted by the area under the curve/MIC ratio, since repeated exposure to the fluoroquinolone causes damage cumulatively. The length of time that fluoroquinolone levels in plasma exceeded the MIC had no influence on survival.

The emergence of fluoroquinolone resistance with respect to *Staphylococcus aureus* and *P. aeruginosa* has been well-documented (53). This major problem is due to a wide variety of fluoroquinolone-resistance mechanisms (54,55), particularly the mutation of DNA gyrase (56). While this type of resistance generally results in MICs only four- to eight-fold higher than the susceptible isolate, recent studies have reported the development of high-level resistance (e.g., ciprofloxacin MIC for *P. aeruginosa* of 1,024 mg/L) mediated by efflux pumps targeting multiple antibacterial agents (57,58). These multidrug efflux pumps could be overcome by high fluoroquinolone concentrations, some of which, however, would not be clinically achievable.

A rabbit meningitis model further demonstrates how the inability to achieve peak concentration/MIC ratios >10:1 influences the postantibiotic effect. In an in vivo study, an exposure to ciprofloxacin at the MIC had minimal impact (59), underscoring the value of bactericidal activity with respect to fluoroquinolone therapy. The greater the activity of the fluoroquinolone, the more likely the agent will achieve serum or tissue levels that are >10 times the MIC, which in turn determines the secondary bactericidal and postantibiotic effects. Consequently, newer fluoroquinolones such as gemifloxacin (60) and others now under development have markedly increased activity compared with traditional agents. For example, ciprofloxacin has MICs against *Streptococcus pneumoniae* of approximately 0.5 mg/L, while gemifloxacin has MICs of approximately 0.03 mg/L.

An important issue associated with the use of fluoroquinolones in the therapy of respiratory tract infections is the fact that fluoroquinolones also are used to treat other infections. The use of these agents for other infections means that the population already had been exposed to fluoroquinolones before their widespread use in respiratory tract infections. Exposure of normal flora in these patients to subbactericidal concentrations of fluoroquinolones may allow resistant strains to emerge. Cross-resistance is a well-recognized problem with fluoroquinolones (55), and the enormous prior exposure of the population to these agents may have created resistant strains in the normal flora of the mucosal surfaces, skin, gastrointestinal tract, and reproductive tract. In addition, prior exposure may result in increasing MICs due to subtle mutations of topoisomerases, which then may leave the microorganism only one step from a mutation that will produce overt resistance (55). An example of such subtle topoisomerase mutation is seen with fluoroquinolones such as levofloxacin, which have been recommended and widely used for the therapy of pneumococcal pneumonia when penicillin resistance to *S. pneumoniae* is a problem (61). Unfortunately, the population has had considerable prior exposure to earlier fluoroquinolones, which has allowed rapid emergence of fluoroquinolone resistance in *S. pneumoniae* (62). Failure of treatment of pneumococcal pneumonia due to resistance to levofloxacin recently has been described (63). This example confirms the problem of cross-resistance and further mutations resulting in increased resistance and suggests that newer fluoroquinolones such as gemifloxacin may be less effective or even ineffective against *S. pneumoniae.*

Macrolides, Lincosamides, and Streptogramins (MLS)

Another class of antibacterials containing newly developed bactericidal agents are the macrolides. The term macrolide was originally applied to specific compounds produced by various *Streptomyces* species containing, as part of their structure, a macrocyclic lactone to which various deoxy sugars are attached. These bacteriostatic compounds bind to bacterial 50S ribosomes, inhibiting protein synthesis without a concomitant inhibition of nucleic acid synthesis. The classification has since been modified to include other structurally diverse agents, such as lincosamides and streptogramins, which are also produced by *Streptomyces* species and target the 50S ribosome. The term MLS (macrolide, lincosamide, and streptogramin) has become the accepted nomenclature for this important group of antibacterial agents—except when the emphasis is on structural similarity, in which case the erythromycin congeners (erythromycin-A, clarithromycin, and azithromycin) are often referred to as the "true macrolides" (64). Although antibacterial agents in the MLS class have been largely bacteriostatic, newer members demonstrate bactericidal activity. These new agents include quinupristin/dalfopristin and telithromycin.

The bactericidal mechanism of action of quinupristin/dalfopristin, a combination of two streptogramins, is unique (65). Dalfopristin is an olefinic macrolactone that binds to the 50S subunit of the prokaryotic ribosome and interferes with the function of peptidyl transferase, thereby inactivating the donor and acceptor sites of the ribosome. In addition, dalfopristin triggers a conformational change in the ribosome that greatly increases the affinity of quinupristin, a peptidic macrolactone, which also binds to the 50S subunit of the ribosome and halts peptide chain elongation. Consequently, protein synthesis is not only halted transiently by either component used alone but also halted permanently by the two components in combination, resulting in synergistic and concentration-independent bactericidal activity against many pathogens. This binding of both macrolactones distinguishes quinupristin/dalfopristin from other antibiotic classes (66,67), with the attendant prolongation of the postantibiotic effect (68) representing a distinct advantage over older agents (69). Quinupristin/dalfopristin is bactericidal against staphylococci and streptococci such as *S. pneumoniae*, generally bacteriostatic against *Enterococcus faecium,* and inactive against *E. faecalis* (70). Because quinupristin/dalfopristin is available only in an intravenous formulation, its utility for treating respiratory tract infections is limited to hospitalized patients. Moreover, clinical data on quinupristin/dalfopristin therapy of pneumococcal pneumonia caused by macrolide resistant strains of *S. pneumoniae* is lacking.

Ketolides: Telithromycin

Telithromycin, the first of a new class of antibacterials, the ketolides, is approved for use in Europe and is currently being reviewed by the U. S. Food and Drug Administration (71). The clinical use of telithromycin in Germany, as well as safety data presented to the Food and Drug Administration, suggests that the toxicity and adverse reactions are similar to those of clarithromycin. This similarity is not surprising, as the ketolides are novel semisynthetic erythromycin-A derivatives structurally similar to clarithromycin. The C6-hydroxyl of erythromycin-A has been replaced by a methoxy group, as in clarithromycin, improving acid stability. The main structural innovation is the lack of the neutral sugar, cladinose, in position C3. The 3-L-cladinose sugar moiety is removed, and the

resulting 3-hydroxy group is oxidized to a 3-keto group, which is responsible for preventing induction of macrolide resistance (72). Telithromycin is produced through substitution at positions 11 and 12 of the erythronolide A ring with a butyl imidazolyl pyridinyl side chain. The resulting C11,C12 carbamate extension facilitates a distinctly different and more effective interaction with domain II of the 23S rRNA than occurs with erythromycin-A or clarithromycin (73) and is responsible for increased activity against erythromycin-A–resistant gram-positive cocci, such as *S. pneumoniae*, which develop resistance due to increased efflux (74,75). Moreover, the bactericidal action is effective against a number of other key respiratory pathogens, such as *H. influenzae* and *C. pneumoniae* (76–78), other gram-negative bacilli, such as *M. catarrhalis* and *Bordetella pertussis,* and the intracellular pathogen *Legionella pneumophila* (79).

Unlike erythromycin-A (which interacts with bacterial 23S ribosomal RNA by contacts limited to hairpin 35 in domain II of the ribosomal RNA and to the peptidyl transferase loop in domain V^{49}), telithromycin is not a true macrolide because the L-cladinose moiety at position C3 has been replaced by a keto group and by alkylaryl side chains at positions C11,C12. Although both erythromycin-A and telithromycin bind to the peptidyl transferase loop (the site of methylation by resistant bacteria), telithromycin binds much more avidly to hairpin 35 than erythromycin-A. In fact, telithromycin interacts strongly with two domains of the bacterial 23S rRNA (domains II and V), which fold together in the tertiary 23S rRNA to form a single drug-binding pocket (80,81). The lack of the L-cladinose moiety, as well as the enhanced binding at domain II and V, may explain why ketolides are associated with a low potential for inducing resistance (82,83) and contributes to telithromycin's sustained activity against MLS_B-resistant strains (in particular those with domain V modifications) (73).

These features, in addition to the MIC and the amount of drug delivered to the infection site, are considered strong predictors of a positive outcome (29,30). However, the population has had enormous exposure to earlier macrolides. This exposure has an influence on the normal flora of mucosal surfaces. This influence means that resistance due to efflux or methylation of the 23S ribosome (domain V) may have already occurred in a large number of pneumococcal isolates. Macrolide-resistant strains of *S. pneumoniae* to date have had a low incidence of cross-resistance to telithromycin (82,83). However, increased efflux or other mutations might result in resistance to ketolides. To date, only two ketolide-resistant strains of *S. pneumoniae* have been identified (84) in over 10,000 pneumococcal isolates screened by the PROTEKT study (10). The MIC of one of these isolates was 1 mg/L; the other was 256 mg/L. Clearly, careful monitoring for ketolide resistance by surveillance studies such as the PROTEKT study will need to be continued.

Conclusion

Meeting the challenge presented by the increasing numbers of bacterial pathogens resistant to common antibiotic treatments will require new types of antibacterial agents. Therapies that maximize bactericidal effects are important because they reduce the development of bacterial resistance mechanisms. Therefore, the use of bactericidal agents such as telithromycin for therapy of respiratory tract infections may well ensure that the antibacterial era endures long into the 21st century. However, careful monitoring of resistance will be needed to ensure that this agent remains active against common pathogens.

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Geographic Translocation of Bats: Known and Potential Problems

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Natural, accidental, and intentional translocation of bats, both intra- and intercontinentally, has been documented. Some bats have been translocated while incubating infectious diseases, including rabies or related lyssavirus infections; others have escaped confinement en route to or at their destinations, while others have been released deliberately. Known events and potential consequences of bat translocation are reviewed, including a proposed solution to the attendant problems.

Among the many potential consequences resulting from the geographic translocation of life forms is the spread of infectious disease organisms harbored by that life form. This consequence was demonstrated long ago by the early devastation of native American human populations caused by pathogens inadvertently introduced by European explorers. Similarly, wildlife rabies outbreaks occurred recently in the United States after foxes, coyotes, and raccoons were translocated to restock areas where these animals are hunted for sport. Wild populations of introduced species can also become common disease vectors where few or none existed before, such as the current role of Indian mongooses (*Herpestes javanicus*) in rabies transmission on Caribbean islands (1), or they can become predators of native species, for example, the wildlife destruction that occurred after ferrets and stoats were introduced into New Zealand (2).

Bats have been translocated through natural, accidental, and deliberate means. Pathogens associated with bats, such as *Rabies virus* (RABV) and related lyssaviruses (3–6), can cause disease after protracted incubation periods, ensuring the extended survival of the host and parasite during periods of translocation. Many bat species enter a hibernationlike state in a cold environment, which further prolongs survival. In this article, I describe some occurrences of bat translocation (published, as well as previously unreported) and the potential consequences of that translocation, as the basis for suggesting preventive measures to alleviate the problems that accompany the relocation of bats across the world.

Translocation of Bats

Natural Translocation

Some species of bats hibernate at the approach of cold weather; other species migrate to warm areas instead. Bats that migrate along coastlines take shortcuts over water and are apparently blown far out to sea at times. Many North American migrant bats have been found in Bermuda, 1,046 km east of North Carolina, United States, during fall and spring migrations, evidently having been blown there by wind along with waves of migratory birds (7). These translocated bats include Hoary Bats *(Lasiurus cinereus),* Red Bats *(L. borealis),* Seminole Bats *(L. seminolus*), and Silver-haired Bats (*Lasionycteris noctivagans*), all species from which RABV has been isolated (8). Hoary Bats are also occasionally found in rabies-free Iceland, also possibly blown there by the wind; one bat was captured in the Orkney Islands, off rabies-free Scotland (9). Similarly, Hoary Bats are sometimes found in the Galapagos Islands, 966 km off the west coast of South America (10).

Translocation after Landing on Ships

Exhausted bats flying far at sea both individually and in flocks have been reported to alight on ships and be transported to unintended destinations. Most records are from the North Atlantic Ocean and involve Red Bats and Silver-haired Bats (11). A Southern Yellow Bat (*Lasiurus ega*) landed on a ship over 322 km from the coast of Argentina (12). A "fruit-destroying bat" was reported sleeping in the rigging of a ship upon arrival in Hawaii from the Philippines (13), and a frugivorous bat (*Vampyressa pusilla*) evidently boarded a vessel passing through the Panama Canal and was later found aboard when the ship was between Australia and Tasmania (14).

Translocation after Using Ships for Shelter

Bats sometimes roost in or on ships in port and may be transported as a consequence. Silver-haired bats were discovered hibernating in hulls of ships, and numbers of them found various refuges on ships and yachts in New York (15). Little Brown Bats (*Myotis lucifugus*) roosted aboard a ship that frequently traveled from Canada to Europe, flying ashore after arrival in the Netherlands and England (16). The presence of individual Little Brown Bats in rabies-free Iceland (9) and Kamchatka, Russia (17), has been attributed to travel by ship. RABV, other viruses, and *Histoplasma capsulatum* have been found in this species (3,8).

On January 21, 1997, a stevedore working in the hold of a ship being unloaded in Long Beach, California, after its arrival from Korea, was bitten on the back of his neck by a bat. A fluorescent rabies antibody test was negative for RABV infection. On February 1, I received the bat for evaluation and determined it to be a Serotine bat *(Eptesicus serotinus),* which is similar to the Big Brown Bat (*E. fuscus*) but with a slightly more massive

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skull. The Serotine has been reported in North Africa and England and across Europe and Asia to Korea. Hundreds of ill or dead Serotines have been found infected with the RABV-related *European bat lyssavirus 1* (EBLV-1) in Europe, where one or two persons have died of the infection after bat bites (5). The rabies conjugate used in the rabies test on the Serotine bat's brain reportedly reacts with this virus as well.

Translocation in Shipping Containers

Translocation of bats by ship also occurs when bats are closed inside shipping containers. Free-tailed bats from the tropics are occasionally transported long distances in fruit shipments (18). A Pallid Bat (*Antrozous pallidus*) was discovered in Victoria, British Columbia, in a shipment of lettuce from California (19), where RABV-infected Pallid Bats have been identified. A Big Brown Bat was found hibernating in a timber container from Canada when it was unloaded in the Netherlands (16). An Asiatic Pipistrelle bat *(Pipistrellus javanicus)* was discovered in a container transported by ship from Japan to New Zealand (20). Sasaki et al. (21) reported the arrival in rabies-free Hawaii of a RABV-infected Big Brown Bat found flying in an automobile container from California. Subsequent study indicated that previously the bat had been transported to California either from Florida in the shipping container or from Michigan in an automobile.

In October 1995, a group of live bats was observed hanging in a dark corner within a large shipping container that had just arrived at a Los Angeles port from Puerto Rico, but the bats escaped as capture was attempted and no further reports of these bats were made. Histoplasmosis, apparently absent in California except for imported human infections, has been diagnosed in some Puerto Rican bats.

Translocation by Aircraft

Bat translocation by aircraft has been reported several times. A Little Brown Bat was found clinging to a seat in an airplane at the end of a flight in Canada (22). An Eastern Pipistrelle bat (*Pipistrellus subflavus)* was recovered from a plane that had just arrived in Texas from Mexico (23); RABV-infected bats of this species have been identified in the United States and Canada. The carcass of a Little Brown Bat, presumably from Tacoma, Washington, was found on a runway at an Air Force base on rabies-free Guam (24). Stebbings reported the arrival in England of a Silver-haired Bat aboard a U.S. Air Force cargo plane from Delaware (25). Observed flying in the plane en route, the bat was captured later while sleeping in a crew member's bed in the aircraft.

An Asiatic Pipistrelle bat was captured May 25, 1993, aboard an airliner en route from Tokyo to San Francisco. This bat was negative for RABV. The next month a Yuma Myotis bat (*Myotis yumanensis)* was discovered flying aboard a U.S. Air Force cargo plane en route from California to Hawaii. This bat was also negative for RABV, although rabies has been diagnosed in the species in California. Evidently the bat was loaded into the aircraft within a shipment of fruit.

In early March 1995, a traveler who had just arrived in Los Angeles by aircraft from South Africa opened his suitcase and observed a bat fly out. The suitcase had been closed three days earlier during darkness in a hut within Kruger National Park. The bat was negative for RABV, and the frozen carcass was sent to me 2 months later with the history of origin in a Los Angeles County community. At first glance, the bat appeared to be a common local free-tailed bat (*Tadarida brasiliensis*), but closer inspection indicated differences, although the bat belonged to a family with similar representatives in warm areas worldwide. After extensive study, I determined the specimen to be a Wrinkle-lipped bat *(Chaerephon pumila),* known throughout sub-Saharan Africa, Madagascar, and southern Arabia. Further research disclosed the transported bat's African origin. This species supports experimental replication of Ebola virus without showing disease signs (26); the remainder of the carcass was immediately sent to a federal laboratory for Ebola virus tests, which proved negative. Several other viruses have also been isolated from the salivary glands of this species in Africa (3).

In June 1997, a woman was bitten by a bat hiding in clothing she was packing before an airline flight from Costa Rica to California. The live bat was restrained in a plastic bag during the flight; it was dead on arrival. The bat was negative for RABV and was identified as a Sinaloan Mastiff Bat (*Molossus sinaloae*), an insectivorous species in which RABV has been reported (5).

Translocation for Confinement

Bats have been transported varying distances, sometimes worldwide, to be maintained in captivity as research animals, as live specimens in zoos or other exhibits, and as pets. Transport for research purposes is not noteworthy except in unusual circumstances. A Big Brown Bat in the incubational stages of rabies was among live bats sent from Canada to a laboratory in Germany, where the bat developed clinical rabies (27). Similarly, six Big Brown Bats that were incubating RABV were in a group sent from the United States to a laboratory in Denmark (28). However, recipient laboratories understood the risks and had taken necessary precautions.

RABV-infected individual bats of the tropical American common Vampire Bat *(Desmodus rotundus)* have been reported throughout their geographic range, which extends from northern Mexico south to Chile and Uruguay (8,29). RABV has also developed in Vampire Bats after being transported to laboratories. In addition, during the 1970s, a group of these bats sent from Mexico to a laboratory in the United States presumably escaped en route, because only the empty shipping container arrived.

Increasing interest in bats has resulted in displaying of more varieties of these mammals, including Vampire Bats, to the public (5). One such display presented a problem I investigated in 1988 after four of eight Vampire Bats escaped their flight cage within a cavelike structure at a southern California zoo 1 month after their arrival from Mexico through a Texas

supplier. Two escaped bats were found dead, possibly due to starvation or unusually cold weather. One dead bat had nearly escaped the building, and the other was outside. Neither bat was infected with RABV. The apparent escape route to the outside was through a fragile false cave ceiling, which could not be inspected. This ceiling may have contained the carcasses of the remaining two missing bats, possibly a male and a female. I found no bat bites on zoo animals and no bats or bat feces in likely hideaways in the zoo.

The large fruit-eating bats (genus *Pteropus)* live on land masses, including islands, from Madagascar, India, Southeast Asia, the East Indies, the Philippines, and Australia to the Samoan and Cook Islands of the South Pacific Ocean. They have been popular zoo attractions for many years. RABV was reported in a *Pteropus* in India (8), and RABV-related lyssaviruses were reported in four species of *Pteropus* and an insectivorous species in Australia, where two persons died of these infections (30).

Three additional viruses (*Paramyxoviridae* family) ascribed to *Pteropus* origin have proven pathogenic or fatal to people and domestic animals. Four species of Australian *Pteropus* bats in Queensland carry Hendra virus without developing symptoms. These bats disseminate virus in urine or placental fluid during birthing, and the virus is later ingested by pregnant horses that amplify the virus, which then spreads to people and causes a fatal pneumonia (13/20 horses were infected in a 1994 outbreak, which resulted in two human deaths) (30). The second virus, Menangle virus, is considered to be spread to pigs in Australia by the same four species of *Pteropus* bats, producing stillbirths with deformities in 1998 in 27% of litters, as well as an influenzalike illness in humans (30). The third virus, Nipah virus, identified in urine and saliva of *Pteropus* bats in Malaysia, apparently spreads the virus to pigs and destroyed that country's swine industry in 1998. The virus spread from pigs to hundreds of industry workers; approximately 40% of these workers died of severe viral encephalitis caused by the agent (31).

Importation of fruit-eating bats has long been severely restricted to protect the fruit industry in the United States. The Egyptian Rousette bat (*Rousettus egyptiacus)* is a widespread Old World fruit bat that readily reproduces in captivity; thus colonies occur in some zoos. This species has been implicated in several viral infections in Africa (3). An error occurred in 1994, when thousands of these and other bat species were permitted entry into the United States for sale as pets or for exhibition (28); this procedural mistake resulted in a policy change to prevent recurrence. Antibodies to *West Nile virus* (WNV) had been reported in the *R. egyptiacus* species in Uganda and Israel (3), and the virus had been isolated in India from the nearly indistinguishable *R. leschenaulti*, which overlaps geographically with *R. egyptiacus* in Pakistan (32). The entry of *R. egyptiacus* into the United States in 1994 suggests a remote connection with the subsequent outbreak of WNV there, first observed 5 years later among captive and wild birds at a zoo in New York (33).

In 1997, two *R. egyptiacus* bats died with rabies-like symptoms in a Denmark zoo; they were later found to be infected with EBLV-1 subtype A, a RABV-related agent known to have caused deaths in European insectivorous bats and in humans. The two infected bats had arrived recently from a Netherlands zoo, where the source captive bat population subsequently was destroyed (34). A replacement colony was similarly destroyed after a bat originating from a Belgian zoo was also determined to be infected (35).

Persons concerned about sick and injured wildlife often try to rehabilitate disabled bats, sometimes transporting the animals a considerable distance from sites of discovery. Unfortunately, an average of 10% of disabled bats tested in North America are found to be infected with RABV, exposing those trying to rehabilitate the bats to rabies. If they have received preexposure rabies prophylaxis in advance, these persons are advised to take booster shots of vaccine; otherwise, they are advised to take both antirabies globulin as well as the full vaccine treatment.

Often, attempting to reverse the negative image of bats usually held by the public, persons trying to rehabilitate sick bats may suppress warnings of rabies hazards, doing both bats and the public a disservice. Moreover, to avoid the embarrassment of repeated exposures to rabid bats, some persons working in bat rehabilitation are known to arrange submission of rabies-suspect bats to a variety of different laboratories in different geographic areas, thus disguising the true history of the bat; this practice may protect the rehabilitator but prevent other persons or pets exposed earlier from receiving adequate antirabies management.

Translocation for Release

Bats have been translocated and released in attempts to establish bat populations in new areas for reasons such as insect control and experimental study. Such efforts are sometimes supplemented by providing living quarters or shelters for bats ranging from elevated boxlike structures to tunnels. Before the knowledge that some insectivorous bats might be infected with rabies or other pathogens, bats were sometimes transported great distances over land or overseas and released in efforts to establish populations at the new location. Tomich (13) assembled historical records about the importation and release in rabies-free Hawaii of Asiatic Pipistrelle bats from Japan and free-tailed bats (*Tadarida brasiliensis)* from California during the late 1800s to establish bat populations for insect control purposes, but the attempts were evidently unsuccessful.

Observing that destruction of old-growth forests eliminated the tree hollow homes of Polish bats, Krzanowski (36) recommended the introduction into Poland of Red Bats and Hoary Bats from the United States because these species take shelter in tree foliage rather than hollows, and they migrate at the approach of cold weather rather than hibernate in tree cavities. However, rabies was discovered simultaneously in North American insectivorous bats, including these two species, discouraging further consideration of the proposal.

The homing abilities of bats have routinely been studied by transporting and releasing marked bats up to 805 km from their home roost, which is then monitored for the return of the marked bats (37). RABV infection has now been identified in 11 of the 12 North American species studied, and histoplasmosis is known in 6; RABV-related lyssavirus infections have been reported in 5 of 12 European species studied (8).

During World War II, field trials were conducted in the southwestern United States to determine the effectiveness of disseminating thousands of free-tailed bats *(T. brasiliensis)* in the air, each transporting a small time-activated fire bomb. The objective was to start thousands of simultaneous fires in adversary target areas, achieved after each bat had sought out a hideaway in various available structures (38). As a participant in the project, I observed that each bomb or dummy bomb, attached by a short string and surgical clip to the bat's abdominal skin, was disengaged after the bat alighted in a refuge and chewed through the string. Thousands of bats were transported ≤1,609 km distant from source bat caves in Texas and New Mexico to test areas in California, New Mexico, and Utah. Frequently, the tests were postponed, and the freshly captured bats were released unencumbered at or near test sites. Unknown at the time, RABV is now known to occur in 0.5% of bats in the source caves (8), so the virus was almost certainly translocated with the bats. *H. capsulatum,* the causative fungus of histoplasmosis, also has been isolated from these bats and their guano in the source caves, but neither bats nor guano have yielded the agent in extensive surveys in California, which is regarded as free of the fungus; no cases of indigenous origin have been detected (8).

Discussion

Bats and the pathogenic organisms they sometimes harbor are being transported by humans within and between continents, and sometimes these transported bats escape. Because bats reproduce slowly (usually only one or two offspring are produced annually by a female), the chances of successful introduction of the species are minimized. Populations would more likely develop should large numbers be freed in places favorable to survival. Although a single escaped bat might not survive long or reproduce, it would seek shelter in places frequented by local bats to which it might transmit pathogens. As has been observed, introduced pathogens include RABV, other lyssaviruses, or various other agents.

Vampire Bats can be especially problematic in view of their possible colonization in warm climates and their dependence on a diet of blood, thus necessitating their biting vertebrates, including man and domestic animals. As reported, in addition to their known role as biologic vectors of rabies to humans and domestic animals and surra *(Trypanosoma evansi)* to horses and cattle, Vampire Bats can also be temporary biologic as well as mechanical vectors of *Venezuelan equine encephalomyelitis virus* and foot-and-mouth disease. They are likely effective mechanical vectors if not biologic vectors of any bloodborne pathogen, including the AIDS virus (29). Various species of fruit-eating bats are infected at times with pathogens destructive to other bats, humans, and domestic animals. However, their entry to many areas is restricted due to concern that their escape would lead to populations destructive to fruit crops.

Accidental or planned translocations of bats between land masses happens almost certainly with far greater frequency than is reported. Such events can be embarrassing, and although incidents that result in successful containment are more likely to be reported, failed efforts can remain unpublicized. Relevant reporting requirements do not exist. Personnel involved in the various described incidents generally have performed very well in efforts to resolve the problems, often with immediately contrived solutions. Inspectors at entry centers are usually exceptionally competent because they must cover a broad array of subject areas, but their competency must be taxed at times. For example, most bats are exceptionally adept at avoiding capture, and even bat scientists with special equipment frequently are outmaneuvered. Some inspectors contact specialists for help in emergencies, but help is not always available or is displaced by previous commitments and economic necessities. Previous contractual arrangements with institutions such as universities, natural history museums, zoos, or specialized commercial services could dispel most relevant problems, including funding, and maintain program continuity. Unaffiliated specialized personnel would be expected to maintain or acquire relevant competency, but incidents, such as those cited here, show some lapses. Ideally, the services of a bat expert are required. For example, if bats are to be excluded from any vehicle of conveyance, the usual procedures and equipment should be reviewed by responsible persons very familiar with bats, their capabilities, their capture, their confinement, and their exclusion in order to recognize flaws that permit bats to be transported. Thus, experts can help establish and maintain more effective programs.

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After the 1953 discovery of bat rabies in the United States, Dr. Constantine established the Southwest Rabies Investigations Station in New Mexico for the Centers for Disease Control and Prevention and developed its program to investigate the problem and control bat rabies. Now retired, he continues research in the field.

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Foot and Mouth Disease in Livestock and Reduced Cryptosporidiosis in Humans, England and Wales

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During the 2001 epidemic of foot and mouth disease (FMD) in livestock in England and Wales, we discovered a corresponding decrease in laboratory reports of cryptosporidiosis in humans. Using a regression model of laboratory reports of cryptosporidiosis, we found an estimated 35% (95% confidence interval [CI] 20% to 47%) reduction in reports during the weeks spanning the period from the first and last cases of FMD. The largest reduction occurred in northwest England, where the estimated decrease was 63% (95% CI 31% to 80%). Genotyping a subgroup of human isolates suggested that the proportion of *Cryptosporidium* genotype 2 strain (animal and human) was lower during the weeks of the FMD epidemic in 2001 compared with the same weeks in 2000. Our observations are consistent with livestock making a substantial contribution to *Cryptosporidium* infection in humans in England and Wales; our findings have implications for agriculture, visitors to rural areas, water companies, and regulators.

Cryptosporidium is a genus of enteric parasites, a leading cause of infectious diarrhea in humans and livestock. Infection is accompanied by fecal shedding of large numbers of highly infectious and environmentally persistent oocysts (1). Transmission occurs through the fecal-oral route in animal-to-human or human-to-human contact, by recreational exposure to contaminated water or land, or by consumption of contaminated water and food. Infection is also frequently associated with travel to high incidence countries (1). In England and Wales, most isolates are characterized as genotype 1 (which infects only humans) and genotype 2 (which infects both livestock and humans) (2).

Cryptosporidium oocysts have also been recognized as a continuing challenge to water treatment during the last 20 years. Because *Cryptosporidium* organisms tend to become widely distributed in surface waters and are resistant to chlorination, if coagulation and filtration are inadequate in public water supplies, the contaminated water can cause large outbreaks (1,3,4). *Cryptosporidium* in water supplies was studied by three expert committees commissioned by U.K. departments of health and environment in the 1990s, which made recommendations on improving management of slurry, on human hygiene relating to livestock, and on best practices in water treatment and outbreak investigation (5–7). Most cases of cryptosporidiosis, however, are not associated with recognized outbreaks and the sources of these infections remain uncertain (1,7).

Human cryptosporidiosis outbreaks in the U.K. are recognized as being bimodal, peaking in the spring and fall (1). Spring peaks vary by year and location and have been attributed to lambing, calving, and the application of slurry, combined with high rainfall, leading to run-off from agricultural land into surface water and drinking water catchments (1). Autumn peaks have been attributed to persons' summer travel to countries with higher incidence (1).

Surveillance of human enteric infection in England and Wales (which encompass 89% of the U.K. population [8]) is conducted by the voluntary reporting of positive laboratory test results from individual case-patients and outbreak summaries to the Public Health Laboratory Service–Communicable Disease Surveillance Centre (PHLS–CDSC) in Colindale, London. A computer database of individual laboratory reports was established in 1975; a database of outbreak summaries was established in 1992 (9,10). England and Wales have 229 microbiology laboratories, of which 47 are public health laboratories (11,12).

In 2001, in all regions of the U.K., an epidemic of foot and mouth disease (FMD) in livestock occurred (13) (Table 1). The following measures were taken to control the epidemic: excluding visitors from the countryside, extensive culling of affected herds and flocks of farm animals, and limiting the movement of animals for trade and to and from pastures in affected areas (13). These measures likely reduced the direct and indirect exposure to livestock of the overall population of England and Wales. Therefore, we examined laboratory reports of human infection with *Cryptosporidium* for evidence of changes that may have occurred during the period of the FMD epidemic.

Methods

From the national laboratory database, we downloaded laboratory reports to PHLS–CDSC of *Cryptosporidium* oocysts identified in fecal smears with dates for obtaining specimens between January 1, 1991, and December 31, 2001. The download was performed on June 20, 2002, to ensure that all data for 2001 were complete.

The data were aggregated into counts by week the specimen was obtained. To make all weeks exactly 7 days long, we excluded reports with specimen dates on December 31 of every year and on December 30 of every leap year. Intervals between date of illness onset, specimen date, and reporting

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Table 1. Key events during the foot-and-mouth-disease epidemic in livestock, United Kingdom, 2001[a]

Dates	Cumulative cases	Event
2001		
19 February	0	FMD case suspected at an abattoir in Essex, southeast England.
20 February	1	Index case confirmed.
21 February	2	Animal movements banned within infected area. Ban on moving susceptible animals and nontreated animal products from the U.K. imposed by the European Commission.
23 February	6	Case confirmed in Northumberland, northeast England. Environment Agency and Ministry of Agriculture, Fisheries and Food issue joint statement that disposal of animal carcasses produced by culling constitutes an emergency under the terms of the Environmental Protection Act 1990.
25 February	7	Case confirmed in Devon, southwest England.
27 February	16	Special rights to close footpaths and rights of way outside infected areas granted to local government. First case in Wales (Anglesey).
28 February	24	First case in Cumbria, northwest England.
1 March	31	First case in Scotland (Dumfries and Galloway).
2 March	38	Animals intended for human consumption permitted to be moved under license.
6 March	80	Environment Agency announces disposal hierarchy, placing rendering and incineration first.
15 March	250	Policy of culling sheep within 3 km of an infected premise announced by Minister of Agriculture.
20 March	394	Prime Minister initiates daily interdepartmental meetings chaired by Ministry of Agriculture, Fisheries and Foods.
23 March	514	First meeting of Cabinet Office Briefing Room, chaired by the Prime Minister. Government Chief Scientific Officer proposes a 24-h infected premises/48-h contiguous cull policy. 101 Logistics Brigade of the Army deployed at Ministry of Agriculture, Fisheries and Food headquarters.
26 March	644	First mass burial of animal carcasses at Great Orton, northeast England.
30 March	829	Largest number of new cases (50) reported in a single day.
15 April	1,320	14% of footpaths open.
7 May	1,563	Last carcasses buried at Great Orton. Last day of incineration of carcasses. Backlog of animals awaiting disposal cleared.
8 June	1,714	Prime Minister announces new Department for Environment, Food and Rural Affairs replacing Ministry of Agriculture, Fisheries and Food.
22 June	1,773	Department for Environment, Food and Rural Affairs announces intention to revoke most footpath closures.
30 September	2,026	Last confirmed case of foot and mouth disease in Cumbria, northwest England.
28 November	2,026	Last foot and mouth disease–infected area designations lifted from parts of Cumbria, northwest England, north Yorkshire, and County Durham, northeast England.
7 December	2,026	Guidance to lift remaining footpath restrictions issued.
2002		
14 January		Northumberland, northeast England, last county declared to be foot and mouth disease–free.
22 January		U.K. regains international foot and mouth disease–free status, clearing way to resume normal trade in animals and animal products.
21 June		National Audit Office report published "The 2001 Outbreak of Foot and Mouth Disease." Six million animals slaughtered. Direct cost to public sector, 3 billion pounds (U.S. $4.7 billion). Cost to private sector, 5 billion pounds (U.S. $7.9 billion), mostly in the tourism sector. Up to 100,000 animals slaughtered and disposed of each day.

[a]Source, National Audit Office, U.K. (13).

date were reviewed to assess consistency of reporting over time.

The exposure interval for the FMD epidemic was defined as weeks 8–39 of 2001, which corresponded with the first FMD case on February 20 and last case on September 30 (13) (Table 1). We plotted the series of *Cryptosporidium* reports for England and Wales over time and reviewed data from Wales and each region in England individually.

The weekly counts of reports were used as the dependent variable in a negative, binomial regression model. Explanatory variables were region, season (weeks 1–7, weeks 8–39, or weeks 40–52), year (1991–2001), FMD interval (weeks 8–39 in 2001), and a binary variable for a batch reporting error in 1995. We used negative binomial regression rather than Poisson regression because the variance of the count was not approximately equal to the mean of the count (14). An esti-

mate of the reduction in the reports of cryptosporidiosis during the FMD interval was obtained from this model; this estimate was derived from the ratio of the mean count within weeks 8–39 in 2001 to the mean count during the same interval in all other years, adjusted for all other explanatory variables. For each year considered, the mean counts A, B, C, and D were summarized in a 2 x 2 table (Table 2). The rate ratio for the FMD interval = (A/B)/(C/D). A similar model was used to estimate a rate ratio for the FMD interval in each region separately and to estimate rate ratios for weeks 8–39 in 1991, 1992, and all other years.

Fecal specimens positive for *Cryptosporidium* species received by the PHLS Cryptosporidium Reference Unit in Swansea with dates of specimen between January 1, 2000, and December 31, 2001, were genotyped by using polymerase chain reaction and restriction fragment length polymorphism analysis of a region of the *Cryptosporidium* oocyst wall protein gene (15). The proportion of isolates of genotype 2 was examined. Monthly rainfall data for England and Wales were also examined for trends during the surveillance period.

Table 2. Mean counts of reports for weeks and years of foot-and-mouth-disease epidemic and weeks and years of non–foot-and-mouth-disease epidemic used to calculate risk ratios

	Yr 2001	Remaining yrs 1991–2000
Wks 8–39	A	B
Wks 1–7 and 40–52	C	D

Results

The data set comprised 51,322 reports of *Crytosporidium*. We concluded that an outlying count of 387 reports with a specimen date of October 6, 1995, was a batch-reporting error of cases from a large waterborne outbreak in Torbay, in southwest England, which occurred during July–September 1995 (9). Annual reporting rates varied from 6.7–11/100,000 for England and Wales as a whole. Rates were generally <5/100,000 in London; in several recent years, the highest rates occurred in northwest England (Table 3).

The timing of clearly identifiable spring and autumn peaks of *Cryptosporidium* laboratory reports was fairly consistent from year to year (Figure 1). Weeks 8–39 of each year included all spring peaks and the first half of all autumn peaks. However, spring and autumn peaks were not always clearly identifiable in each year. The year 1993 was notable for the absence of an autumn peak; in 1996 and 1997, identifying either a spring or autumn peak was difficult (Figure 1). In 2001, weekly counts remained lower than in previous years until approximately week 35, when an autumn peak comparable to previous years was observed (Figure 1).

By modeling counts, a significant decrease in overall seasonal and yearly associations was noted only in 1992 and in 2001 (Table 4). Weeks 8–39 in 1992 showed an estimated 22% decrease, whereas weeks 8–39 in 2001, corresponding to the FMD epidemic, showed an estimated 35% (95% confidence interval [CI] 20% to 47%) decrease in England and Wales as a whole (p=0.001). We estimated that the FMD epidemic interval was associated with some reduction of human cryptosporidiosis in all regions of England and in Wales, but the largest association was in northwest England, which showed decrease of approximately 63% (95% CI 31% to 80%) (p=0.001) (Table 5).

The age distribution for persons with reported cryptosporidiosis for weeks 8–39 in 2001 was similar for the same time of year in each of the preceding 10 years. A history of foreign travel preceding diagnosis was given in 5% of reports and showed a single autumn peak in weeks 31–41 of each year, while those reports from case-patients without a history of foreign travel showed both spring and autumn peaks (Figure 2).

Genotyping results were available for approximately half of the reported cases of cryptosporidiosis made to PHLS–CDSC in 2000 and 2001. When specimens from persons with a history of recent foreign travel were excluded, the proportion of genotype 2 cases was generally higher in the first half of both years (Figure 3). In weeks 21–24 of 2001 (May 21–June 17), the proportion of genotype 2 cases was significantly lower than for the same weeks of 2000 (Figure 3). In weeks 8–39 as a whole, the proportion of genotype 2 cases was significantly

Table 3. Annual rate of reported *Cryptosporidium* species per 100,000 population, England and Wales, 1991–2001[a]

Region in England	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
Eastern	7.8	9.4	9.3	6.6	12.3	5.0	11.3	5.1	7.5	9.1	8.2
London	7.8	3.6	2.6	2.7	3.6	2.4	2.3	1.9	2.9	3.8	2.3
Northwest	11.7	17.6	12.9	10.6	11.7	10.1	15.8	11.9	19.8	20.9	7.5
Northern and Yorkshire	10.2	13.0	11.4	12.6	12.1	10.4	8.2	7.9	9.6	8.2	6.9
Southeast	11.5	6.8	8.2	8.8	10.2	5.5	6.9	5.9	6.6	8.7	6.7
Southwest	15.3	14.2	12.1	12.7	22.0	8.9	10.2	10.2	12.7	14.7	8.7
Trent	9.5	9.6	8.9	10.5	11.2	7.0	9.1	7.8	10.1	14.1	7.1
West Midlands	6.5	7.6	8.6	4.9	7.6	6.4	5.7	5.6	8.6	10.3	7.3
Wales	12.1	11.3	12.0	11.5	12.5	7.9	7.2	9.3	11.5	12.1	8.3
Total (England and Wales)	10.2	10.1	9.2	8.7	11.0	6.9	8.4	7.0	9.6	11.0	6.7

[a]Mid-year population estimates from the Office for National Statistics, U.K.

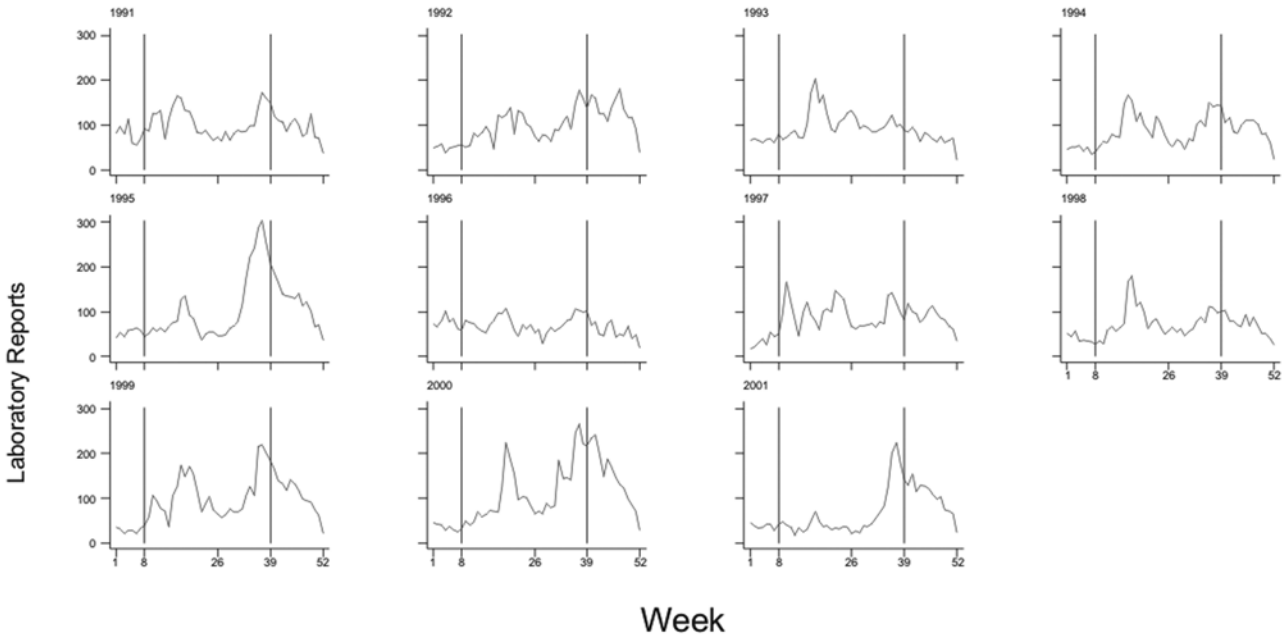

Figure 1. Laboratory reports of *Cryptosporidium* species to Public Health Laboratory Service–Communicable Disease Surveillance Centre, by specimen week, England and Wales, 1991–2001. Reporting artifact on October 6, 1995 not shown.

lower in 2001 than in 2000 (49%, 338/696 in 2001; 63%, 977/1,558 in 2000; p<0.00005).

Mean rainfall in England and Wales between January and June (to approximately week 26) was <60 mm in 1992 and 1996 (Table 6): in these years, the spring peak in *Cryptosporidium* reporting was also below normal (Figure 1). In contrast, rainfall in the first 6 months of 2001 was above the 1961–1990 average and showed a pattern more like that that observed in 1998, 1999, and 2000: in these years, a spring peak in *Cryptosporidium* reporting was conspicuous (Figure 1; Table 6).

Discussion

Cryptosporidium reports from England and Wales decreased substantially during the FMD epidemic in livestock in 2001, with a marked attenuation of reports in the first half of the year. Modeling counts of *Cryptosporidium* reports showed that the observed decrease was unlikely to be explained by seasonal and yearly associations. In none of the previous 10 years were deviations from overall yearly and seasonal associations as large as those estimated for weeks 8–39 in 2001.

For a number of reasons, this decrease cannot be attributed to errors in transmission and entry of reports into the PHLS–CDSC database. Counts returned to normal levels by about week 35 of 2001, followed by an autumn peak comparable in size to that in most previous years. A similar pattern of decrease was not apparent for *Salmonella*, *Campylobacter*, or *Giardia* reports, which were received and stored in a manner similar to those for *Cryptosporidium* (PHLS data). Lag times between onset of illness, specimen collection, and reporting dates were stable throughout the data set, except for the single batch-reporting artifact in 1995 (for which adjustment was made in the regression analysis). No change or disruption in reporting methods or data storage was known to have occurred over the study period.

Genotyping of specimens from 2000 and 2001 showed that most submitted were of genotype 2 (livestock and human strain) in the first half of each year, whereas most were of genotype 1 (human-only strain) in the second half of each year. During the FMD epidemic interval, the proportion of genotype 2 isolates was lower than that for the same time of year in 2000.

The presence of an autumn peak in case-patients reported to have recently traveled abroad, coincident with an autumn

Table 4. Rate ratios for weeks 8–39 of each year adjusted for yearly and seasonal effects, England and Wales, 1991–2001

Yr	Rate ratio	95% confidence intervals	p value
1991	1.10	0.89 to 1.35	0.38
1992	0.78	0.64 to 0.96	0.02
1993	1.27	1.03 to 1.57	0.02
1994	1.10	0.89 to 1.35	0.38
1995	0.97	0.79 to 1.20	0.81
1996	1.02	0.83 to 1.26	0.82
1997	1.20	0.97 to 1.47	0.10
1998	1.11	0.90 to 1.38	0.32
1999	1.07	0.87 to 1.31	0.55
2000	0.93	0.76 to 1.14	0.47
2001	0.65	0.53 to 0.80	0.001

Table 5. Rate ratios associated with foot-and-mouth-disease epidemic, England and Wales, 2000

Region	Rate ratio	95% confidence intervals	p value
Northwest	0.37	0.20 to 0.69	0.001
Eastern	0.57	0.31 to 1.06	0.08
Northern and Yorkshire	0.60	0.32 to 1.11	0.10
Southeast	0.66	0.36 to 1.21	0.17
London	0.67	0.35 to 1.31	0.24
Southwest	0.72	0.39 to 1.34	0.30
Trent	0.90	0.48 to 1.68	0.74
West Midlands	0.90	0.48 to 1.69	0.75
Wales	0.70	0.37 to 1.33	0.28
Total (England and Wales)	0.65	0.53 to 0.80	0.001

peak in case-patients not known to have recently traveled abroad, is consistent with substantial underreporting of travel abroad and the association of the autumn peak with such travel. Conversely, the absence of a spring peak among case-patients with reported recent travel abroad suggests that the spring peak is predominantly due to exposures occurring within the U.K.

The absence of clear spring peaks in human *Cryptosporidium* reports in 1992 and 1996 may have been related to below average rainfall in England and Wales in the first 6 months of these years. However, the rainfall levels in the first 6 months of 2001 were similar to those in 1998, 1999, and 2000, years in which spring peaks were conspicuous. Therefore, no strong evidence suggests that the exceptionally low number of reports observed during the FMD epidemic interval could be explained by below average rainfall.

The low reporting rate for *Cryptosporidium* in London may be explained by the historic low number of public health laboratories in the capital. Public health laboratories, which provide approximately half of all laboratory reports to PHLS–CDSC, examine all fecal specimens for *Cryptosporidium* oocysts and report all positive results to PHLS–CDSC (10). Recent surveys indicate that 20% of laboratories in the northwest region of England and Wales and 40% of laboratories in the east and southeast regions of England continue to use varied criteria to select a subset of submitted fecal specimens for examination for *Cryptosporidium* oocysts, and may not always report positive test results (17,18): similar variation may be expected in other regions. However, we have no evidence to suggest that these laboratories systematically changed their practices during the study period. The recent comparatively high reporting rates of *Cryptosporidium* infections in the northwest region cannot be entirely explained by the fact that a higher proportion of laboratories now have policies requiring the examination of all fecal specimens for *Cryptosporidium* oocysts and the reporting of all positive results. The northwest region of England has experienced comparatively frequent confirmed and suspected waterborne outbreaks of cryptosporidiosis (19); therefore, a genuinely higher incidence is a more likely explanation. Throughout England and Wales, a substantial decrease in reports was observed, coinciding with the start of the FMD epidemic in 2001, followed by a return to normal levels by about week 35. A systematic change in testing and reporting by over 200 laboratories does not explain this observation.

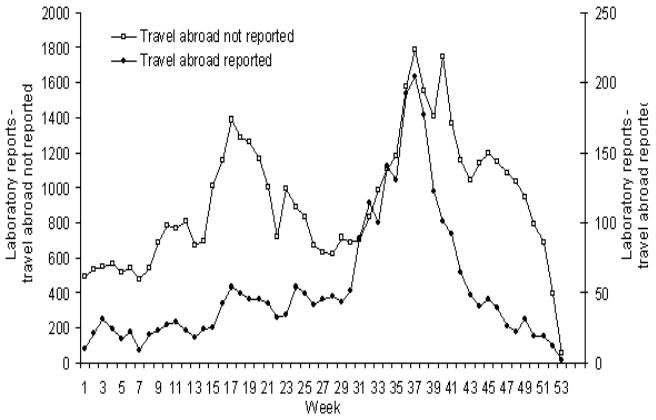

Figure 2. Laboratory reports of *Cryptosporidium* species to Public Health Laboratory Service–Communicable Disease Surveillance Centre, by specimen week, including reports with and without foreign travel, England and Wales, 1991–2001. FMD, foot and mouth disease.

On the whole, our results suggest that a decrease in genotype 2 *Cryptosporidium* infection in humans was associated with a decrease in human exposure to reservoirs of infection in livestock in England and Wales during the FMD epidemic interval. That the FMD epidemic interval was associated with a decrease in all English regions and Wales, including London, may have been because visitors from throughout England and Wales had decreased access to affected regions.

That contamination of water supplies was decreased through removal of livestock from drinking water catchments by slaughter or containment elsewhere is also plausible. The FMD epidemic was estimated to have had the largest effect in northwest England, which is consistent with the particularly large change in animal husbandry and livestock numbers associated with the FMD epidemic in this region. Livestock fecal contamination of an unfiltered surface water reservoir may

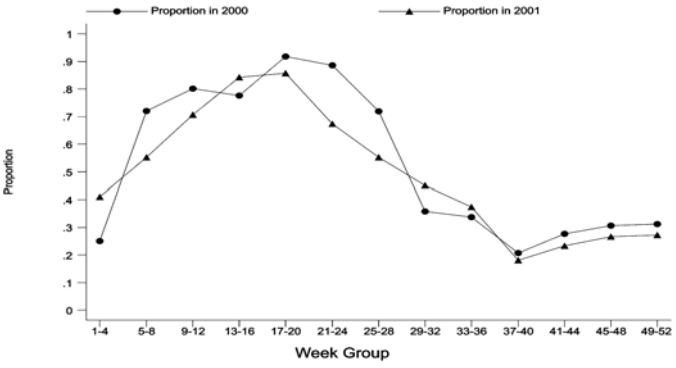

Figure 3. Laboratory isolates of *Cryptosporidium* species, proportion of genotype 2, by specimen week, England and Wales, 2000 and 2001.

Table 6. Monthly precipitation values (mm), England and Wales, 1991–2001[a]

		Yr											
Month	Approx wks	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	1961–90 mean
Jan	1–4	97.5	48.7	115.3	131	162.6	65.9	16.4	121.2	127.7	46.5	84.4	91.0
Feb	5–8	64.3	44.8	13.8	85.2	114.8	83.3	115.9	20.1	49.1	95.1	105.3	65.0
Mar	9–13	74.3	82.2	26.6	94.0	70.6	43.2	30.7	88.0	69.6	32.7	107.5	74.0
Apr	14–17	70.9	75.9	94.8	76.3	28.1	51.0	24.6	132.6	76.3	142.6	100.0	61.0
May	18–21	13.6	51.4	89.3	71.1	48.5	58.4	72.8	35.4	55.5	98.0	42.1	65.0
Jun	22–26	103.0	38.0	68.6	36.1	20.2	29.6	136.7	119.8	89.2	43.0	44.4	65.0
Jan–June mean	1–26	70.6	56.8	68.1	82.3	74.1	55.2	66.2	86.2	77.9	76.3	80.6	70.2
Jul	27–30	70.7	89.7	88.6	45.0	37.6	43.6	45.9	56.5	26.1	63.8	73.2	62.0
Aug	31–35	27.8	134.6	54.4	75.7	9.1	79.9	104.0	47.2	115.8	65.9	86.3	77.0
Sep	36–39	64.9	96.9	119.6	106.2	123.3	34.0	34.3	102.1	120.6	132.6	82.9	78.0
Oct	40–43	72.1	90.5	94.4	103.5	52.0	87.8	72.3	154.7	86.5	188.0	135.4	87.0
Nov	44–48	93.4	148.5	75.9	87.9	82.8	134.3	122.1	88.8	67.3	182.1	65.1	92.0
Dec	49–52	49.3	78.6	172.0	138.0	91.1	55.5	108.5	96.8	142.4	137.2	43.5	95.0
Jul–Dec mean	27–52	63.0	106.5	100.8	92.7	66.0	72.5	81.2	91.0	93.1	128.3	81.1	81.8
Annual mean	1–52	66.8	81.7	84.4	87.5	70.1	63.9	73.7	88.6	85.5	102.3	80.8	76.0

[a]Including the 1961–1990 mean. Source, University of East Anglia (16).

have decreased in the English Lake District, which serves approximately one-third of the population of the northwest region with drinking water (19).

Water companies in England and Wales have been required to conduct risk assessments of their water sources for *Cryptosporidium* and to undertake real-time monitoring of treated water for oocysts at high-risk works since April 1, 2000 (20). However, introduction of this regulation was not associated with a decrease in *Cryptosporidium* reporting between April 1, 2000, and the beginning of the FMD epidemic on February 20, 2001.

The surveillance patterns observed suggest that exposure to livestock and their excreta may contribute a substantial fraction of human cryptosporidiosis in England and Wales. Our observations support continued concern over the presence of *Cryptosporidium* oocysts in public water supplies, especially in northwest England, and suggest that policies and the economics for the management of water catchments and water treatment in England and Wales, especially the northwest region, require further review.

The impact of the FMD epidemic in livestock on *Cryptosporidium* infection in humans can be characterized as complex. Long-lasting changes to farming practices and restructuring of rural economies occurred and will continue. Additionally, water companies continue to improve the microbiologic safety of public water supplies, supported by strict legal limits for *Cryptosporidium* oocyst concentrations in treated water. Whether the decline in *Cryptosporidium* reporting coincident with the FMD epidemic will be sustained in future years will be interesting to observe.

Changes in livestock-mediated exposure to *Cryptosporidium* would not correspond precisely with the interval between the first and last confirmed cases of FMD. A delay was expected between the start of the FMD epidemic in livestock and a change in livestock-mediated *Cryptosporidium* exposure in humans and its consequent detection by the national laboratory surveillance system. Key components of this delay include the incubation period of *Cryptosporidium* in humans; the amount of time before seeking medical attention, and the time required for giving a fecal test, examining the specimen, and reporting and entering positive test results into the national database. We expected the degree of change in livestock-mediated *Cryptosporidium* exposure to vary by time and place because of variation in livestock densities, the intensity of animal culling, and differences in the containment of animals from traditional pastures between different areas of the U.K. Nonetheless, the coincidence between the FMD epidemic and decline in human cryptosporidiosis is striking and suggests that the FMD epidemic in livestock has changed the ecology between humans, livestock, and *Cryptosporidium* in England and Wales.

Further studies to define the contribution of key components of the FMD epidemic on human *Cryptosporidium* infection may be of value in appropriate geographic areas such as northwest England. Such studies could include modeling the independent effect of changes in livestock densities, farm access, and rural access and adjusting for water supply to residences, changes in water treatment, and rainfall.

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Congenital Transmission of *Trypanosoma cruzi* Infection in Argentina

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Trypanosoma cruzi, the causative agent of Chagas disease, infects 10–18 million people and may be transmitted to the newborn. Using various data sources, we estimated that nearly 850 congenital cases occurred in Argentina in 1993, or 6.3 expected cases per each reported case in 1994 and in 1994–2001. The congenital transmission of *T. cruzi* constitutes a sizeable public health problem in the region.

Trypanosoma cruzi, the causative agent of Chagas disease, infects 10–18 million people in the Americas (1), half of whom most likely are women who may eventually transmit *T. cruzi* to their newborns. As residual spraying of insecticides against cone-nosed bugs (*Triatoma infestans)* and screening of blood donors have produced a decreasing trend in human incidence of *Trypanosoma cruzi* in Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay, the relative magnitude and visibility of vertical transmission have been gradually emerging.

Congenital cases of *T. cruzi* are mostly asymptomatic or monosymptomatic and seriously affect the newborn's survival and illness rate (2,3). Such cases cannot be prevented because the available drugs have adverse effects, but early detection and prompt treatment are frequently successful (3). However, as screening of pregnant women and newborns has not been routinely conducted in most *T. cruzi*–endemic countries, the magnitude of the congenital transmission of this pathogen as a public health problem has not been established. Having such an estimate would be important for making policy recommendations and health service planning. Our study estimates the annual number of congenital Chagas cases that occurred in Argentina recently and compares them with official case reports.

Materials and Methods

For a given province, year, and maternal age group, the estimated number of live patients with congenital *T. cruzi* infection was computed as the total number of live newborns (f), times the probability of a woman's being infected with *T. cruzi* (p), times the probability of transmitting *T. cruzi* to the live newborn (t). Province and age-specific numbers of live births and maternal seroprevalence rates of infection are needed to provide a countrywide annual estimate.

The total number of live births in 1991 (694,776 newborns), according to the mother's age group and province (4), changed very little during the 1990s. We categorized births uniformly within each maternal 5-year age class because data on births of single-year age classes were not available; we did not count the very small number of births in other countries or with unspecified birthplace.

The probability of a woman's being infected with *T. cruzi* varies with age; however, it may be estimated from the seroprevalence for *T. cruzi* in men of the same age group because no evidence of a gender-related excess risk of infection has been detected (5,6). For Argentina, the available province-specific seroprevalence rates of *T. cruzi* infection for young men drafted into military service in 1965–1969 (when they were 21 years of age) and annually from 1981 to 1993 (when they were 18 years) showed quite different temporal trends (7). Each recruit age class was a randomly selected birth cohort; unhealthy persons were excluded after blood samples were taken. To reconstruct maternal seroprevalence rates in 1993, we assumed a closed, steady-state population between year of diagnosis and 1993. This assumption meant that a) the fraction infected in each birth cohort remained stable because most *T. cruzi* infections were acquired during childhood, and specific treatment of infected adults was uncommon except for male recruits and legal immigrants; and b) men and women ages 15–50 had similar age-specific death and emigration rates because most findings of *T. cruzi*–specific pathology in women occur at postreproductive ages (8). Potential differences in cohort-specific rates of recruitment or loss of infected women through differential migration associated with *T. cruzi* infection were ignored, although internal migrations may modify the expected number of cases, depending on the interplay between fertility, *T. cruzi* infection, age at migration, and source and destination of migrants. We ignored the contribution of increasing numbers of adult immigrants from neighboring *T. cruzi*–endemic countries since the 1950s. We tentatively assumed that the chance of being pregnant and having an uneventful pregnancy was not affected by infection with *T. cruzi*, but present evidence is controversial.

The province-specific maternal prevalence of *T. cruzi* infection in 1993 comprised the partial contributions of mothers who were 18 years old in 1993 (born in 1975) and to whom we assigned the seroprevalence rate of 18-year-old men

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assessed in 1993, and so on through mothers 30 years of age in 1993 (born in 1963) and to whom we assigned the seroprevalence rate of 18-year-old men assessed in 1981. For mothers >30 years of age in 1993, we assigned the rate from 1965–1969 data to 1967 and assumed that the prevalence followed a linear trend between 1967 and 1981. The few 14- to 17-year-old mothers were assigned the 18-year-olds' seroprevalence assessed in 1993. Tierra de Fuego was excluded from calculations because the time series had numerous missing data. Calculations were carried out in an Excel spreadsheet, available on request.

The probability of congenital transmission from pregnant women seropositive for *T. cruzi* has been extremely variable (range 0.005–0.117) among countries and geographic areas (1–3), and its determinants are little known (9,10). From the latest review (3), we estimated the median t as 0.025 (interquartile range 0.02–0.04). We assumed that t was not modified by the mother's age (9,10) because most mothers were in the indeterminate or chronic phase of infection in which the parasitemia levels are low and age independent. We ignored potential geographic variations and parasite strain effects on t.

Results

The National System of Epidemiological Surveillance reported (11) a total of 1,136 congenital cases of *T. cruzi* in 1994–2001 (annual mean 142; standard deviation 54), with no significant time trend and very large asynchronic variations among and within provinces (Figure 1). As demonstrated for leishmaniasis surveillance in Argentina (12), inconsistencies among provincial, national, and Chagas surveillance reports of congenital cases were frequent and led to substantial underreporting.

A total of 846 congenital cases were estimated for 1993 (Figure 2A). An example of the calculations for the province of Buenos Aires is given in the Appendix. The expected annual number of congenital cases peaked in Chaco (153 cases) and the province of Buenos Aires (96 cases); the latter had rare domiciliary triatomine infestations and a large number of immigrants from Chagas-endemic provinces during 1947 to the 1970s. Santiago del Estero, with high seroprevalence and fertility rates but very low human population, ranked 3rd (90 cases). The ratio between our conservative estimate of congenital cases in 1993 (846 cases) and official notifications in 1994 (135) and 1994–2001 (142 cases) was 6.3: 1. A very rough calculation that used countrywide averages (f = 687,051; p = 0.019; t = 0.025) yielded 326 congenital cases, or 38% of the above estimate.

Extreme differences between expected and reported mean numbers of congenital cases occurred in Chaco and Santiago del Estero, followed by Formosa, Tucuman, and Mendoza (Figure 2A), suggesting strong underreporting. Santa Fe, Jujuy, Salta, and the province of Buenos Aires reported most cases. The ranked province-specific total numbers of cases estimated for 1993 and reported officially from 1994 to 2001 were significantly correlated (Figure 2B) and thus provided a qualitative hierarchic ordering of provinces in terms of the likely burden of congenital cases.

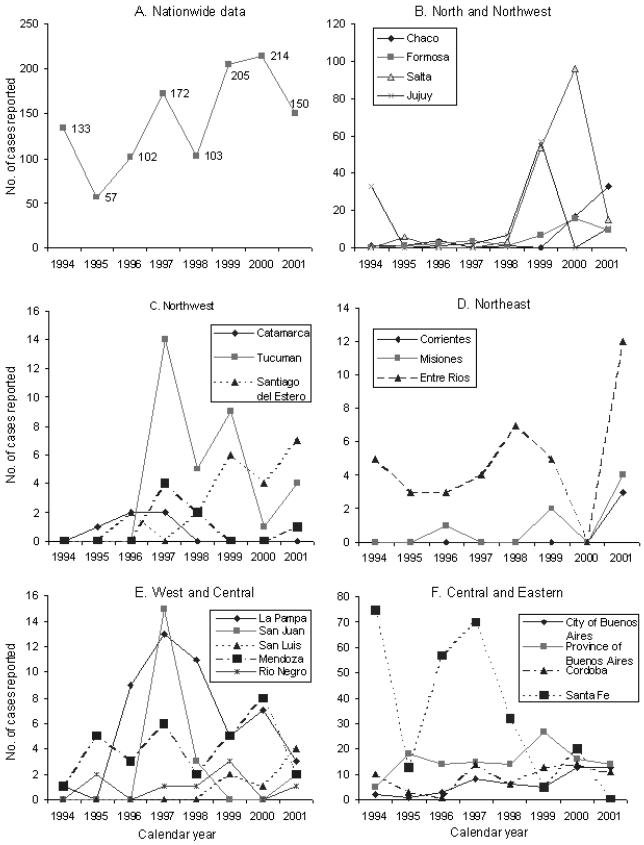

Figure 1. Nationwide and province-specific numbers of reported cases of congenital *Trypanosoma cruzi* infection notified to the Ministry of Health of Argentina. Mean coefficient of variation among provinces over time, 266%; range 39% to 283%. For the city and Province of Buenos Aires, we used the 1994–1999 data corrected by the Chagas National Surveillance System.

Additional calculations tend to support our rough estimates. A pilot control program in a public maternity facility, where 37.8% of all births in Tucuman took place, detected 32 congenital cases over 28 months (mean 13.7 cases per year) (13). If we assume this was a random sample, the annual number of congenital cases projected to the whole province was 36.2 cases, which is roughly close to the expected number of 46.7 cases.

Schmuñis (1) estimated that 1,593 congenital cases of *T. cruzi* occurred annually in Argentina around 1985, on the assumptions that the maternal seroprevalence equaled the overall seroprevalence of *T. cruzi* in blood banks (6.96%); that t = 0.03; and that no age-specific variations in fertility and prevalence of infection occurred. When we used data on the seroprevalence of *T. cruzi* among 131,909 pregnant women (4.4%) from 15 Argentine provinces in 2000, and among 153,266 women (5.7%) from 20 provinces in 2001 (Sonia Blanco, unpub. data), where f = 700,000 and t = 0.025, a similar calculation yields 770 and 997 estimated congenital cases for 2000 and 2001, respectively. However, because mean rural parity in 1991 was positively and significantly associated with

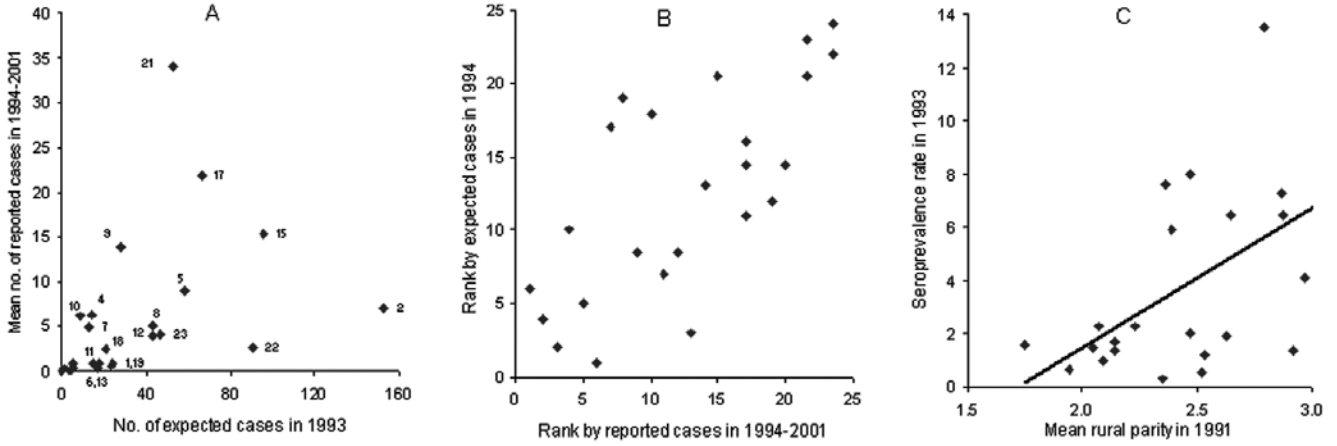

Figure 2. (A) Numbers of expected (in 1993) and reported (mean of 1994–2001) cases of congenital *Trypanosoma cruzi* infection. (B) Ranked ordering of provinces according to numbers of expected (in 1993) and total reported cases (1994–2001) of congenital *T. cruzi* infection; Spearman's correlation coefficient (R) = 0.711, n=24, p<0.0001. 1, Catamarca; 2, Chaco; 3, Chubut; 4, City of Buenos Aires; 5, Cordoba; 6, Corrientes; 7, Entre Rios; 8, Formosa; 9, Jujuy; 10, La Pampa; 11, La Rioja; 12, Mendoza; 13, Misiones; 14, Neuquen; 15, Province of Buenos Aires; 16, Rio Negro; 17, Salta; 18, San Juan; 19, San Luis; 20, Santa Cruz; 21, Santa Fe; 22, Santiago del Estero; 23, Tucuman. (C) Relationship between seroprevalence rates of *T. cruzi* infection in military recruits in 1993 and mean rural parity in 1991 (r=0.541, n=23, p<0.01). The City of Buenos Aires, which does not have a rural area, was excluded from analysis.

T. cruzi seroprevalence in military recruits in 1993 at a province level (Figure 2C), fertility and maternal infection also may be positively associated at the individual level in rural settings. Therefore, the use of average, province-wide fertility rates would underestimate both the number of newborns from infected women and the occurrence of congenital cases.

Discussion

The congenital transmission of *T. cruzi* appears to be a sizeable public health problem in Argentina, where it has already surpassed the number of vector-mediated acute cases by a factor of 10, and probably elsewhere in the region. Despite a long-term decreasing trend in the human prevalence of *T. cruzi*, for which we need increased prevention measures, infected women of reproductive age will still give birth in the foreseeable future. Available data favor a short-term policy of antenatal diagnosis of pregnant women for *T. cruzi* infection and follow-up of their newborns. Increased international migrations from *T. cruzi*–endemic Latin American countries suggest the need for an increased awareness among obstetricians, neonatologists and pediatricians. Effective, nontoxic drugs that may be administered to prospective mothers or pregnant women to reduce the likelihood of congenital transmission are clearly needed.

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Appendix

Appendix Table. Example of the calculations involved in the estimate of the number of congenital cases of *Trypanosoma cruzi* infection for the Province of Buenos Aires, 1993

Mother's birth year	Seroprevalence of *T. cruzi*	No. of live newborns	No. of live newborns from infected women	No. of expected congenital cases
1946	4.70	281	13.22	0.3
1947	4.57	281	12.85	0.3
1948	4.44	281	12.49	0.3
1949	4.31	1,322	56.99	1.4
1950	4.18	1,322	55.28	1.4
1951	4.05	1,322	53.57	1.3
1952	3.92	1,322	51.86	1.3
1953	3.79	1,322	50.15	1.3
1954	3.66	4,623	169.42	4.2
1955	3.54	4,623	163.44	4.1
1956	3.41	4,623	157.46	3.9
1957	3.28	4,623	151.47	3.8
1958	3.15	4,623	145.49	3.6
1959	3.02	8,628	260.37	6.5
1960	2.89	8,628	249.20	6.2
1961	2.76	8,628	238.04	6.0
1962	2.63	8,628	226.87	5.7
1963	2.50	8,628	215.70	5.4
1964	2.39	12,703	303.61	7.6
1965	2.31	12,703	293.44	7.3
1966	0.18	12,703	22.87	0.6
1967	0.15	12,703	19.05	0.5
1968	1.19	12,703	151.17	3.8
1969	1.04	12,571	130.74	3.3
1970	1.08	12,571	135.77	3.4
1971	0.77	12,571	96.80	2.4
1972	0.68	12,571	85.48	2.1
1973	0.65	12,571	81.71	2.0
1974	0.71	6,160	43.74	1.1
1975	0.70	6,160	43.12	1.1
1976	0.70	6,160	43.12	1.1
1977	0.70	6,160	43.12	1.1
1978	0.70	6,160	43.12	1.1
1979	0.70	695	4.86	0.1
Total				95.6

Molecular Surveillance System for Global Patterns of Drug Resistance in Imported Malaria

Annie-Claude Labbé,* Samir Patel,* Ian Crandall,* and Kevin C. Kain*

Analysis of imported malaria in travelers may represent a novel surveillance system for drug-resistant malaria. We analyzed consecutive falciparum malaria isolates from Canadian travelers from 1994 to 2000, for polymorphisms in *pfcrt, dhfr,* and *dhps* linked to chloroquine and pyrimethamine/sulfadoxine resistance. Forty percent of isolates possessed the K76 *pfcrt* allele, suggesting that many imported falciparum infections are still responsive to chloroquine. Travelers who had recently taken chloroquine had a significantly increased risk of harboring isolates with *pfcrt* resistance alleles (odds ratio = 4.47; p=0.03). The presence of two or more mutations in *dhfr* or *dhps* was found in 64.8% (95% confidence interval [CI] 54.6 to 73.9) and in 30.4% (95% CI 21.7 to 40.3) of isolates, respectively, and increased significantly over the course of the study. These molecular markers indicate that pyrimethamine/sulfadoxine resistance is increasing and is now too high to rely on this drug as a routine therapeutic agent to treat malaria in travelers.

Drug-resistant malaria is increasing, and novel strategies to monitor for resistance are needed. Over 50 million persons from the industrialized world visit malaria-endemic countries annually, and record numbers of imported malaria cases are being reported in North America and Europe (1). The first well-documented cases of chloroquine-resistant and sulfadoxine-pyrimethamine (SP)–resistant *Plasmodium falciparum* malaria were identified in tourists visiting East Africa in the late 1970s and early 1980s, which suggests that travelers may represent an important sentinel population to monitor for drug-resistant malaria (2,3). Although assessing travelers for malaria treatment and prophylaxis failures may be an effective strategy for detecting emerging drug resistance, traditional methods of detecting resistance, including in vivo treatment trials and in vitro drug susceptibility testing, are time- and labor-intensive and are not well suited to large-scale surveillance of travelers (4).

Molecular methods that detect genetic markers of drug resistance in parasites are potentially powerful tools to detect and track drug-resistant malaria. The molecular basis of resistance to antifolate drugs such as SP has been well characterized. High-level pyrimethamine resistance results from the accumulation of mutations in the *dhfr* gene, principally at codons 108, 59, and 51 (5,6). Similarly, point mutations in *dhps* have been associated with decreased susceptibility to sulfadoxine in vitro (7). Chloroquine resistance has been linked to mutations in two genes, *pfmdr1* and *pfcrt*, that encode the digestive vacuole transmembrane proteins Pgh1 and PfCRT, respectively (8–13). Transfection studies with *pfmdr1* suggest that mutations in Pgh1 may modulate the chloroquine resistance phenotype in vitro; however, in vivo studies have shown an inconsistent association between mutations in Pgh1 and chloroquine resistance (9–12). More recently, a series of point mutations in *pfcrt* have been associated with chloroquine resistance (13). One mutation at position 76 (K76T) was present in all in vitro resistant parasites and has been proposed as a molecular marker for surveillance of chloroquine-resistant falciparum malaria, particularly in nonimmune populations such as travelers (10,13).

The objectives of this study were to establish a molecular surveillance system for imported malaria, to determine and track the prevalence of putative molecular markers of drug resistance, and to examine risk factors for infection with isolates bearing resistance markers.

Materials and Methods

From January 1, 1994, to June 30, 2000, patients seen at the Toronto General Hospital or the Hospital for Sick Children in Toronto, Canada, with microscopically confirmed falciparum malaria were enrolled. Patient interviews were conducted or medical charts were reviewed for potential risk factors for infection and drug resistance by using a standardized data extraction form. This study was approved by the Institutional Review Boards of the Toronto General Hospital and the Hospital for Sick Children.

Falciparum isolates were characterized by polymerase chain reaction-restriction fragment length polymorphism analysis and sequencing for allelic variants of *pfcrt* and *pfmdr1*, *dhfr,* and *dhps* as described (5–17). Proportions were compared by using the chi-square test or Fisher exact test, as appropriate. The chi-square test for trend was used as required for variables that involved ordered categories. For the purpose of this analysis, we considered mixed isolates (e.g., isolates containing parasites with mutant and wild-type alleles) to be mutant ones and compared them against those possessing only wild-type alleles.

Results

During the study period, 105 consecutive cases of imported falciparum malaria were recorded (69 males, 36 females; age range 1–70 years [mean 30.5]). The geographic regions in which these persons acquired their infections are shown in Table 1.

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The prevalence of mutations in chloroquine-resistance markers (*pfmdr1* and *pfcrt*) was significantly higher in isolates acquired in East Africa compared with West Africa (Table 1). Patients who acquired malaria infection in East Africa had a 4.5-fold higher risk of being infected by an isolate possessing the K76T mutation in *pfcrt* (odds ratio [OR] 4.53 [95% confidence interval (CI) 1.26 to 16.02]; p=0.03) than those visiting West Africa. The N86Y mutation in *pfmdr1* was also found more often in isolates acquired in East Africa than in those acquired in West Africa (OR 3.56 [95% CI 1.16 to 10.80]; p=0.03). A linear trend for increasing prevalence of N86Y mutant isolates was evident during the study period (OR per year =1.21; p=0.07) (Table 2). We also examined the association between past chloroquine exposure and the prevalence of chloroquine-resistance markers. Nineteen (18.1%) patients had used chloroquine for prophylaxis (n=13) or treatment (n=6) while abroad. These persons had a significantly increased risk of being infected by an isolate harboring the K76T mutation when compared with other travelers (OR=4.47 [95% CI 1.13 to 25.45]; p=0.03).

We grouped the parasite *dhfr* and *dhps* genotypes into four categories on the basis of the cumulative number of mutations that have been linked to escalating SP resistance (Table 1). More mutations in *dhps* were found in isolates from travelers returning from West Africa versus East Africa (p=0.001, Fisher exact test). We found that the proportion of isolates with at least two mutations increased during the study period in both *dhfr* (OR for a 1-unit increase in year = 1.28; p=0.02, chi-square test for trend) and *dhps* (OR=1.29; p=0.03) (Table 2).

Discussion

In this study, we demonstrate "proof-of-principle" that a molecular surveillance strategy based on imported malaria in travelers can be used to detect and track drug-resistant malaria. Monitoring travelers for imported drug-resistant malaria is a surveillance strategy that offers several potential advantages. Recommendations regarding treatment regimens and chemoprophylaxis for travelers should ideally be made on the basis of the efficacy of these drugs in nonimmune travelers rather than on partially immune persons residing in malaria-endemic

Table 1. Prevalence of molecular markers of drug resistance by region of malaria acquisition

	Area of endemicity n (%)					Total
Genotypes	West Africa[a]	East Africa[b]	Central Africa[c]	Southern Africa[d]	Other[e]	n (%; 95%CI)[f]
pfmdr1						
N86 (wild)	40 (59.7)	5 (29.4)	1 (20.0)	1 (33.3)	6 (75.0)	53 (53.0; 42.8 to 63.1)
86Y (mutant)	27 (40.3)	12 (70.6)	4 (80.0)	2 (66.7)	2 (25.0)	47 (47.0; 36.9 to 57.2)
pfcrt						
K76 (wild)	32 (49.2)	3 (17.6)	3 (60.0)	0	1 (11.1)	39 (39.8; 30.0 to 50.2)
76T (mutant)	33 (50.8)	14 (82.4)	2 (40.0)	2 (100)	8 (88.9)	59 (60.2; 49.8 to 70.0)
dhfr[g]						
Wild-type	17 (25.0)	3 (17.6)	3 (60.0)	1 (33.3)	2 (22.2)	26 (25.5; 17.4 to 35.1)
Single mutants	6 (8.8)	0	0	0	3 (33.3)	9 (8.8; 4.1 to 16.1)
Double mutants	25 (36.8)	8 (47.1)	1 (20.0)	2 (66.7)	3 (33.3)	39 (38.2; 28.8 to 48.4)
Triple mutants	20 (29.4)	6 (35.3)	1 (20.0)	0	1 (11.1)	28 (27.5; 19.1 to 37.2)
dhps[h]						
Wild-type	3 (4.4)	7 (41.2)	0	2 (66.7)	8 (88.9)	20 (19.6; 12.4 to 28.6)
Single mutants	40 (58.8)	5 (29.4)	5 (100)	1 (33.3)	0	51 (50.0; 40.0 to 60.1)
Double mutants	21 (30.9)	5 (29.4)	0	0	0	26 (25.5; 17.4 to 35.1)
Triple mutants	4 (5.9)	0	0	0	1 (11.1)	5 (4.9; 1.6 to 11.1)
No. of infected patients	71 (67.6)	17 (16.2)	5 (4.8)	3 (2.9)	9 (8.6)	105

[a]Two patients had visited more than one country: Ghana (45 patients), Nigeria (21), The Gambia (2 patients), Sierra Leone (3 patients), Burkina Faso (1 patient), Mali (1 patient), and Guinea (1 patient).
[b]Three patients had visited more than one country: Kenya (9 patients), Uganda (6 patients), Tanzania (3 patients), Rwanda (1 patient), and Burundi (1 patient).
[c]Central African Republic (2 patients), Congo (2 patients), and Cameroon (1 patient)
[d]Angola (2 patients) and Madagascar (1 patient).
[e]India (5 patients), Malaysia (1 patient), Bali/New Guinea (1 patient), Brazil (1 patient), and Haiti (1 patient).
[f] CI, confidence interval.
[g]*dhfr*: Wild-type: parasites with A16 / C50 / N51 / C59 / S108 / I164 (n = 26). Single mutants: isolates with the S108N alone (n=9). Double mutants: parasites with mutations at codons N51I and S108N (n=11), C59R and S108N (n=27), or A16V and S108T (n=1). Triple mutants: parasites with the genotypes of N51I / C59R / S108N (n=27) or C50R / N51I / S108N (n=1). Of note, the falciparum isolate with the A16V/S108T mutations was acquired in 1996 by a 12-year-old in Ghana. Those mutations in *dhfr* were not accompanied by the mutant codon I164L, previously associated with pyrimethamine and cycloguanil resistance (17).
[h]*dhps*: Wild-type parasites: parasites with S436 / A437 / K540 / A581 / A613 (n=20). Single mutants: isolates with the S436A (n=19) or A437G (n=32) mutation alone. Double mutants: parasites with mutations at codons S436A and A437G (n=18), A437G and K540E (n=6), or S436F and A613S (n=2). Triple mutants: parasites with S436A / A437G / A613S (n = 3), S436A / A437G / A581G (n=1), or A437G / K540E / A581G (n=1). Note: Some isolates could not be amplified at all loci and account for occasional missing values.

Table 2. Proportions of falciparum isolates with chloroquine- or sulfadoxine-pyrimethamine-associated resistance markers by year of acquisition

	Proportions of mutant isolates				Proportions of isolates with at least 2 mutant codons			
Year	*pfmdr1* (N86Y)[b]		*pfcrt* (K76T)		*dhfr*[c]		*dhps*[d]	
1994	42.9%	9/21	71.4%	15/21	45.5%	10/22	14.3%	3/21
1995	25.0%	2/8	55.6%	5/9	62.5%	5/8	33.3%	3/9
1996	31.6%	6/19	50.0%	9/18	52.4%	11/21	25.0%	5/20
1997	52.9%	9/17	66.7%	10/15	88.2 %	15/17	35.3%	6/17
1998	64.3%	9/14	50.0%	7/14	85.7%	12/14	21.4%	3/14
1999	50.0%	7/14	64.3%	9/14	78.6%	11/14	57.1%	8/14
2000[e]	71.4%	5/7	57.1%	4/7	50.0%	3/6	42.9%	3/7
Total	47.0%	47/100	60.2%	59/98	65.7%	67/102	30.4%	31/102
(95% CI)	(36.9 to 57.2)		(49.8 to 70.0)		(54.6 to 73.9)		(21.7 to 40.3)	

[a]OR, odds ratio; CI, confidence interval.
[b]OR for a 1-unit increase in year = 1.21 (95% CI 0.99 to 1.49); p=0.07, chi-square test for trend.
[c]OR for a 1-unit increase in year = 1.28 (95% CI 1.0 to 1.59); p=0.02, chi-square test for trend.
[d]OR for a 1-unit increase in year = 1.29 (95% CI 1.03 to 1.61); p=0.03, chi-square test for trend.
[e]Data for year 2000 are from January 1 to June 30.

areas. However, to date there has been little information on the rates of drug resistance in cases of imported malaria. Using travelers as a sentinel system provides a mechanism to study large numbers of persons returning from diverse malaria-endemic areas. In contrast, traditional studies have often been based on relatively small numbers of persons residing in geographically restricted areas. Travelers are generally nonimmune, facilitating the interpretation of treatment and prophylaxis studies since outcome measures are not confounded by reinfections and by the varying degrees of immunity present in residents of malaria-endemic areas. Similarly, correlating the molecular mechanisms of drug resistance to treatment outcome in travelers may be more straightforward since these confounding variables can largely be excluded. Knowledge of the resistance genotypes of malaria parasites obtained from returning travelers can provide credible and complementary data for evidence-based recommendations for both chemoprophylaxis and therapy of malaria in travelers.

The high correlation between mutations in DHFR and DHPS and in vitro resistance to pyrimethamine and sulfadoxine, further supported by site-directed mutagenesis and transfection experiments, suggests that the epidemiology of antifolate resistance in *P. falciparum* can be monitored by molecular techniques (5–7, 14–17). Furthermore, evidence exists for an association between a stepwise increase in the number of mutations in DHFR and DHPS and a corresponding increase in the level of clinical resistance to SP. In recent in vivo studies in partially immune persons in Cameroon and Kenya, multiple mutations in DHFR (e.g., triple mutation at codons 108, 59, and 51) were associated with early treatment failure, suggesting that these could be useful markers for predicting the in vivo efficacy of SP (18–20).

Using molecular markers of antifolate resistance, our study provides important data on the appropriateness of drugs such as SP that are currently recommended in North America and Europe for treatment or self-treatment of malaria in travelers. We observed that 75%, 66%, and 28% of consecutive imported isolates had at least one, two, and three mutations in DHFR, respectively. In DHPS, corresponding figures were 80%, 30%, and 5%. Furthermore, we found a linear trend for increasing prevalence mutations in *dhfr* and *dhps* during this study. These results suggest that antifolate resistance in imported falciparum malaria is now common and escalating over time. These observations question the rationale of continued recommendation of SP as either standby therapy or combination therapy with quinine for the treatment of *P. falciparum* malaria in travelers. However, some caution is needed in extrapolating our data to predict the in vivo efficacy of SP. Additional prospective in vivo studies, especially in the nonimmune host, are required to definitively link antifolate molecular markers with in vivo resistance (18–20).

We have also collected data on the occurrence of mutations associated with chloroquine resistance in consecutive imported falciparum isolates. The overall prevalence of the N86Y mutation in *pfmdr1* and K76T mutation in *pfcrt* was 47.0% and 60.2%, respectively. Recent in vivo studies have assessed the association between *pfcrt* mutations and chloroquine response and determined that the K76 allele correctly predicted successful outcome (9–11). A rapid assay to detect *pfcrt* K76T in travelers' malaria may be useful, since the presence of the K76 allele would indicate the probable effectiveness of treatment with chloroquine alone. On the basis of these findings, we anticipate that at least 40% of our patients would have responded to chloroquine. However, in the absence of a rapid test, current recommendations from the World Health Organization and the Centers for Disease Control and Prevention must be applied, and *P. falciparum* infections acquired in areas of known chloroquine resistance should not be treated with chloroquine.

Chloroquine and SP resistance has been selected by drug pressure (5,6,10,21). In Mali, the K76T mutation in *pfcrt* was observed in persons with persistent or recurrent infection after

chloroquine therapy, indicating selection for this mutation. Our study extends these observations to travelers; those who had taken chloroquine for prophylaxis or treatment had a 4.5-fold higher risk of being infected with an isolate carrying the K76T mutation. Although the number of isolates studied was relatively small, our results also indicate that the prevalence of genotypes associated with chloroquine resistance was significantly higher in isolates acquired in East Africa than in those acquired in West Africa. This observation is consistent with currently reported epidemiologic patterns (22). The distribution of chloroquine- and SP-resistant parasites and their degree of resistance are far from uniform, and regular assessment of the therapeutic efficacy of chloroquine and SP, such as obtained with World Health Organization in vivo studies, is required. Studies such as ours, using travelers as sentinels, can contribute in a novel and complementary way to the continuous monitoring and tracking of geographic drug-resistance patterns. A network of digitally linked sites in the developed world that are performing these analyses in cases of imported malaria could provide global and timely monitoring.

In summary, our study demonstrates that a molecular surveillance strategy based on imported malaria in travelers can be used to detect and track patterns of drug-resistant malaria. Given the high prevalence of observed mutations in *dhfr* and *dhps*, our data provide evidence that raises questions about the rationale of continued use of SP to treat falciparum malaria in returned travelers. Our data also indicate that a considerable proportion of imported falciparum infections are still responsive to chloroquine.

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Cost Effectiveness of a Potential Vaccine for *Human papillomavirus*

Gillian D. Sanders* and Al V. Taira*

Human papillomavirus (HPV) infection, usually a sexually transmitted disease, is a risk factor for cervical cancer. Given the substantial disease and death associated with HPV and cervical cancer, development of a prophylactic HPV vaccine is a public health priority. We evaluated the cost-effectiveness of vaccinating adolescent girls for high-risk HPV infections relative to current practice. A vaccine with a 75% probability of immunity against high-risk HPV infection resulted in a life-expectancy gain of 2.8 days or 4.0 quality-adjusted life days at a cost of $246 relative to current practice (incremental cost effectiveness of $22,755/quality-adjusted life year [QALY]). If all 12-year-old girls currently living in the United States were vaccinated, >1,300 deaths from cervical cancer would be averted during their lifetimes. Vaccination of girls against high-risk HPV is relatively cost effective even when vaccine efficacy is low. If the vaccine efficacy rate is 35%, the cost effectiveness increases to $52,398/QALY. Although gains in life expectancy may be modest at the individual level, population benefits are substantial.

Cervical cancer is one of the most common malignancies in women: this year in the United States, approximately 13,000 new cases will be diagnosed, and >4,000 women will die of the disease. Fortunately, cervical cancer is highly preventable with regular Papanicolaou (Pap) testing. Between 1973 and 1995, the Surveillance, Epidemiology, and End Results (SEER) Program (sponsored by the National Cancer Institute) documented a 43% decrease in incidence and a 46% decrease in death from cervical cancer. Such reductions, however, have not been observed in locations or countries where cytologic testing is not widely available. Epidemiologic research strongly implicates *Human papillomavirus* (HPV) as the major risk factor for cervical cancer. Therefore, methods of prevention, diagnosis, and treatment of HPV infection have been pinpointed as a means of reducing the incidence of cervical cancer.

HPV comprises >100 different types of viruses; approximately 40 of these are transmitted sexually. Although most HPV infections proceed and resolve without symptoms, some types of HPV (such as 6 and 11) may cause genital warts, whereas other types (such as 16 and 18) are associated with certain types of cancer. HPV infections are recognized as the major cause of cervical cancer: >90% of women who have cervical cancer also have been infected with HPV (1–7). HPV types that are correlated with the development of cancer are referred to as high-risk. Although no medical means currently exist to eliminate HPV infection, precancerous lesions and warts caused by these viruses can be treated.

Given the substantial disease and death associated with HPV and cervical cancer, research to develop a prophylactic HPV vaccine is ongoing (8). Vaccines for HPV-16 and HPV-18 are currently being studied in clinical trials; the Phase I trial results are encouraging (9,10). The cost effectiveness of such vaccines, however, has not been studied sufficiently. Therefore, we evaluated the effectiveness and cost effectiveness of a prophylactic vaccine.

Data and Methods

We used a decision model to estimate the length of life and expenditures for vaccination of adolescent girls for high-risk HPV types (Figure 1). We adhered to the recommendations of the Panel on Cost Effectiveness in Health and Medicine (11)

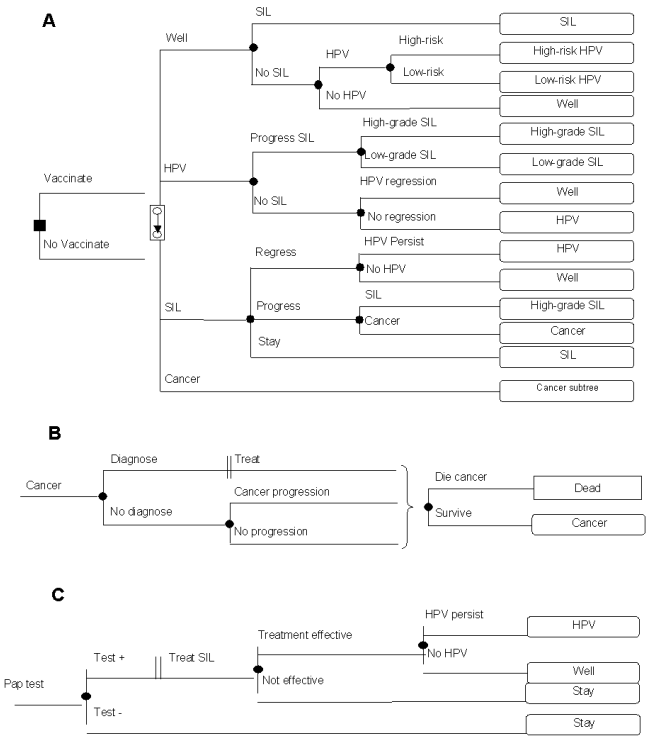

Figure 1. Schematic representation of the decision model. In panel A, the square node at the left represents the vaccination decision. The woman's health thereafter is simulated by a Markov model. Each month, women are at risk of developing *Human papillomavirus* (HPV) infection, SIL (squamous intraepithelial lesions), or cervical cancer. Women who contract HPV may be infected by a low- or high-risk type. Panel B demonstrates cervical-cancer diagnosis, treatment, and natural history. Throughout a woman's lifetime, her HPV, SIL, or cervical cancer status can be discovered either through development of symptoms or through routine Pap tests. Panel C shows that women who undergo a Pap test may test negative or positive for SIL.

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for conducting and reporting a reference-case analysis. We expressed our results in terms of costs, life-years, quality-adjusted life-years (QALYs), and incremental cost-effectiveness (ICE) ratios. We performed one-way sensitivity analyses on all model variables, as well as multi-way sensitivity analyses on selected variables.

Patient Population

The target population for this analysis was all adolescent girls in the United States. Our base-case analysis considered a hypothetical cohort of 12-year-old girls. A recent study by the Centers for Disease Control and Prevention (CDC), indicated that although 3% of girls have had sexual intercourse before reaching age 13, 18.6% are sexually active by age 15, and 59.2% by age 18 (12). We therefore believed that vaccinating 12-year-old girls would capture most girls before they are sexually active and are at risk for HPV infection. We examined the optimal vaccination age in sensitivity analyses.

Our analysis assumes a universal vaccination strategy for adolescent girls. Although risk factors for HPV infection are identifiable, we chose to evaluate a universal vaccination program for several reasons. Previous vaccination programs aimed to reduce incidence of *Hepatitis B virus* (HBV) infection have tried to target the risk groups that account for most cases (13). These high-risk groups, however, are difficult to vaccinate for a variety of reasons, including inaccessibility, noncompliance, and the inability to identify people at risk. Also, because >30% of HBV-infected persons show no identifiable risk factor for infection (13,14), they would not be included in such a targeted immunization strategy. Similarly for HPV infection, the broad range of risk factors and the difficulty identifying these behaviors inhibit targeting such risk groups. We evaluated the cost-effectiveness of targeting high-risk girls (assuming a reduced compliance) in sensitivity analyses.

Decision Model

We used Decision Maker software (Pratt Medical Group, Boston, MA, v2002.07.2) to develop a Markov model that followed the girls over their lifetimes. For each strategy, our model included probabilities of occurrence and progression of HPV, of squamous intraepithelial lesions (SIL), and of cervical cancer, as well as the probability of death, costs, and quality of life associated with the various health states. Whenever possible, we based our probability estimates (Appendix) on large, high-quality studies reported in the literature.

Our model (Figure 1A) tracks a cohort of girls who are either vaccinated against specific HPV types or who receive the current standard of care. Based on hepatitis B vaccination completion rates among U.S. adolescents, we assumed that 70% of the targeted girls would be vaccinated successfully (Appendix). We assumed that girls who were not vaccinated would receive the current standard of care.

Every month, each girl is at risk of developing high- or low-risk HPV, SIL, or cervical cancer. Over time, an infected woman's HPV infection can regress, persist, or progress to either low- or high-grade SIL. SIL can also exist independent of an HPV infection. High-grade SIL can progress to cervical cancer. The diagnosis, treatment, and natural history of cervical cancer are modeled in Figure 1B.

We assumed that the current standard of care included routine Pap tests for compliant patients every 2 years starting at age 16. Throughout a woman's lifetime, her HPV, SIL, or cervical cancer status can be discovered and treated either because symptoms have developed or through routine Pap tests (Figure 1C). We assumed that 10% of woman diagnosed with low-grade SIL would undergo cryotherapy and that all would receive a 6-week reexamination, and Pap tests at 3, 6, 12, and 18 months after cryotherapy. Treatment of high-grade SIL was assumed to include loop electrosurgical excision procedure (LEEP), and subsequent reexamination and Pap tests (15,16).

A woman may also choose to have a benign hysterectomy reducing her risk of cervical cancer. In addition to being at risk for death because of cervical cancer, all women are at risk for age-specific death unrelated to HPV or cervical cancer.

Data and Base-Case Assumptions

HPV Infection

Incidence of HPV infection was based on Myers' mathematical model of HPV infection (Appendix) (17). In our base-case analysis, annual incidence began at age 15 (10%), peaked at age 19 (18%), and dropped off quickly after age 29 (1%). We assumed that no prevalent HPV infections existed in the initial cohort of 12-year-old girls but varied this assumption in sensitivity analyses. Given HPV infection, regression rates were highest for women <25 years (46%/yr) and lowest for women >30 years (7%/yr), reflecting a preponderance of more persistent infections in the older age group (Appendix).

Low- Versus High-Risk HPV

Because of a lack of significant HPV genotype cross-immunity, any vaccine developed probably will be effective against a limited number of HPV types (18,19). HPV types 16, 18, 45, and 31 together are the most commonly associated with cervical cancer, with evidence of these four types apparent in >75% of women who have cervical cancer (20). In our model, HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 were considered high risk; all other HPV types were categorized as low risk (20–22). Based upon this classification in the general population, 59% of HPV infections are caused by high-risk types (Appendix).

Low- and High-Risk Rates of HPV Progression

To evaluate potential vaccination strategies, we modeled different disease-progression rates in women infected with low- and high-risk HPV. By combining data on overall progression rates of HPV infection to cancer, with prevalence data on women infected with low- and high-risk HPV who had low- or high-grade SIL or cervical cancer, we estimated sepa-

rate progression rates for low- and high-risk HPV infections. Data from seven articles were considered of high enough quality to be included in our analysis (1–7).

High-risk HPV infections were significantly more common in women who had cervical cancer than in women who had precursor lesions. Based on the results of seven studies (N=1609), high-risk HPV infection was detected in 56% of women who had low-grade SIL, in 83% of women who had high-grade SIL, and in 90% of women who had cervical cancer. Low-risk HPV infection was detected in 22%, 8%, and 3% of these women, respectively. No evidence of HPV infection was found in 22%, 9%, and 7% of these women, respectively (1–7). We then calculated relative progression rates for transition from high-risk, low-risk, or no HPV infection to low-grade SIL; from low-grade to high-grade SIL; and from high-grade SIL to cervical cancer (Appendix).

Cancer Surveillance, Treatment, and Progression

We estimated that 71% of the adult female population received biennial Pap testing. Pap test sensitivity and specificity results were based on a meta-analysis conducted by the Duke Evidence-Based Practice Center (17,23). Diagnosis of asymptomatic cervical lesions depended on a woman's likelihood of having a Pap test and on the sensitivity and specificity of this test.

Assessment of treatment effectiveness for cervical lesions was based on a review of 13 studies that detailed treatment effectiveness by lesion stage (Appendix). Initial treatment effectiveness was estimated at 97% and 94% for low-grade and high-grade SIL, respectively. Unsuccessful initial treatment of cervical lesions was followed with a repeat treatment (cryotherapy or LEEP) (77%), cone biopsy (18%), or hysterectomy (5%) (24), increasing treatment success. We based cancer progression rates, annual patient survival rates, and probability of symptoms by cancer stage on an analysis by Myers et al. (Appendix) (17). Myers et al. validated their data by comparing predicted distribution of cancer by stage for an unscreened population with data from studies of women who had had no prior screening.

Benign Hysterectomy

We considered women who did not have cervical cancer but who had hysterectomies to be fully protected from cervical cancer. We tested this assumption in sensitivity analyses. Age-specific hysterectomy rates were based on data from the Hospital Discharge Survey of the National Center for Health Statistics (Appendix).

HPV Vaccine Characteristics

In our model the HPV vaccine was administered by using a series of three injections in a school-based immunization program. Because vaccine longevity is uncertain, we assumed that successful vaccination conferred immunity for 10 years but that repeated booster shots every 10 years were required to maintain the vaccine's efficacy. We evaluated the need for more frequent booster shots or a vaccine that conferred lifetime immunity in sensitivity analysis. For our base-case analysis, vaccine efficacy against high-risk HPV types was estimated at 75%. We tested the complete range of vaccine effectiveness (from 0% to 100%) because of the absence of efficacy data from Phase III clinical trials and because future marketed vaccines may target only a subset of the high-risk HPV types.

Quality of Life

HPV infection and cervical cancer can markedly affect quality of life and therefore can affect a woman's quality-adjusted life expectancy. Accordingly, we incorporated adjustments for quality of life associated with current health, HPV, SIL, and with cervical cancer and its treatment.

Utilities for health states were based on a report by the Institute of Medicine on Vaccines for the 21st Century, which used committee-consensus Health Utility Indices levels for relevant health states (Appendix). Undiagnosed HPV and cervical lesions were considered to be asymptomatic and to have no utility decrement. Diagnosed and treated low- and high-grade SIL were assigned lower utilities (0.97) for a 1-year duration. Treatment for locally invasive cancer was assigned a low utility (0.79–0.80) during 4 months of initial treatment, with a moderate utility (0.90–0.97) during a 2- or 3-year follow-up. For more advanced cancer, a woman's utility was decreased to 0.62 during both treatment and follow-up to reflect the severity of her disease and its effects on quality of life. We based current health utilities on the gender- and age-specific data from the Beaver Dam study (25).

Costs

We converted all costs to 2001 U.S. dollars by using the gross-domestic-product deflator. Pap-testing costs were $81 per test, including a 10% rescreen rate. We estimated the cost of the vaccine materials, personnel, and administration at $300, based on school-based HBV vaccination programs (Appendix). We assumed a three-injection protocol with a booster shot ($100) required every 10 years.

Treatment costs of low- and high-grade SIL were based on Medicare average reimbursements and resource-based cost estimates. We estimated the cost of treatment of low-grade SIL from the cost of an initial colposcopy and biopsy, cryotherapy (in 10% of patients), a 6-week reexamination, and Pap tests at 3, 6, 12, and 18 months after treatment. The cost of treatment of high-grade SIL was based on cost of initial colposcopy and biopsy, LEEP, and subsequent reexamination and Pap tests. Cost of cancer treatment varied, depending on the stage at which cancer was diagnosed. Costs were based on Medicare average reimbursement rates (26) and cross-checked with a 1999 HMO case-control full-cost analysis (27) (Appendix).

Sensitivity Analysis

We performed one-way and multi-way sensitivity analyses to account for important model uncertainties. For clinical vari-

ables, our ranges for sensitivity analyses represent our judgment of the variation likely to be encountered in clinical practice, based on the literature and on discussion with experts. The ranges for costs represent variation by 25% above and below the base-case estimate. To determine ranges for utilities, we used clinical judgment.

Results

Model Validation

We evaluated outcomes in the current practice arm of the model to ensure that they reflected the frequency of events from the Surveillance, Epidemiology and End Results (SEER) registry. Our model's annual rates of cervical-cancer cases and cervical-cancer-related deaths match 2001 SEER estimates, as well as those calculated by the Myers model (17) (data available from the authors).

Base-Case Analysis

A prophylactic vaccine against high-risk HPV types is more expensive than current practice but results in greater quality-adjusted life expectancy (Table 1). HPV vaccination of 12-year-old girls improves their life expectancy by 2.8 days or 4.0 quality-adjusted life days at a cost of $246 relative to current practice (ICE of $22,755/QALY).

Vaccinating the present U.S. cohort of 12-year-old girls (population approximately 1,988,600) averts >224,255 cases of HPV, 112,710 cases of SIL, 3,317 cases of cervical cancer, and 1,340 cervical-cancer deaths over the cohort's lifetime. Prevention of one case of cervical cancer would require vaccination of 600 girls (Table 2).

Table 1. Health and economic outcomes of HPV vaccination[a]

Outcome	No vaccination	HPV vaccination
Cost, $	39,682	39,928
Incremental cost, $		246
Life expectancy, yrs	28.785	28.793
Incremental life expectancy, days		2.8
Quality-adjusted life expectancy, yrs	27.720	27.731
Incremental quality-adjusted life expectancy, days		4.0
Incremental cost effectiveness		
$/life year		32,066
$/quality-adjusted life year		22,755

[a]HPV, *Human papillomavirus*.

Sensitivity Analyses

Figure 2 shows the ICE ratios of one-way sensitivity analyses of the vaccination strategy compared to current practice. We explored those variables with the greatest effect on the ICE ratio by running more extensive sensitivity analyses. Given the uncertainty surrounding the vaccine efficacy, pricing, and mechanism, we performed extensive sensitivity analyses using vaccine-related variables.

In our base-case analysis, we estimated that an HPV vaccine would provide immunity against high-risk HPV types in 75% of the girls vaccinated. At early stages of vaccine development or given a vaccine that targets only selected high-risk HPV types, the efficacy may prove to be lower. Sensitivity analyses on the vaccine efficacy and cost showed that even if the efficacy was reduced to 40% or the vaccine cost was increased to $600, vaccination costs <$50,000/QALY, relative to current practice (Figures 3 and 4).

We assumed that vaccination required a one-shot booster every 10 years. We also considered that vaccination could provide lifetime immunity, in which case the ICE improved to $12,682/QALY. Vaccinating the present U.S. cohort of 12-year-old girls with such a lifetime vaccine would avert >272,740 cases of HPV, 174,208 cases of SIL, 7,992 cases of cervical cancer, and 3,093 cervical-cancer deaths over the cohort's lifetime. Prevention of one case of cervical cancer would require vaccination of 250 girls. Even if a booster shot is required every 3 years, the vaccine compared to current practice remained fairly cost effective ($45,599/QALY) (Figures 3 and 4). Our model assumes that a vaccination program would target 12-year-old girls for vaccination. Waiting until girls are 15 years old to provide vaccination results in a slightly lower life expectancy (reducing quality-adjusted life expectancy by 0.2 days) though at a reduced cost ($20). Vaccination of 12-year-old girls as compared to 15-year-old girls costs $40,440 per additional quality-adjusted life year gained.

Although the estimates used in our analysis reflect current Pap-test characteristics and compliance, if every woman obtained a Pap test every 2 years (base-case estimate is 71% compliance every 2 years), the ICE of vaccination increases to $33,218/QALY. Our base-case analysis assumes that vaccinated women would continue to receive Pap tests at the same

Table 2. Intermediate health outcomes of HPV vaccination[a,b]

Outcome	HPV vaccination	No vaccination	Lifetime cases averted	No. needed to vaccinate to prevent one case
HPV	1,460,699	1,684,954	224,255	9
SIL	417,549	530,259	112,710	18
Cervical cancer	13,374	16,690	3,316	600
Cervical-cancer deaths	5,121	6,461	1,340	1,484

[a]Assumes program that successfully administers a vaccine against high-risk HPV to the current U.S. cohort of 12-year-old girls.
[b]HPV, *Human papillomavirus*; SIL, squamous intraepithelial lesions.

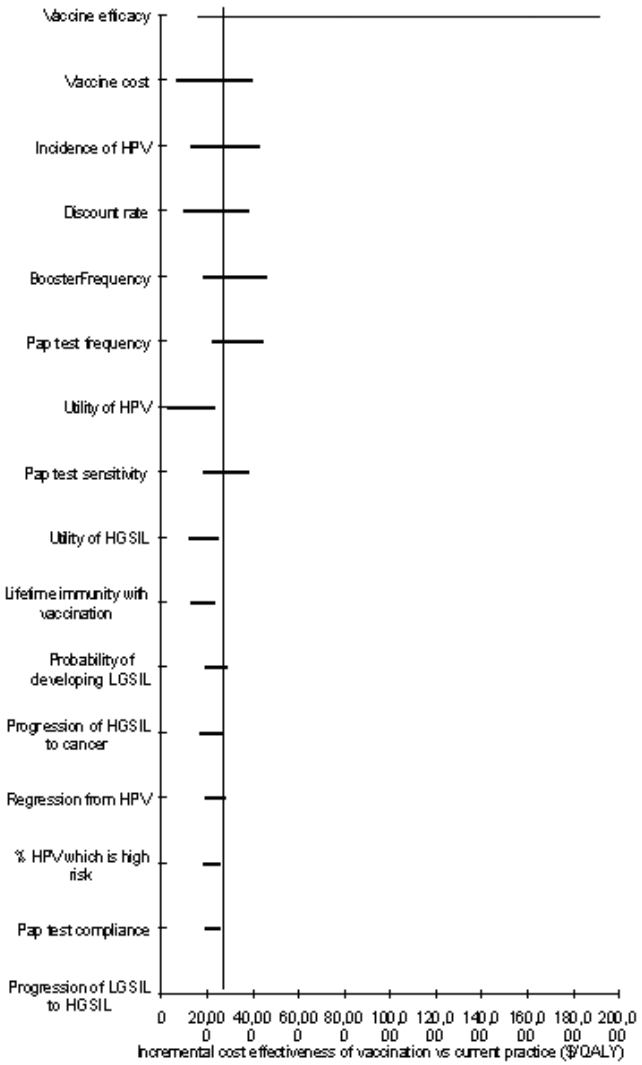

Figure 2. Sensitivity analysis. Tornado diagram representing the incremental cost-effectiveness ratios of one-way sensitivity analysis on the vaccination strategy compared to current practice. The vertical line represents the incremental cost-effectiveness ratio under base-case conditions.

frequency as unvaccinated women. HPV vaccination and the resulting reduction in cervical-cancer risk, however, might decrease frequency of Pap testing. Figure 5 shows how the costs and quality-adjusted life expectancy are influenced by the frequency of Pap tests in the vaccinated cohort. A strategy in which vaccinated women have Pap testing every 4 years increases life expectancy while reducing costs compared to current practice. While providing more frequent Pap tests to vaccinated women does increase a woman's quality-adjusted life expectancy, it also increases costs. The cost-effectiveness ratios of more frequent testing are shown in Figure 5.

Our results were sensitive to several of our base-case assumptions (Figure 2). Vaccination saved 11.4 quality-adjusted life days and cost $290 over current practice when costs and benefits were not discounted (ICE of $9,286/QALY). At a discount rate of 5%, vaccination cost $37,752/QALY. Some women may be quite alarmed by being diagnosed with high-grade SIL; decreasing the utility of high-grade SIL to 0.8 lowers the cost-effectiveness ratio to $16,927/QALY. Varying the underlying incidence of HPV from 0.5 to 2 times our base-case values resulted in cost-effectiveness ratios ranging from $43,088 to $12,664 per QALY, respectively. Sensitivity analyses with other variables did not change our results substantially (Figure 2).

Discussion

We evaluated the usefulness of a potential vaccine against high-risk HPV types administered to adolescent girls and found it to be cost effective as compared to current practice ($22,755/QALY). Although the increase in quality-adjusted life expectancy from a vaccination program is modest for the individual, the increase aggregates to substantial numbers of HPV infections, cases of cervical cancer, and prevented cancer-related deaths (Table 2). Furthermore, the life-expectancy gains are similar to those realized by current vaccination programs. Vaccination against high-risk HPV saved 2.8 life days and 4.0 quality-adjusted life days per person. In comparison, vaccinations against measles, mumps, rubella, and pertussis each save 2.7, 3.0, 0.3, and 3.3 life days, respectively (28,29). Sensitivity analyses found that the HPV vaccine would be cost effective, even assuming vaccine efficacy as low as 40% or that booster shots would be required every 3 years.

The only previous analysis of the cost effectiveness of a vaccine against HPV was published by the Institute of Medicine (IOM) (30). That analysis also showed an HPV vaccine to be cost effective. Our analysis differs from the IOM's, however, in that we modeled a vaccine specific to high-risk types of HPV because such vaccines are under development and in clinical trials. In addition, our progression and recurrence rates are HPV-type specific.

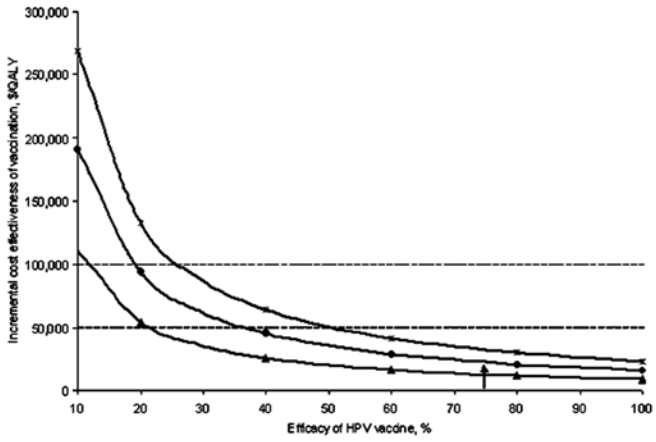

Figure 3. Sensitivity analysis: Vaccine efficacy. Effect of a change in *Human papillomavirus* (HPV) vaccine efficacy on the cost effectiveness of vaccination compared with current practice under varying assumptions of vaccine immunity. The triangles represent a vaccine which provides lifetime immunity to high-risk types of HPV. The circles represent a vaccine which requires booster shots every 10 years to remain effective (base-case assumption). The hatches represent a vaccine that requires booster shots every 5 years to remain effective. The dashed lines indicate the $50,000and $100,000 per quality-adjusted life year cost-effectiveness thresholds. The base-case value of 75% efficacy is indicated by the arrow.

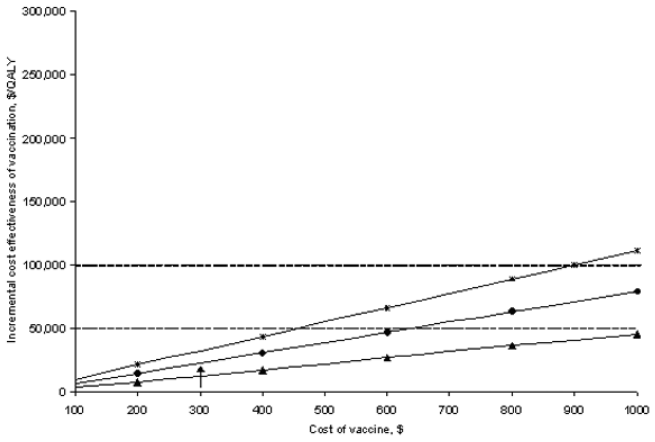

Figure 4. Sensitivity analysis: Vaccine cost. Effect of a change in *Human papillomavirus* (HPV) vaccine cost on the cost effectiveness of vaccination compared with current practice under varying assumptions of vaccine immunity. The triangles represent a vaccine that provides lifetime immunity to high-risk types of HPV. The circles represent a vaccine that requires booster shots every 10 years to remain effective (base-case assumption). The hatches represent a vaccine that requires booster shots every 5 years to remain effective. The dashed lines indicate the $50,000 and $100,000 per quality-adjusted life year cost-effectiveness thresholds. The base-case value of $300 is indicated by the arrow.

Our analysis does have limitations. We analyzed the benefits and costs of vaccinating only adolescent girls against HPV. Because HPV is sexually transmitted, reducing the prevalence of HPV in the population will also affect the prevalence of HPV in women's sexual partners. Although HPV is most commonly associated with cervical cancer, it may also play a role in cancers of the anus, vulva, vagina, and penis. The benefits of HPV vaccination associated with reductions in these types of cancers are not included in our analysis. Including them should make HPV vaccination even more favorable. The decision whether to vaccinate adolescent boys as well is more complex; therefore, in future work we plan to extend our analysis to incorporate such costs and benefits. In addition, the costs and benefits used in this analysis are tailored to the population and health-care environment of the United States. As Figure 5 demonstrates, the availability of HPV vaccines may justify less frequent Pap tests. This effect may be particularly relevant in developing countries that must decide how best to allocate their limited health-care resources.

We make several assumptions about the target vaccination population and program implementation that need discussion in terms of their political and social feasibility. First, we propose a school-based vaccination program rather than a clinic-based one. School-based immunization programs address several challenges encountered when vaccinating adolescents. First, school-based programs provide an infrastructure in which to vaccinate adolescents. Adolescent health-care visits are often not routine, and given scheduled visits, adolescents are often noncompliant with appointments. In addition, we believe that fitting the three-dose HPV vaccination regimen into the academic year will increase compliance while containing costs. Several school-based programs have documented completion rates of >90%. In contrast, lower rates of completion (11% to 87%) have been found in more traditional health-care settings (31–34). Second, we propose providing universal vaccination rather than targeting specific high-risk groups. Certain groups of women are at higher risk for HPV infection, and the cost effectiveness of vaccinating such target groups may be more favorable than a universal vaccination program. Experience with *Hepatitis B* vaccination in adolescents, however, has demonstrated how such groups may be those that are hardest to reach (13,14,35), and that many risk factors for infection (such as number of partners) may not be readily identifiable (13,14). Finally, we propose vaccinating girls at an early adolescent age (12 years). Although the lifetime cost of vaccinating 12-year-old girls is slightly greater than that of vaccinating 15-year-old girls, earlier vaccination costs <$50,000 per QALY when compared to costs of vaccinating older adolescents. A significant proportion of adolescents are sexually active by 15 years of age; therefore, vaccination at 12 years of age aims to include as many girls as possible before sexual activity begins and HPV infection risk increases. In addition, studies using *Hepatitis B* vaccines as a proxy have found better immune responses in younger persons and have shown that younger children require lower doses (36,37). Finally, we believe a 3-dose school-based vaccination program aimed at 12-year olds will result in greater compliance because adolescents of this age have more consistent school attendance (13,38–40). Before a HPV vaccination program is successfully implemented, social and political issues will need to be addressed and agreed upon by stakeholder groups, including pediatricians, public health officers, parents, adolescents, school administrators, and community leaders.

Several institutions, including Merck Research Laboratories, MedImmune Inc., GlaxoSmithKline, and the National Cancer Institute (NCI), are developing and testing prophylactic HPV vaccines. Researchers at NCI and Johns Hopkins have developed a virus-like particle vaccine with promising initial results (9,10). If the results of the recently completed Phase II

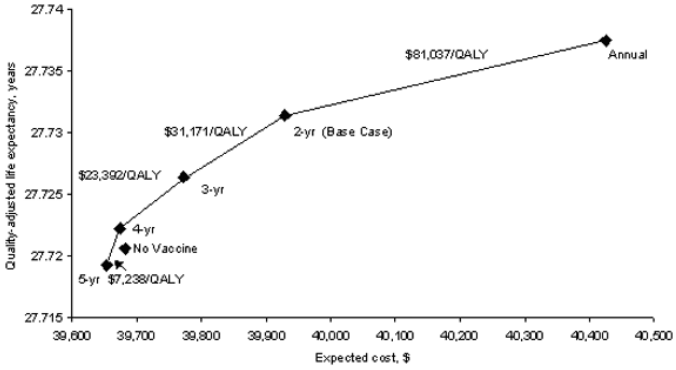

Figure 5. Sensitivity analysis: Frequency of Pap tests in vaccinated women. Effect of changing the frequency with which vaccinated women receive a Pap test. The diamonds represent Pap testing vaccinated women annually, every 2 years (base case), every 3 years, every 4 years, and every 5 years. The x-axis represents the lifetime expected cost of the vaccination strategy; the y-axis is the quality-adjusted life expectancy in years. The incremental cost effectiveness of increasing the frequency of Pap testing for vaccinated women is indicated numerically above the cost-effectiveness frontier.

study in the United States and Costa Rica confirm the Phase I results, a Phase III trial in Costa Rica involving 10,000 women will begin. Nonetheless, a vaccine probably will not be approved for widespread use for 3–5 years. Meanwhile, the need for continued cervical-cancer screening and treatment programs remains high.

Our study suggests that vaccination of girls with a HPV vaccine is cost effective when compared to many other generally acceptable health interventions. Although HPV vaccines are still under development, our assessment of the cost effectiveness, however, is robust across a wide range of vaccine mechanisms and efficacies. Although several hurdles to an HPV vaccine must be overcome before it is widely disseminated, our analysis suggests that a vaccine against high-risk HPV would have substantial public health benefit and emphasizes the importance of ongoing vaccine research and development.

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Appendix

Appendix Table. Input variables and sources[a]

Input variable	Base-case estimate	Range	Source
Demographic variables			
Population starting age, y	12	0–25	Assumed
Vaccine variables			
Vaccine effectiveness, %	75	0–100	Estimate
Vaccine compliance, %	70	30–100	(1,2)
Booster shot frequency, y	10	3–lifetime	Assumed
Treatment variables			
Initial treatment efficacy, given high-grade SIL, %	95	88–97	(3–6)
Treatment efficacy (including retreat), given high-grade SIL, %	99.5	99–100	(3–7)
Probability HPV infection persists, given effective treatment of high-grade SIL	10	0–25	Assumed
Initial treatment efficacy, given low-grade SIL, %	98	93–100	(8–10)
Treatment efficacy (including retreatment), given low-grade SIL, %	99.5	99–100	(7–10)
Probability HPV infection persists, given effective treatment of low-grade SIL	10	0–25	Assumed
Surveillance variables			
Pap test sensitivity for SIL (both low- and high-grade)	51	40–80	(11,12)
Pap test specificity for SIL (both low- and high-grade)	97	95–98	(11,12)
Compliance with Pap testing, %	71	60–80	(13,14)
Pap testing frequency in unvaccinated population, months	24	12–60	(13,14)
Pap testing frequency in vaccinated population, months	24	12–60	(13,14)
HPV variables			
Prevalence of HPV in initial cohort population, %	0	0–25	(12,15)
Annual incidence of HPV infection, given woman aged (yrs):		0.5–2x	(12)
0–15	0		
15–16	0.1		
17	0.12		
18	0.15		
19	0.17		
20	0.15		
21	0.12		
22–23	0.10		
24–29	0.05		
30–49	0.01		

Appendix Table continued. Input variables and sources[a]

Input variable	Base-case estimate	Range	Source
50+	0.005		
Proportion of high-risk HPV infections, %[a]	59	52–72	(16–19)
Annual probability (%) of HPV infection resolving, woman aged (yrs):			(12,18,20,21)
0–24	45.7	40–55	
25–29	32.9	30–37	
30+	6.8	4–10	
SIL variables			
Annual probability of SIL, given no HPV infection, %	0.025	0.02–0.03	(22–26)
Annual probability of SIL, given low-risk HPV infection, %	3.6	3–5	(12,14,22–27)
Annual probability of SIL, given high-risk HPV infections, %	6.5	5–8	(12,14,22–27)
Low-grade SIL, given no HPV infection, %	100	90–100	Assumed
Low-grade SIL, given HPV infection, %	90	80–100	(12)
Annual probability (%) of low-grade SIL regressing, given woman aged (yrs):			(27–36)
0–34	14.2	12–16	
35–44	5.8	4–8	
45+	2.7	2–8	
Probability of low-grade SIL regressing to previous state of HPV infection, given regression occurs, %	10	0–20	(12)
Annual probability (%) of high-grade SIL regressing, given woman aged (yrs):			(27,28,30,31)
0–44	5.8	3–7	
45+	3.7	3–7	
Probability of high-grade SIL regressing to well state, given regression, %	45	40–50	(12)
Probability of high-grade SIL regressing to previous state of HPV infection, given regression, %	5	0–10	Assumed
Probability of high-grade SIL regressing to low-grade SIL given regression, %	50	40–60	(12)
Annual probability (%) of developing high-grade SIL from low-grade SIL with no HPV infection, women aged (yrs):			(12,14,22–27)
0–34	0.5	0.3–0.7	
35–44	3.1	2–5	
45+	4.5	3–6	
Annual probability (%) of developing high-grade SIL from low-grade SIL when low-risk HPV infection is present, women aged (yrs):			(12,14,22–27)
0–34	0.4	0.2–0.6	
35–44	2.7	2–4	
45+	3.8	3–5	
Annual probability (%) of developing high-grade SIL from low-grade SIL when high-risk HPV infection is present, women aged (yrs):			(12,14,22–27)
0–34	2.0	1–3	
35–44	15.6	7–20	
45+	31.3	15–35	
Annual probability of developing cervical cancer, given high-grade SIL and no HPV infection, %	2.6	2–4	(12,14,22–27)
Annual probability of developing cervical cancer, given high-grade SIL developed through low-risk HPV infection, %	1.0	0.7–1.5	(12,14,22–27)
Annual probability of developing cervical cancer, given high-grade SIL developed through high-risk HPV infection, %	3.8	3–6	(12,14,22–27)
Cervical cancer variables			
Annual probability of progressing from undiagnosed Stage I cervical cancer to Stage II cervical cancer, %	43.7	40–45	(12)
Annual probability of progressing from undiagnosed Stage II cervical cancer to Stage III cervical cancer, %	53.5	50–55	
Annual probability of progressing from undiagnosed Stage III cervical cancer to Stage IV cervical cancer, %	68.3	65–70	
Annual probability of symptoms with undiagnosed Stage I cervical cancer, %	15	12–18	
Annual probability of symptoms with undiagnosed Stage II cervical cancer, %	22.5	20–25	

Appendix Table continued. Input variables and sources[a]

Input variable	Base-case estimate	Range	Source
Annual probability of symptoms with undiagnosed Stage III cervical cancer, %	60	67–73	
Annual probability of symptoms with undiagnosed Stage IV cervical cancer, %	90	87–93	
Annual probability of survival after diagnosis, by stage:			(12)
Stage I			
Year 1	0.9688	0.95–0.99	
Year 2	0.9525	0.93–0.97	
Year 3	0.9544	0.93–0.97	
Year 4	0.9760	0.95–0.99	
Year 5	0.9761	0.95–0.99	
Stage II			
Year 1	0.9066	0.88–0.92	
Year 2	0.8760	0.85–0.89	
Year 3	0.9225	0.90–0.94	
Year 4	0.9332	0.91–0.95	
Year 5	0.9604	0.94–0.98	
Stage III			
Year 1	0.7064	0.68–0.72	
Year 2	0.7378	0.71–0.75	
Year 3	0.8610	0.84–0.88	
Year 4	0.9231	0.90–0.94	
Year 5	0.9142	0.89–0.93	
Stage IV			
Year 1	0.3986	0.37–0.41	
Year 2	0.4982	0.47–0.51	
Year 3	0.7638	0.74–0.78	
Year 4	0.8652	0.84–0.88	
Year 5	0.8592	0.83–0.87	
Time to remission, yrs	5		
Five-year survival after diagnosis, by stage, %			(12)
Stage I	83.9		
Stage II	65.66		
Stage III	37.87		
Stage IV	11.27		
Costs, $			
Vaccine	300	100–500	(37–40)
Booster shot	100	30–130	Assumed
Cost of treatment for cervical cancer, Stage I	14,979	11,234–18,724	(41,42)
Cost of treatment for cervical cancer, Stage II	21,811	16,358–27,264	(41,42)
Cost of treatment for cervical cancer, Stage III	21,811	16,358–27,264	(41,42)
Cost of treatment for cervical cancer, Stage IV	24,004	18,003–30,005	(41,42)
Cost of Pap test (w/10% retest)	81	61–101	(42)
Cost of treatment for high-grade SIL	1,218	914–1523	(42–45)
Cost of treatment for low-grade SIL	630	473–788	(42–45)
Cost of treatment for a false-positive SIL	230	172–288	(43,44,46)
Cost of hysterectomy	7,883	5912–9854	(11,45)

Appendix Table continued. Input variables and sources[a]

Input variable	Base-case estimate	Range	Source
Annual probability of hysterectomy by age (yrs), %		0.25–2x	(47)
15–24	0.04		
25–29	0.35		
30–34	0.60		
35–39	0.99		
40–44	1.29		
45–54	0.99		
≥55	0.33		
Utilities			(48)
Low-grade SIL	0.97	0.8–1	
High-grade SIL	0.97	0.5–1	
Low-risk HPV infection	1.00	0.9–1	
High-risk HPV infection	1.00	0.8–1	
Cervical cancer, treatment phase			
Stage I	0.79	0.25–1	
Stages II–IV	0.62	0.25–1	
Cervical cancer, follow-up			
Stage I	0.90	0.25–1	
Stages II–IV	0.62	0.25–1	
Well	1.00		Age-specific utilities based on (49)
Other Variables			
Markov model cycle length, months	1		Assumed
Discount rate, %	3	0–5	(50)

[a]HPV, *Human papillomavirus*; SIL, squamous intraepithelial lesion. High risk HPV is defined as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68; low-risk HPV is defined as all other types. All probabilities are annual unless otherwise noted. All costs are in 2001 U.S. dollars.

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Natural Enzootic Vectors of *Venezuelan equine encephalitis virus*, Magdalena Valley, Colombia

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To characterize the transmission cycle of enzootic *Venezuelan equine encephalitis virus* (VEEV) strains believed to represent an epizootic progenitor, we identified natural vectors in a sylvatic focus in the middle Magdalena Valley of Colombia. Hamster-baited traps were placed into an active forest focus, and mosquitoes collected from each trap in which a hamster became infected were sorted by species and assayed for virus. In 18 cases, a single, initial, high-titered mosquito pool representing the vector species was identified. These vectors included *Culex* (*Melanoconion*) *vomerifer* (11 transmission events), *Cx*. (*Mel*.) *pedroi* (5 transmissions) and *Cx*. (*Mel*.) *adamesi* (2 transmissions). These results extend the number of proven enzootic VEEV vectors to 7, all of which are members of the Spissipes section of the subgenus *Melanoconion*. Our findings contrast with previous studies, which have indicated that a single species usually serves as the principal enzootic VEEV vector at a given location.

Venezuelan equine encephalitis (VEE) is an emerging zoonotic arboviral disease that affects equines and humans in the Americas (1). *Venezuelan equine encephalitis virus* (VEEV) has caused sporadic outbreaks since the early part of the 20th century, with some epidemics affecting ≥100,000 persons. For many years, the source of the epizootic/epidemic VEEV strains belonging to subtypes IAB and IC viruses remained unknown. After antigenically related but distinct, equine-avirulent, enzootic strains of VEEV were isolated in the 1960s, researchers hypothesized that epizootic/epidemic strains evolve from enzootic VEEV progenitors (2). The first genetic evidence supporting this hypothesis came from RNA fingerprinting studies that indicated a close relationship between subtype ID–enzootic VEEV strains from Colombia and epizootic/epidemic isolates belonging to subtype IC (3). Later, sequencing (4) and phylogenetic (5,6) studies also supported the evolution of the epizootic/epidemic serotype IAB and IC strains from enzootic ID VEEV progenitors. Recently, comprehensive phylogenetic analyses have indicated that the epizootic/epidemic strains evolved independently on at least three occasions from a single lineage of ID VEEV that circulates in eastern and central Colombia, western Venezuela, and northern Peru (7–10). Other ID-like VEEV lineages that occur in Panama, Amazonian Peru, southwestern Colombia, coastal Ecuador, north-central Venezuela, and Florida have not generated any of the epizootic/epidemic strains sequenced (10–12).

Enzootic VEEV (subtypes ID–IF, II–VI) circulate nearly continuously in sylvatic or swamp habitats in various tropical and subtropical locations in the New World (1,13). These viruses generally use small mammals as their reservoir hosts and are transmitted by mosquitoes. Enzootic mosquito vectors have been identified for four VEEV variants: 1) *Culex* (*Melanoconion*) *portesi* transmits *Mucambo virus* (VEE complex subtype IIIA) in Trinidad (14), 2) *Cx.* (*Mel.*) *cedecei* transmits *Everglades virus* (VEE complex subtype II) in southern Florida (15), 3) *Cx.* (*Mel.*) *aikenii sensu lato* (*ocossa* and *panocossa*) transmits subtype ID VEEV in Panama (16,17), and 4) *Cx.* (*Mel.*) *taeniopus* (formerly *opisthopus*) is the primary enzootic vector of subtype IE VEEV in Guatemala (18). More than 70% of enzootic field isolations have come from the subgenus *Melanoconion*, suggesting that these mosquitoes are the principal vectors of most or all enzootic VEE complex strains (17).

The infrequency of VEE emergence is probably determined by the infrequent, simultaneous occurrence in time and space of viral mutations that mediate host range changes, combined with ecologic and epidemiologic conditions that permit efficient amplification (1). To understand the mechanisms of VEE emergence from enzootic progenitors in Colombia and Venezuela, we are studying the hosts in which epizootic mutations may occur and in which the selection of epizootic strains may follow. However, the vector and reservoir hosts of the particular subtype ID VEEV lineage implicated in epizootic emergence have not been identified. Using an efficient system of vector identification employing hamster baited traps, we identified *Cx*. (*Mel.*) *vomerifer*, *Cx*. (*Mel.*) *pedroi*, and *Cx*. (*Mel.*) *adamesi* as natural enzootic vectors in an active focus of subtype ID VEEV in the middle Magdalena Valley of Colombia.

Methods

Study Area

The study was carried out from 1999 to 2000 in the Monte San Miguel Forest in the middle Magdalena Valley of Colombia (6° 23′ 30″N; 74° 21′ 41″ W; 50 m elevation). This is a lowland tropical rainforest surrounded by cattle ranches cre-

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ated by deforestation. Mean minimum and maximum daily temperatures are 23°C and 33°C (overall mean of 29°C), respectively, and annual rainfall averages 2,700 mm. Mean relative humidity is 80%. Generally, the peaks of the rainy seasons occur in April–May and October–November. Numerous previous isolations of subtype ID VEEV from sentinel hamsters (9) indicate that this forest site is a stable enzootic focus.

Mosquito Traps

Hamster-baited traps were used for detection of natural VEEV vectors. These traps were a version of the Trinidad No. 10 trap (19) with the following modifications: 1) the metal can comprising the trap opening was replaced by a polyvinyl chloride pipe, 10 cm in diameter; 2) the cylindrical animal cage was enlarged to 11 cm in diameter and 12 cm in height; 3) the roof was constructed from plexiglass; and 4) the opening for mosquito aspiration was a simple buttonhole sewn into the polyester collection net (Figure). The traps were baited with adult golden Syrian hamsters obtained from a colony maintained at the Instituto Nacional de Salud in Bogota. Baited traps were suspended approximately 1.5 m above the ground and placed in transects at 10-m intervals. Carrots and rat chow were provided for food and water. The traps were checked each morning between 0600 and 0800 h, and some were also checked in the evening between 1700 and 1900 h. Mosquitoes were removed from the traps by using an aspirator, and the daily or semi-daily collections from each trap were frozen as a single pool in a plastic bottle immersed in liquid nitrogen vapor. When hamsters within the traps became moribund or died, serum samples were obtained by cardiac puncture or their hearts were dissected aseptically and frozen for virus isolation.

Detection of Natural Transmission to Hamsters

To confirm VEEV infection in dead or moribund hamsters, virus was isolated from a 10% heart tissue suspension in Eagle's minimal essential medium (MEM), supplemented with 20% fetal bovine serum (FBS) and antibiotics. The suspension was prepared in a Ten Broeck tissue grinder and centrifuged at 15,000 x *g* for 5 min; 200 µL of the supernatant was added to a 25-cm^2 flask containing a monolayer of Vero cells and adsorbed for 1 h at 37°C; 6 mL of additional MEM containing 2% FBS was then added. Cultures were incubated at 37°C for 5 days or until cytopathic effects were evident.

Mosquito pools from traps in which hamster infection with VEEV was confirmed were assayed for infectious virus. Pools containing 1–40 individuals of each mosquito species were triturated with a Minibeadbeater (BioSpec Products, Inc., Bartlesville, OK) or a Ten Broeck tissue grinder containing 1.0 mL of MEM supplemented with 20% FBS, penicillin, streptomycin, and amphotericin B. The triturated pool was centrifuged for 5 min at 15,000 x *g*, and 200 µl of the supernatant was added to a 10-mL plastic tube or a 25-cm^2 cell culture dish containing a monolayer of Vero cells and 2–5 mL of MEM. Cultures were monitored for cytopathic effects for 5 days.

Genetic and Antigenic Characterization of VEEV Isolates

Viruses isolated from hamster heart tissue suspensions and mosquito pools were characterized antigenically by using immunofluorescence of infected cells and a panel of monoclonal antibodies described previously (20). Subtype ID VEEV isolates were further characterized genetically by reverse transcription–polymerase chain reaction (PCR) amplification of an 856-nucleotide portion of the PE2 (sometimes called p62) envelope glycoprotein precursor gene as described previously (8), followed by single-stranded conformation polymorphism (SSCP) or sequence analysis (9). For SSCP analysis, PCR products were purified on agarose gels by using the QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA). A 2-µl volume of the PCR amplicon DNA suspension was mixed with 8 µl of SSCP loading buffer (95% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanol). The DNA was heated to 95°C for 5 minutes, rapidly cooled on ice, loaded onto an 8% polyacrylamide gel, and underwent electrophoresis in 1X Tris-borate EDTA buffer at room temperature for 20 h at 8 mA. Single-stranded DNA products were visualized by using silver staining (21). SSCP patterns were compared by measuring the migration of single-stranded DNA of the various isolates in comparison to one another and to a standard DNA ladder.

Results

For vector identification studies, 87 hamsters were exposed in traps within the Monte San Miguel Forest for 5–7 days. Of these, 38 became moribund or died and were processed for virus isolation. VEEV was isolated from 37 hamsters, and the mosquito collections from the corresponding traps were assayed for virus.

In 18 of the traps yielding infected hamsters, a vector species was identified by using the following criteria: 1) the ham-

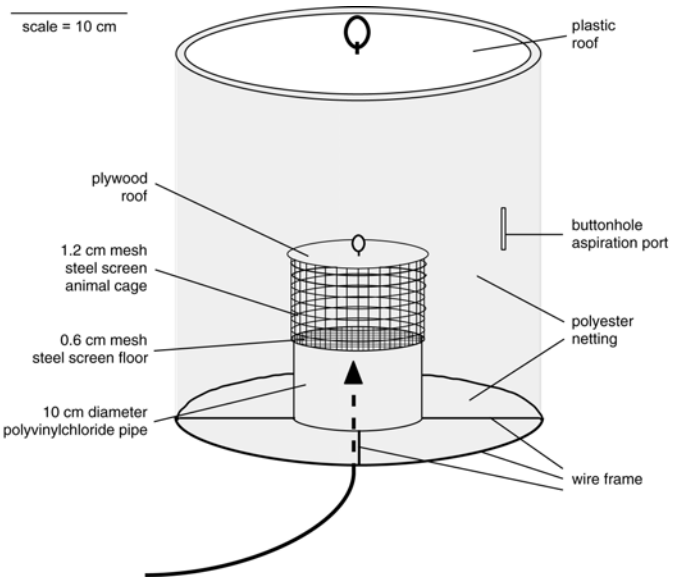

Figure. Major features of hamster-baited traps used to identify vectors of *Venezuelan equine encephalitis virus*. Arrow shows entry route of mosquitoes.

ster died at least 24 h after the collection of the presumed vector, consistent with the incubation time of VEEV in hamsters (22); 2) during the first day in which infected mosquitoes were collected from the trap, only one species pool had a high titer (>5.0 $\log_{10}$ PFU/pool) consistent with an infectious mosquito, as determined by previous experimental studies of enzootic VEEV vectors (18,23–26); 3) the remaining pools, from the first day in which infected mosquitoes were collected, were uninfected, or had low titers (<5.0 log) shown previously to be inconsistent with an infectious mosquito (18,23–26); 4) the mosquito collections on the days subsequent to that of the vector collection were mostly infected, reflecting hamster viremia and the ingestion of infectious blood by mosquitoes biting ≥12 h after the transmission event; and 5) virus isolates from the hamster and corresponding vector were indistinguishable antigenically and genetically with SSCP analysis, sequencing, or both. In 18/37 infected hamster events studied, these criteria were fulfilled, vector was identified unambiguously. Typical data for one of these transmission events (hamster 164) is shown in Table 1. In this example, transmission by *Cx. vomerifer* occurred ≤ 24 h after exposure of the trap, and the vector pool had a titer of 5.8 $\log_{10}$ PFU/pool. The other two infected pools from day 2, *Cx. pedroi* and *Aedes serratus*, had log titers ≤3.3, indicating that they were not capable of transmission. These pools presumably contained one or more mosquitoes that engorged on the hamster after viremia began, probably just before the daily trap collection. On the next day, all mosquito pools contained infectious virus in their midguts, representing viremic hamster blood ingested by mosquitoes within the trap.

A total of 18 transmission events were characterized as described above. The most common interval of collection of the identified vector was 24–48 h after exposure, reflecting a very high level of enzootic VEEV transmission in the Monte San Miguel Forest. *Cx. vomerifer* was implicated in 11 of these events, *Cx. pedroi* in 5, and *Cx. adamesi* in 2 transmissions (Table 2). The minimum infection/transmission rate for the mosquitoes we collected could not be determined directly because we did not identify the mosquito collections for traps where transmission to the hamster did not occur. However, rates on the order of 1/200–1/1000 can be estimated for these three vector species if the species composition is assumed to be similar in traps where transmission did not occur. Even if this assumption is incorrect, the error in this estimate should not be more than twofold because VEEV transmission occurred in most traps.

Discussion

Use of Hamster-Baited Traps for Arbovirus Vector Identification

Traditional criteria for arthropod vector identification include the following: 1) demonstration of feeding or other effective contact with pathogen's host; 2) association in time and space of the vector and pathogen; 3) repeated demonstration of natural infection of the vector, and 4) experimental transmission of the pathogen by the vector (27). Infection rates for arbovirus vectors tend to be relatively low, usually <1%. Therefore, fulfillment of these criteria for arbovirus vectors usually relies on the capture of large numbers of arthropods for virus isolation, followed by experimental laboratory transmission studies to ensure that species found infected in nature are competent vectors. Although this strategy is the most comprehensive and unbiased, it is extremely costly and time consuming, accounting for the relative paucity of information on natural vectors of many arboviruses. Some studies of VEEV vectors have also relied on oral infection from experimentally infected hamsters with viremia levels of very high titer, on the order of 8 $\log_{10}$ PFU/mL (28,29), a titer at least 100–1,000 times greater than that generated by experimentally infected rodent reservoir hosts (30,31), equines (13,30,32), or naturally infected humans (8,33) (Some studies of equine viremia have

Table 1. Mosquito collections from hamster-baited trap no. 164

April 8, 1999			April 9, 1999			April 10, 1999		
Species	Fraction of pools positive	Pool titers	Species	Fraction of pools positive	Pool titers	Species	Fraction of pools positive	Pool titers
Culex (Melanoconion) pedroi	0/1	NT[a]	*Cx. (Mel.) pedroi*	1/1	3.3	*Cx. (Mel.) pedroi*	1/1	5.9
Cx. (Mel.) spissipes	0/1	NT	*Cx. (Mel.) spissipes*	0/1	NT	*Cx. (Mel.) spissipes*	1/1	4.7
Cx. (Mel.) vomerifer	0/1	NT	*Cx. (Mel.) crybda*	0/1	NT	*Cx. (Mel.) ferreri*	1/1	4.8
Cx. (Mel.) adamesi	0/1	NT	*Cx. (Mel.) vomerifer*[b]	1/1	5.8	*Cx. (Mel.) vomerifer*	1/1	5.5
Aedes serratus	0/1	NT	*Cx. (Mel.) adamesi*	0/1	NT	*Cx. (Mel.) adamesi*	1/1	5.1
Cx. (Cx.) nigripalpus	0/1	NT	*Ae. serratus*	1/1	<2	*Ae. serratus*	1/1	5.2
Cx. (Ae.) amazonensis	0/1	NT	*Cx. (Cx.) nigripalpus*	0/1	NT	*Cx. (Cx.) nigripalpus*	1/1	5.2
Coquilletidia venezuelensis	0/1	NT	*Cx. (Ae.) amazonensis*	0/1	NT	*Cx. (Ae.) amazonensis*	1/1	4.9
Ae. fulvus	0/1	NT	*Ae. fulvus*	0/1	NT	*Cx (Ae.) accelerans*	1/1	4.5
						Mansonia titillans	1/1	5.2

[a]NT, not tested.
[b]Incriminated vector pool.

Table 2. Mosquito vector species identified in transmission of *Venezuelan equine encephalitis virus* to hamsters

Hamster no.	Vector species	Titer of vector pool[a]	Collection interval of vector pool (h)	Titer of other mosquito pools in the same collection as the vector[a]	Fraction of mosquito species infected on the subsequent day's collection[a]
65	*Culex pedroi*	5.2	0–24	<2	6/10
66	*Cx. vomerifer*	5.0	48–72	<2	18/18
144	*Cx. vomerifer*	5.4	24–48	<2	NA
150	*Cx. adamesi*	5.5	24–48	<2	9/9
164	*Cx. vomerifer*	5.8	0–24	≤3.3	10/10
172[b]	*Cx. vomerifer*	5.4	24–48	≤3.8	9/9
184	*Cx. pedroi*	5.1	0–24	≤2.3	6/6
186	*Cx. vomerifer*	6.1	0–24	<2	9/9
264	*Cx. vomerifer*	5.4	0–24	≥3.9	12/16
272	*Cx. pedroi*	5.1	0–24	<2	11/11
277	*Cx. adamesi*	5.3	0–24	<2	18/18
279	*Cx. vomerifer*	6.1	24–48	<2	14/16
286	*Cx. vomerifer*	5.5	120–144	<2	14/15
287	*Cx. pedroi*	5.4	0–24	<2	15/15
296	*Cx. vomerifer*	5.7	0–24	≥4.9	10/10
290	*Cx. pedroi*	5.4	0–24	<2.8	13/15
304	*Cx. vomerifer*	5.3	48–72	<2	8/8
305	*Cx. vomerifer*	5.7	2	≤2.1	8/8

[a]Log_{10} Vero PFU per pool
[b]A second infected pool of *C. vomerifer* with a log titer of 3.8 was collected on day 1, but was presumed not to have been transmitted to the hamster due to its low titer.

yielded titers of $>10^8$ suckling mouse intracerebral 50% lethal doses, but this method for quantifying VEEV titers is 100- to 1000-fold more sensitive than PFU [30,34]). Results from these studies are therefore inconclusive regarding natural transmission potential.

Other investigators have streamlined the vector identification process by collecting suspected vectors and sorting them according to species, then exposing single-species pools to naïve animals in a field or laboratory setting to detect transmission (16,18). We have taken this approach one step further by combining collection and transmission detection using hamster-baited traps. This method simplifies the vector identification process in several ways: 1) Hamster-baited traps attract and capture only arthropod species that are attracted to small mammals, the natural reservoir hosts of the enzootic VEEV (*Proechimys* spp. spiny rats in the case of subtype ID VEEV circulating in this focus [35]), minimizing collection and mosquito processing efforts. 2) Arthropod collections from traps where no transmission occurs do not need to be sorted, greatly reducing a laborious step in the vector identification process. 3) Only a small number of arthropod pools must be tested for virus, eliminating much of the cost, labor, and biosafety hazard associated with traditional vector identification approaches. In addition, the hamster-baited traps can serve as sentinels for detection of active virus circulation in a forest and reveal the presence of other viruses in a focus. However, unlike other sentinel enclosures that allow arthropods to escape after biting a viremic bait animal and thereby initiate artificial amplification, the hamster-baited traps capture most of the arthropods that bite the viremic host and prevent most or all artificial amplification. A similar strategy for detecting transmission of western equine encephalitis and St. Louis encephalitis viruses to chickens in baited traps was described by Reeves et al. (36).

Using these hamster-baited traps alone, we were not able to measure directly the capture efficiency of our traps. However, in the case of five infected hamsters, the lack of any collections with a single or few high titer mosquito species pools on the day preceding total infection of collected mosquitoes indicates that the arthropod responsible for transmission may have escaped. In other cases, two or more mosquito pools collected on the first day virus was detected had titers consistent with infectious vectors, precluding vector identification. We are currently experimenting with funnel-shaped openings to reduce the frequency of vector escape from this trap design. As with any passive trap design, a compromise between ease of vector entry and frequency of escape must be sought to maximize collections.

Enzootic Vectors of Venezuelan Equine Encephalitis Complex Viruses

Previous studies of VEE complex enzootic transmission have each identified a single, principal mosquito species in a given geographic region. All of these species, including *Cx. portesi* (14), *Cx. cedecei* (15), *Cx. aikenii sensu lato* (*ocossa* and *panocossa*) (16,17), and *Cx. taeniopus* (18) are members

of the Spissipes section of the subgenus *Melanoconion* within the genus *Culex* (37). Previous studies of enzootic VEEV transmission in the Catatumbo region of northeastern Colombia also suggested that *Cx. pedroi* might be the principal vector, based on abundance in active foci (38). *Cx. vomerifer* from Iquitos, Peru, also has been shown to be susceptible to infection by several strains of VEEV (28), but was only tested after mosquitoes ingested 8 $\log_{10}$ PFU/mL from viremic hamsters, a viremia titer at least 100 times greater than that generated by experimentally infected rodent reservoir hosts (30,31). Our findings of at least three enzootic vectors of subtype ID VEEV in Colombia contrast with the findings of all previous studies of enzootic VEEV vectors, which suggested that enzootic VEEV strains are each adapted to a single, principal vector species (13,18,39–41). In Colombia, subtype ID VEEV appears to utilize efficiently both *Cx. vomerifer* and *Cx. pedroi* in the Magdalena Valley. *Cx. adamesi*, which is usually less abundant in the Monte San Miguel Forest, appears to serve as a secondary vector.

All three of the mosquito species that we identified as VEEV vectors are members of the Spissipes section of the subgenus *Culex* (*Melanoconion*), bringing the total to seven confirmed vectors within this section of closely related mosquitoes. The genetic or ecologic basis for the exclusive use of these mosquitoes by enzootic VEE complex viruses deserves further study. Hypotheses to explain this phenomenon include possible shared, derived characteristics of the Spissipes section, such as particularly high susceptibility to infection by enzootic VEE complex viruses, a particularly high degree of association with the *Proechimys* spp. (35) and other small mammalian reservoir hosts (13), or both. Mosquito longevity and population sizes in habitats that support large populations of reservoir hosts may also favor transmission by members of the Spissipes section (35).

Role of Enzootic Vectors in VEEV Emergence and Disappearance

Identification of the principal enzootic vectors (*Cx. vomerifer* and *Cx. pedroi)* of subtype ID VEEV strains believed to be closely related to epizootic progenitors will allow us to assess the role of these mosquitoes in the generation of mutations that mediate VEE emergence by enhancing equine viremia and infection of epizootic mosquito vectors such as *Ochlerotatus taeniorhynchus*. The hypothesis that epizootic VEEV is not recovered from sylvatic foci because these strains lose their fitness for the enzootic vectors (25) can also be tested in the two principal vectors that we identified.

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Highly Endemic, Waterborne Toxoplasmosis in North Rio de Janeiro State, Brazil

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In Campos dos Goytacazes, northern Rio de Janeiro state, Brazil, reports of uveitis consistent with toxoplasmosis led to a survey of the prevalence and risk factors for *Toxoplasma gondii* infection in 1997–1999. The survey population was selected randomly from schools, randomly chosen communities, and an army battalion. Serum samples from 1,436 persons were tested. With results adjusted for age, 84% of the population in the lower socioeconomic group was seropositive, compared with 62% and 23% of the middle and upper socioeconomic groups, respectively (p<0.001). When multivariate analysis was performed, drinking unfiltered water was found to increase the risk of seropositivity for the lower socioeconomic (odds ratio [OR]: 3.0, 95% confidence interval [CI] 1.3 to 6.9) and middle socioeconomic (OR: 1.7, 95% CI 1.2 to 2.3) populations. We also found a high *T. gondii* seroprevalence in this Brazilian community. Drinking unfiltered water increased the risk of *T. gondii* seropositivity, indicating the potential importance of oocyst transmission in water in this region.

Toxoplasmosis, a zoonotic protozoal disease caused by *Toxoplasma gondii,* is horizontally transmitted to humans by the accidental ingestion of oocysts in cat feces or by eating raw or undercooked meat containing cysts (1). The infection is prevalent throughout the world, affecting a large proportion of adults who usually have no symptoms. Vertical transmission of toxoplasmosis from an acutely infected pregnant woman can cause serious disease in the fetus (2). In immunocompromised persons, a previously acquired *T. gondii* infection can be reactivated and result in severe illness, including encephalitis (3). In some areas of Brazil, the serologic prevalence of *T. gondii* infection ranges from 50% to 80% of the adult population; the highest values are found in some northern and southern states (4). In 1987, a survey of public school students in the capital of Rio de Janeiro State (Rio de Janeiro City) showed that the prevalence increased with age, reaching 71% for persons 16–20 years old (5). However, in the rest of Rio de Janeiro State, little information exists on the epidemiology of toxoplasmosis.

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Water has been identified as a source of *T. gondii* infection in outbreaks (6,7), but it has not been well studied as a risk factor in toxoplasmosis-endemic areas. We report the results of a seroprevalence survey in northern Rio de Janeiro State in which we assessed exposure to known sources of *T. gondii* infection, as well as exposure to various types of water.

Methods

Study Area

Campos dos Goytacazes (Campos), located north of Rio de Janeiro with a population of about 400,000 persons, is the third most economically important city in the state. The Paraiba do Sul River, which supplies approximately 4.8 million persons in Brazil, provides the city with water and divides it into two parts. For this study, Campos was further divided into four geographic regions, according to predominantly urban or suburban (regions 1 and 2) or rural characteristics (regions 3 and 4).

Study Population

A preliminary serologic survey in 1997 showed that 61 (82%) of 74 persons living in an extremely impoverished region of Campos and 15 (55%) of 27 public school children had antibodies to *T. gondii*. In the subsequent 1997–1999 study presented here, we divided the city's population into three socioeconomic strata. The first strata included persons living in poor communities in urban, suburban, or rural areas where neither residential sewage facilities nor municipally treated water were often available; these persons were from households that received less than US$150 per month in income (lower socioeconomic population). Lower middle-class children attending public schools, adult staff of the schools, and soldiers belonging to the county army battalion and adult members of their families constituted population 2 (middle socioeconomic population), in which monthly household income ranged from U.S. $150–$500. Upper and middle class children attending private schools and the schools' staff constituted population 3 (upper socioeconomic population); their households received more than U.S. $500/month. Only some households in the lower socioeconomic population received treated water, whereas all households in the middle and upper socioeconomic populations received treated water.

To ensure a geographically representative sample of persons from the lower socio-economic population, 3 of 15 poor

areas of the city were randomly selected. These areas represented 6% of the total population of Campos de Goytacazes. Households within these communities could not be randomly selected because the areas did not have a systematic spatial organization (such as conventional streets, quarters, or blocks). Therefore, we employed a visual sampling scheme in an attempt to select houses throughout the communities. In rural areas, because the houses were distant from each other, we went to all houses and invited persons found at home to participate.

To obtain a sample of persons from the middle socioeconomic population, we selected all public schools with >300 students (54 [61%] of the 89 public schools in Campos). In each school, all members of each odd-numbered class and all adult staff members were eligible for testing. The middle socioeconomic population also included soldiers from the only army base in Campos, ranging in age from 18 to 30 years, and adult members of their families (spouses and parents); 95% of the soldiers participated in the survey, and 80% brought at least one adult family member.

To obtain representatives of the upper socioeconomic population, we randomly selected two of the six private schools, from which children were chosen by using the same sampling scheme as for the middle socioeconomic population. Participants or their guardians provided written consent. This study was approved by the Brazilian Ministry of Health Ethics Commission (FIOCRUZ) and exempted by the Centers for Disease Control and Prevention Institutional Review Board (for data analysis).

Questionnaire

For the evaluation of risk factors, we used a standard questionnaire, adapted from an outbreak of toxoplasmosis in Vancouver (7), which addressed (in 171 questions) exposure to known sources of *T. gondii* infection, as well as exposure to various types of water. A partial list of questionnaire variables included the following: age; sex; birth location; occupation; area of the city; urbanicity; number of persons in family; cat ownership; feeding cats raw or undercooked meat; where cats are kept (inside or outside); handling stray cats; exposure to cat feces; exposure to dogs and other pets; participation in birthing of animals and type of animal; gardening; exposure to soil; hand washing after soil exposure; hand washing before meals; types and frequency of meat and poultry eaten (including pork, chicken, beef, wild game, lamb, goat, sausage, locally prepared meats, and others); eating undercooked meat (for each type of meat); ingestion of unpasteurized cow's milk, butter, and cheese, unpasteurized goat's milk and cheese, eggs and raw eggs, homemade ice cream, nonleafy and leafy vegetables, and fruits; location of meat purchases; eating food grown in own garden; frequency of eating at restaurants and other locations; type of water used at home (well, unfiltered, filtered, spring [bottled] water); frequency of drinking various types of beverages and type of water used to prepare beverages; drinking directly from a lake, river, or stream; and whether a home water filter was used and how often it was changed. Parents or guardians responded for children <6 years old.

Municipal Water Distribution and Treatment

Campos has two municipal water treatment plants. Since 1930, plant 1 has processed surface water from the river and supplies the major part of region 1 and all of region 2. In the early 1990s, plant 2 was built to supply part of region 1 with water from wells (more than 100 m deep). The rural areas do not receive treated water.

Plant 1 has sand and charcoal filters and treats water by flocculation, decantation, filtration, and chloramination; plant 2 has only sand filters and employs flocculation, decantation, filtration, oxidation of iron, and chloramination. The Brazilian Ministry of Health requires that the turbidity of treated water be below 5 nephelometric turbidity units and recommends that municipal drinking water be filtered at home with commercially available filters or boiled. Because of these recommendations, water from the faucet was considered unfiltered.

Serologic Tests

Serum samples were tested at Núcleo de Apoio Diagnostico from the Federal University of Minas Gerais for toxoplasmosis by using a commercial ultramicro enzyme-linked immunosorbent assay (UMELISA) with fluorometric detection for anti-*Toxoplasma* IgG (UMELISA *Toxoplasma,* La Habana, Cuba). The correlation between the UMELISA and the Sabin-Feldman dye test, as well as the quality control of the UMELISA's system, were evaluated by blindly exchanging 20 collected serum samples with the Research Institute, Palo Alto Medical Foundation, Palo Alto, California, USA. Twenty-six samples were also sent to the World Health Organization's International Collaboration Centre for Research and Reference for *Toxoplasma*, Statens Serum Institut, Copenhagen, Denmark. Results from samples with values lower than 9 IU and higher than 15 IU in UMELISA agreed 100% with results from the dye test, and 99.7% of the survey samples were in this range of detection. Serum titers found by UMELISA (IU) were highly correlated with those found by the dye test (both low and high).

Statistical Analyses

For examination of risk factors for *T. gondii* seropositivity, age was categorized into the following 7 groups: 0–9, 10–14, 15–19, 20–29, 30–39, 40–49, $\geq$50 years, and socioeconomic status was categorized into three groups as described previously. The data were initially examined with univariate analysis. For multivariate analysis, first a Cochran-Mantel-Haenszel summary statistic, controlling for age and socioeconomic status, was calculated for each risk factor examined in the survey (this was done because age and socioeconomic status were strongly associated with seropositivity). Then, factors that were found by the Cochran-Mantel-Haenszel summary statistic to have a significant ($p<0.05$) association with *T. gondii*

seropositivity were entered into backward elimination logistic models, along with standard demographic variables. Variables were allowed to remain in the models at a p<0.1 level. Persons with high serologic test IU values (top 25%) and, separately, children (ages 0–14 years) with high serologic titers, were examined with separate models. Because of the numerous variables entered into each logistic model, p<0.01 was used as the cut-off for statistical significance. To compare seroprevalence among the three socioeconomic groups, the seroprevalence values were age-adjusted by the direct method to the age distribution of the combined study population.

Results

A total of 1,436 persons were enrolled in the study. Of 381 persons from the lower socioeconomic population, 316 (83%) agreed to participate; of 1,059 persons from the middle socioeconomic population, 819 (77%) agreed to participate; and of 372 persons from the upper socioeconomic population, 301 (81%) agreed to participate. The age-adjusted seroprevalence values were: 84%, 62%, and 23% for the lower, middle, and upper socioeconomic populations, respectively. We observed an increasing prevalence with age for all three population groups (Figure). However, the prevalence of *T. gondii* infection among persons in each age group <40 years old was significantly (p<0.05, chi square or Fisher exact test) lower for the upper socioeconomic population than for the middle and lower socioeconomic populations.

Age and socioeconomic status were the strongest predictors of *T. gondii* seropositivity. Table 1 shows the values for statistically significant variables (p≤0.01) in the univariate analysis. All the variables assessing consumption of unfiltered or untreated water were significantly associated with *T. gondii* seropositivity with a 1.6- to 3-fold increased risk of infection. Having spring water in the house was protective (odds ratio [OR] 0.4 confidence interval [CI] 0.3 to 0.5). Increased risk for *T. gondii* seropositivity was also detected for owning a cat and eating unpasteurized butter. However, decreased risk for *T. gondii* seropositivity was detected for living in region 1, eating undercooked meat (general question about all types of meat only, not specific types of meat), eating kibbeh (ground meat with spices), eating food at home entertainment shows, and eating at least some of the time in restaurants. Soil contact was not associated with increased risk of infection, nor was infection associated with other types of meat, other pet exposure, hand washing, soil exposure, gardening, or eating fruits or vegetables.

The results of the backward elimination logistic models for the entire study population are shown in Table 2, and subset analyses for each of the three socioeconomic groups are shown in Table 3. The tables include all the factors that remained in the backward elimination models. In Table 2, the logistic model for the entire population shows that drinking water from a faucet (unfiltered) or directly from a lake, river, or stream was associated with *T. gondii* infection, with ORs of 1.5- and 1.6-fold, respectively.

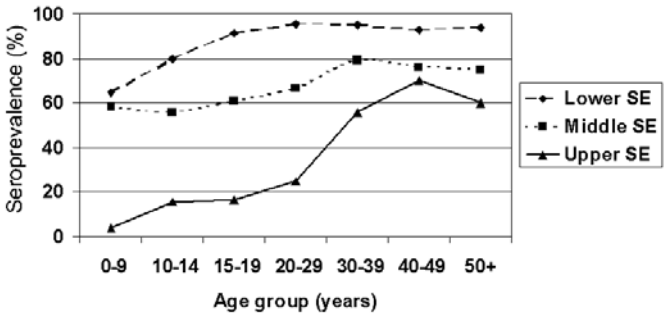

Figure. Serum prevalence by socioeconomic group and age for the lower, middle, and upper socioeconomic (SE) populations.

The logistic model for the lowest socioeconomic population (Table 3) shows that living in rural regions (OR 0.27, CI 0.10 to 0.69) as well as eating in restaurants (OR 0.40, CI 0.19 to 0.83) were protective for *T. gondii* infection; on the other hand, drinking unfiltered water increased the risk for *T. gondii* infection 3-fold (OR 3.04, CI 1.34 to 6.93). For the middle socioeconomic population, no variable was found to be protective for *T. gondii* infection, and as with the entire population, drinking unfiltered water or drinking directly from a lake, river, or stream increased the risk for *T. gondii* seropositivity 1.7- and 1.8-fold, respectively (Table 3). No risk factor other than age was associated with seropositivity for the upper socioeconomic population (Table 3), although living in region 1 of the city was protective in this group (OR 0.05, CI 0.01 to 0.49).

We then examined risk factors for the 360 persons with the strongest 25% of serologic reactions (412–1000 IU), compared with those who were seronegative (<12 IU). In addition to age and socioeconomic status, suburban vs. urban location (OR 2.8, 95% CI 1.8 to 4.2, p<0.001) and drinking unfiltered water (OR 2.0, 95% CI 1.4 to 2.8, p<0.001), or lake, river or stream water (OR=1.5, 95% CI 1.0 to 2.2, p=0.048), or faucet water (OR 1.7, 95% CI 1.2 to 2.5, p=0.001) vs. the absence of these water-related factors, increased the risk for strongly reactive serologic tests. Eating in restaurants (OR 0.4, 95% CI 0.3 to 0.6, p<0.001) and living in region 1 compared with other regions (OR 0.4, 95% CI 0.3 to 0.6, p<0.001) decreased the risk for strongly reactive serologic tests.

We also determined risk factors for children 0–14 years of age; the strongest 25% of serologic reactions were compared to those with seronegative reactions in a logistic model (192 with strongest serologic reactions, 433 seronegative, total N=625). In this analysis, children belonging to the middle socioeconomic population had an increased risk for *T. gondii* seropositivity when they were compared with children in the lower soscioeconomic population (OR 2.0, 95% CI 1.3 to 3.1, p=0.002); those living in a suburban location had a higher risk than those in an urban location (OR 2.8, 95% CI 1.7 to 4.8, p<0.001); and those drinking unfiltered water (OR 2.5, 95% CI 1.6 to 3.9, p<0.001) or drinking lake, river, or stream water (OR 1.9, 95% CI 1.1 to 3.3, p=0.020) had higher risks than children not drinking these types of water. Eating in restaurants (OR 0.4,

Table 1. Univariate results showing *Toxoplasma gondii* seropositivity for statistically significant characteristics[a]

Characteristic	No. persons	% seropositive	Odds ratio	95% CI[b]
Age (yr)				
0–9	189	39.7	Ref.[c]	
10–14	585	45.5	1.3	0.9 to 1.8
15-19	256	63.7	**2.7**	**1.8 to 4.0**
20-29	161	70.8	**3.7**	**2.3 to 5.9**
30-39	91	83.5	**7.7**	**4.0 to 15.2**
40-49	89	83.2	**7.5**	**3.9 to 14.8**
50+	65	83.1	**7.5**	**3.5 to 16.2**
Socioeconomic population				
Lower	316	84.8	Ref.	
Middle	819	61.4	**0.3**	**0.2 to 0.4**
Upper	301	16.9	**0.04**	**0.02 to 0.06**
Location				
Urban	1040	49.9	Ref.	
Suburban	251	79.7	**3.9**	**2.8 to 5.6**
Rural	145	71.0	**2.5**	**1.7 to 3.7**
Regions 2-4	597	73.2	Ref.	
Region 1	839	45.9	**0.3**	**0.2 to 0.4**
Well water in house				
No	1159	54.0	Ref	
Yes	277	70.8	**2.1**	**1.6 to 2.7**
Unfiltered water in house				
No	1050	50.1	Ref.	
Yes	386	74.4	**2.8**	**2.2 to 3.6**
Spring water in house				
No	874	65.6	Ref.	
Yes	562	44.3	**0.4**	**0.3 to 0.5**
Drink at least some unfiltered water				
No	907	48.0	Ref.	
Yes	529	73.2	**3.0**	**2.4 to 3.7**
Drink at least some beverages made with unfiltered water				
No	968	49.5	Ref.	
Yes	468	73.3	**2.8**	**2.2 to 3.5**
Drink water directly from lakes, rivers or streams				
No	1160	55.2	Ref.	
Yes	276	65.9	**1.6**	**1.2 to 2.1**
Drink water directly from faucet				
No	699	46.2	Ref.	
Yes	737	67.7	**2.4**	**2.0 to 3.0**
Drank beverages made with well water in the past 12 months				
No	1173	54.7	Ref.	
Yes	263	68.4	**1.8**	**1.4 to 2.4**
Food exposure				
Ate undercooked meat in past 12 months				
No	877	59.9	Ref.	

Table 1 continued. Univariate results showing *Toxoplasma gondii* seropositivity for statistically significant characteristics[a]

Characteristic	No. persons	% seropositive	Odds ratio	95% CI[c]
Ate undercooked meat in past 12 months				
Yes	559	53.1	**0.8**	**0.6 to 0.9**
Ate kibbeh the past 12 months				
No	1181	59.8	Ref.	
Yes	255	45.5	**0.6**	**0.4 to 0.7**
Ate unpasteurized butter in past 12 months				
No	983	54.5	Ref.	
Yes	453	63.1	**1.4**	**1.1 to 1.8**
Ate food at home entertainment shows in the past 12 months				
No	923	62.6	Ref.	
Yes	513	47.6	**0.5**	**0.4 to 0.7**
Ate at least some of the time in restaurants in the past 12 months				
No	517	73.5	Ref.	
Yes	919	48.1	**0.3**	**0.3 to 0.4**
Owned cat in past 12 months				
No	942	52.0	Ref.	
Yes	494	67.2	**1.9**	**1.5 to 2.4**

[a]p<0.01, in bold.
[b]CI, confidence interval.
[c]Ref., referent.

95% CI 0.3 to 0.6, p<0.001) and living in region 1 compared with living in other regions (OR 0.4, 95% CI 0.2 to 0.6, p<0.001) decreased the risk for *T. gondii* seropositivity.

Discussion

The findings that age and socioeconomic status were the strongest predictors of *T. gondii* seropositivity in this study are consistent with those of other studies (8–11). However, to our knowledge, this study is the first to implicate the consumption of untreated or unfiltered water as a source of *T. gondii* infection in a disease-endemic area.

Most persons in the lower socioeconomic population complement their water supplies by constructing wells on their property. In this region of northern of Rio de Janeiro State, on average, water can be found only 9 m deep, and the well entrances are usually close to the ground. Contamination with oocysts from soil is likely to occur in floods or runoff that are frequent in Campos after rainfall. Since oocysts can survive long periods in water (12), the lower socioeconomic population may frequently be exposed to *T. gondii* oocysts from drinking water. In fact, most persons in the lower socioeconomic population were infected by the age of 15 (Figure).

Anecdotal reports imply that, in the past 15 years, the upper socioeconomic population in the city began to consume bottled spring water, and that, for the past 10 years, nearly 100% of the upper socioeconomic population utilizes this type of water for drinking and to rinse raw vegetables and fruits. This change is attributed to the poor taste of the municipal water, which has not been well accepted by the upper socioeconomic population.

Most *T. gondii* oocysts that contaminate water reservoirs and wells come from soil; however, in this study, contact with soil was not a risk factor for toxoplasmosis. In northern Rio de Janeiro State, water may be a more important means of ingesting oocysts. The lack of association between variables related to owning cats and seropositivity for toxoplasmosis in the logistic regression models is consistent with results of numerous other studies (13–16). Cats often spread oocysts away from their home, and feral cats may be responsible for much of the environmental contamination with oocysts. However, owning a cat was associated with *T. gondii* seropositivity in the univariate analysis and was more common among persons in the lower and middle socioeconomic groups than among the upper socioeconomic group.

Several other factors were associated with *T. gondii* seropositivity by univariate analysis, but not by multivariate analysis, for example, eating unpasteurized butter in the past 12 months. Unpasteurized butter could increase the risk for *T. gondii* infection if the butter was contaminated by oocysts from soil. However, unpasteurized butter was not one of the stronger risk factors, and the association with *T. gondii* seropositivity may be confounded by socioeconomic status, i.e., persons in lower socioeconomic groups (a strong risk factor for *T. gondii* seropositivity) may be more likely to eat unpasteurized butter. In fact, when we checked the data to determine the proportion of persons eating unpasteurized butter stratified by socioeconomic group, we found that persons in the lower socioeconomic group were more likely to report that they had eaten unpasteurized butter than were those in the middle or upper socioeconomic groups (39% vs. 32% and 24%,

Table 2. Risk for *Toxoplasma gondii* seropositivity shown as odds ratios estimated with backward elimination logistic regression for all the three socioeconomic populations combined[a]

Variable	Odds ratio	Wald confidence limits		p value
		Lower	Upper	
Age (yrs)				
0–9	Ref.[b]			
0–14	1.75	1.15	2.68	0.001[c]
15–19	2.84	1.75	4.63	<0.001[c]
20–29	4.36	2.52	7.55	<0.001[c]
30–39	8.84	4.30	18.20	<0.001[c]
40–49	10.81	5.18	22.56	<0.001[c]
50+	8.45	3.75	19.00	<0.001[c]
Socioeconomic population				
Lower	Ref.			
Middle	0.48	0.32	0.72	<0.001[c]
Upper	0.14	0.08	0.24	<0.001[c]
Location				
Urban	Ref			
Suburban	1.56	1.07	2.28	0.022
Other factors (ref. is absence of factor)				
Unfiltered water	1.23	0.97	1.74	0.081
Lake, river, or stream water	1.63	1.16	2.29	0.005[c]
Faucet water	1.54	1.17	2.03	0.002[c]
Kibbeh	0.69	0.50	0.96	0.027
Ice cream (in plastic pop-up bags)	1.32	1.01	1.73	0.046
Attend home shows	0.75	0.57	0.99	0.039
Eat in restaurants	0.69	0.52	0.92	0.011[c]
Region 1 (compared with other regions)	0.74	0.56	0.98	0.037

[a]All variables remaining in the model are shown.
[b]Ref., referent.
[c]Statistically significant ($p \leq 0.01$, rounded).

respectively). Consuming either unpasteurized cow's milk or goat's milk were not found to be risk factors. Drinking spring (bottled) water and attending home entertainment shows were associated with a decreased risk for *T. gondii* seropositivity by univariate analysis; these two factors were, as well as undercooked meat and kibbeh consumption, more common in the upper socioeconomic group than in the middle and lower socioeconomic groups.

In logistic regression, variables such as "living in region 1 of the city" and "eating in restaurants" were protective for the lower socioeconomic population. These findings may reflect differences in social conditions within the lower socioeconomic population. In fact, in region 1 the sanitary conditions of very poor communities are slightly better in comparison with those found in other regions of Campos. Another variable, "living in rural regions" (Table 3) was protective for the lower socioeconomic population. The closer proximity of animals (including cats) to human homes and the smaller space for deposition of animal waste in urban/suburban regions, thus increasing the possibility of water contamination with *T. gondii* ooccysts, might account for this difference within the lower socioeconomic population.

Our study is subject to a number of limitations. Because seropositivity for *T. gondii* persists for many years a direct temporal relationship between behaviors and *T. gondii* infection cannot be established. However, our findings were confirmed in subset analyses of persons who may have been infected in the more recent past, i.e., children and persons with high serologic titers.

Our study and those of others recently published on congenital toxoplasmosis in Brazil (17,18) implicate toxoplasmosis as an important health problem. Toxoplasmosis may be equally important in many other developing countries, where the lack of adequate sanitary conditions expose populations to a variety of diseases. Although some waterborne infections have been more thoroughly evaluated by the public health system, others, such as toxoplasmosis, remain to be investigated further to fully define the risk attributable to waterborne transmission.

Table 3. Risk for *Toxoplasma gondii* seropositivity shown as odds ratios estimated with backward elimination logistic regression for each of the three socioeconomic populations[a]

Variable	Odds ratio	Wald confidence limits		p value
		Lower	Upper	
Lower socioeconomic group				
Age (yrs)				
0–9	Ref.[c]			
15–19	4.31	1.15	16.13	0.300
20–29	9.78	2.10	45.46	0.036
30–39	10.02	2.13	47.20	0.036
40–49	5.83	1.60	21.28	0.076
50+	11.95	2.47	57.86	0.021
Location				
Urban	Ref.			
Rural	0.27	0.10	0.69	0.007[b]
Other factors (ref. is absence of factor)				
Unfiltered water	3.04	1.34	6.93	0.008[b]
Undercooked beef	2.89	1.05	7.96	0.040
Ate in restaurants	0.40	0.19	0.83	0.014[b]
Living in region 1	0.31	0.12	0.78	0.013[b]
Middle socioeconomic group				
Age (yrs)				
0–9	Ref.			
15–19	1.56	1.07	2.26	0.019
20–29	2.06	1.24	3.40	0.005[b]
30–39	4.70	2.10	10.50	<0.001[b]
40–49	4.62	2.03	10.50	<0.001[b]
50+	4.62	1.81	11.79	0.001[b]
Other factors (ref. is absence of factor)				
Female	0.74	0.54	1.01	0.061
Unfiltered water	1.67	1.22	2.30	0.002[b]
Lake, river, or stream water	1.80	1.17	2.76	0.008[b]
Kibbeh	0.64	0.44	0.95	0.026
Ice cream (in pop-up bags)	1.38	1.00	1.92	0.051
Attend home shows	0.72	0.52	0.98	0.037
Living in region 1	0.74	0.54	1.00	0.052
Upper socioeconomic group				
Age (yrs)				
0–9	Ref.			
40–49	18.24	4.36	76.30	<0.001[b]
50+	12.42	1.93	79.89	0.008[b]
Other factors (ref. is absence of factor)				
Female	0.50	0.26	0.99	0.046
Living in region 1	0.05	0.01	0.49	0.011[b]

[a]All variables remaining in the model are shown.
[b]Statistically significant (p<0.01, rounded) factors.
[c]Ref., referent

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Seasonal Dynamics of *Anaplasma phagocytophila* in a Rodent-Tick (*Ixodes trianguliceps*) System, United Kingdom

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We investigated the reservoir role of European wild rodents for *Anaplasma phagocytophila* using polymerase chain reaction (PCR) analysis of blood collected from individually tagged rodents captured monthly over 2 years. The only tick species observed in the woodland study site was *Ixodes trianguliceps*, and ruminant reservoir hosts were not known to occur. *A. phagocytophila* infections were detected in both bank voles and wood mice but were restricted to periods of peak nymphal and adult tick activity. Most PCR-positive rodents were positive only once, suggesting that rodent infections are generally short-lived and that ticks rather than rodents may maintain the infection over winter. Bank voles were more likely to be PCR positive than wood mice, possibly because detectable infections are longer lived in bank voles. This study confirms that woodland rodents can maintain *A. phagocytophila* in Great Britain in the absence of other reservoir hosts and suggests that *I. trianguliceps* is a competent vector.

Anaplasma phagocytophila (formerly *Ehrlichia phagocytophila*, *E. equi*, and the agent of human granulocytic ehrlichiosis [HGE]; [1]) is an obligate intracellular bacterium that targets mainly granulocytes in its mammalian hosts (2). This bacterium has a wide mammalian host range, infecting domesticated animals such as dogs, sheep, cows, and horses (2–5), as well as wildlife species such as deer and rodents (6,7). The discovery of HGE, an acute febrile disease, in the United States and Europe (8,9) has generated increasing public health interest in this organism.

A. phagocytophila is transmitted by ixodid ticks; in the United States, the principal vectors are *Ixodes scapularis* and *I. pacificus* (6,10), while in Europe the main vector is thought to be *I. ricinus* (3). *A. phagocytophila* is transstadially transmitted by all these vector ticks and, to date, no evidence of transovarial transmission has been found (3,6,11,12).

A number of studies have reported *A. phagocytophila* infection in wild rodents in the United States, the United Kingdom, and mainland Europe (6,13,14), but relatively little is known about the precise role that rodents play in its ecology and epidemiology, especially in Europe. Recently, *A. phagocytophila* has been detected in woodland rodents in northwest England, where *I. trianguliceps*, a nidicolous tick that feeds almost exclusively on small mammals, was the only tick species identified on rodents (11). This woodland rodent/*I. trianguliceps*/*A. phagocytophila* system is therefore one of many supporting a tickborne zoonosis, where lack of knowledge of the dynamics of the interacting populations is a major barrier to understanding potential threats to human health. We report the results of a longitudinal study of this system conducted during 2 years from January 1997 to December 1998.

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Materials and Methods

Small Mammals and Sample Collection

The study was conducted in woodland area in northwest England (N53:20:48, W03:02:50). Grazing livestock were excluded by fencing, and no deer are present in the locality. Brown hares (*Lepus europaeus*) and grey squirrels (*Sciurus vulgaris*) occur in the wood at low densities. Although their status as reservoir hosts for *A. phagocytophila* is unknown, these two species are unlikely to be frequent hosts of the nidicolous *I. trianguliceps*. Rodents were trapped as previously described (15,16). Briefly, 200 Longworth traps (Penlon Ltd, Oxfordshire, UK) were placed on a 1-hectare grid over 3 consecutive trap-nights every 4 weeks in 1997 and 1998 for a total of 26 sample periods. Individual animals were identified by a microchip transponder (Avid Pettrac, Sussex, UK), inserted subcutaneously on first capture. At first capture in each sample period, we recorded body mass, numbers of feeding ticks, and evidence of flea infestation and took a blood sample (approximately 50 μL) from the tail tip, after which the rodent was released. On first capture, we also assigned animals to one of three age categories on the basis of mass. Using monthly growth rates estimated from field data and laboratory information on mass at 2 weeks of age, we calculated mass thresholds for juvenile (J; <6weeks), subadult (S; 6–10 weeks) and adult (A; >10 weeks) age categories (unpub. data). Thresholds used were as follows: wood mice, April–July: J = <15 g, S = 15–18 g, A = >18 g; wood mice, August–December: J = <14 g, S = 14–17 g, A = >17 g; bank voles, April–July: J = <14 g, S = 14–17 g, A = >17 g; and bank voles, August–December: J = <12 g, S = 12–14 g, A = >14 g.

This study was intended to be as noninvasive as possible, and ticks were not routinely collected in case this affected the transmission of *A. phagocytophila* in the study site where pilot

studies had suggested that tick densities were low. Small numbers of engorged ticks were, however, collected from rodents at the study site between May 1997 and August 1998. Because of the nidicolous nature of *I. trianguliceps*, no questing ticks were collected.

DNA Extraction and Polymerase Chain Reaction (PCR)

DNA was extracted from blood pellets by alkaline digestion (17). We added 0.5 mL 1.25% ammonia solution to the blood sample in a Sure-Lock microcentrifuge tube (Fisher Scientific, Loughborough, UK) and heated at 100°C for 20 min. After brief centrifugation, the tubes were opened and heated until half the initial volume had evaporated. This solution was then diluted 1 in 10 in sterile deionized, distilled water, and 5 µL was included in the first round of PCR reactions. Sensitivity was compared with that of a commercial kit by extracting DNA from serial dilutions of acutely infected sheep blood; PCR of these dilutions indicated the limit of detection to be approximately two infected leukocytes for both methods, the same as previously reported (11). The same method was used to extract DNA from ticks that had first been macerated in the microcentrifuge tube with a pipette tip. For engorged adult female ticks, however, the initial volume of 1.25% ammonia solution was 1 mL.

A. phagocytophila infection was detected by using a nested PCR specifically targeting the 16S rDNA of *A. phagocytophila*, as described previously (11). Each 50-µL reaction contained 1.5 mM Mg Cl_2, 0.2 mM each of dNTP, 75 mM Tris-HCl (pH8.8), 20 mM $(NH_4)_2SO_4$, 1.25 U *Taq* polymerase (Abgene, Surrey, UK), and 40 pmol of each of the following primers (18): first reaction: EE1: TCCTGGCTCAGAACGAACGCTGGCGGC; EE2: GTCACTGACCCAACCTTAAATGGCTG; second reaction: EE3: GTCGAACGGATTATTCTTTATAGCTTGC; EE4: CCCTTCCGTTAAGAAGGATCTAATCTCC. For the second-round reaction, 1 µL of the first-round product was added as template. Both reactions consisted of 35 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 60 sec, followed by a final extension stage of 72°C for 5 min.

16S rRNA Sequence Analysis

The PCR product from a positive bank vole was cloned by using the TOPO TA cloning kit (Invitrogen Corp., Carlsbad, CA) and sequenced by using an ABI 377 automated sequencer. The sequence (GenBank accession no. AY082656) was compared to previously published *A. phagocytophila* sequences on GenBank by using the BLAST program from the National Center for Biotechnology Information website (available from: URL: http://www.ncbi.nlm.nih.gov/BLAST/).

Statistical Analysis

We investigated two outcome variables, the numbers of ticks counted per rodent and the rodent blood PCR result. Our purpose was to identify the factors that influenced the contact rates of rodents with ticks and the probability that the rodents acquired *A. phagocytophila* infections.

Analysis of Rodent Infestations

Distributions of larval, nymphal, and adult ticks were significantly different from normal and Poisson distributions ($p<0.05$), but none were significantly different from the negative binomial distribution ($p>0.1$). Consequently, factors influencing the numbers of adult, nymphal, and larval ticks counted per rodent were investigated by using negative binomial, linear regression models in STATA for Windows version 6 (19). Rodent ID number was included as a random effect to account for repeated sampling of some of the rodents (20).

The analysis was undertaken in two stages. In the first stage, we investigated any seasonal variations in the abundance of ticks because such variations could superimpose on seasonal variations in rodent demography and confound investigation of animal-level variables. This stage itself involved three steps. First, we tested the null hypothesis that no significant variation existed in the counted numbers of ticks among sample periods. Second, we tested the null hypothesis that variation in the numbers of ticks among sample periods was not different from the specific pattern of seasonal *I. trianguliceps* abundance suggested by the detailed study of Randolph (21) after visual examination of data from a woodland in southern England (N51:01:46; W0:50:11). Other studies on *I. trianguliuceps* conducted in different locations in the United Kingdom at different times have suggested that the seasonal pattern of *I. trianguliceps* abundance observed by Randolph is more general in the United Kingdom (22–24). In this analysis we compared the power of binary variables for year, season (spring, summer, autumn, and winter), and the tick activity periods observed by Randolph (e.g., June and September–January for larvae, and May–August for nymphs) to explain any between-sampling variation in tick abundance observed in the present study. Third, we sought the optimum pattern of between-sampling variation in tick abundance in the present study by investigating binary dummy variables for each sampling period, as well as those for year and seasons. In these models, forward and backward elimination and combination of variables were performed stepwise until a minimal model was reached beyond which the variables could not be combined without significantly affecting model deviance.

In the second stage, we investigated rodent-level factors of species, sex, age category, and mass as explanatory variables for tick infestations in multivariable models that accounted for any seasonal variation in tick abundance deduced in the first stage. Mass and age category were investigated in separate models because of some colinearity. We also investigated interactions between sex and species and between sex and mass as explanatory factors. In addition, evidence for relationships between parasitism with one tick developmental stage and another was investigated by using similar regression models, accounting for any deduced seasonal variation in tick abundance and notable animal-level factors. The critical probability was $p<0.05$ throughout.

Analysis of PCR Result

Rodent species and sex, mass, and age category (in separate models), the presence of fleas (a binary variable), and the numbers of larval, nymphal, and adult ticks counted per rodent were investigated as variables that could explain results of PCR analysis of rodent blood by using logistic regression models in STATA. Interactions between sex and species and between sex and mass were also investigated as explanatory factors. The binary variable age (rodent >6 months old) was investigated in case susceptibility increased in older animals that had not recently received infectious challenge, as occurs in sheep (2). Rodent ID number was again included as a random effect. The likelihood that a rodent encountered a tick of a particular developmental stage in any month of the study was investigated by using variables developed (as described previously) in investigations of the seasonal activity of ticks. However, any infections detected at one sample period may have been acquired from ticks (or other vectors) that attached to rodents in the previous sample period (because of latent detectability of infections) (3,25–27), or from ticks that fed and dropped off without being counted (21). To account for this, we investigated four additional binary variables as explanatory of rodent infections: whether the rodent was infested, either at the time of sample or the previous sample period, with a tick of a given stage or a flea, termed "carried a larva," "carried a nymph," "carried an adult," and "carried a flea." The critical probability was $p<0.05$ throughout.

Results

Rodents Captured and Their Tick Infestations

Over the study period, we captured 690 rodents: 475 wood mice (*Apodemus sylvaticus*) and 209 bank voles (*Clethrionomys glareolus*), plus 6 field voles (*Microtus agrestis*) which, because of the low numbers, were excluded from subsequent analyses. *I. trianguliceps* was the only species of tick found on the rodents during this study. Data on the numbers of captures and ticks are summarized in Table 1.

We found significant differences among sampling periods in the numbers of larvae and nymphs counted on rodents (likelihood ratio statistic chi square=72 and 65 for larvae and nymphs, respectively, df=25, $p<0.0005$). We found no significant differences among sampling periods in the numbers of adults counted on rodents (chi square=22, df=25, $p>0.25$).

The counted numbers of larvae were significantly higher in those sample periods that occurred in months when larvae were most abundant in the studies of Randolph (coefficient=0.311, SE=0.140, p=0.027) (21). This, however, only partly explained the between-sampling variation in larval abundance: in the most parsimonious model, significantly more larvae were counted on the rodents in 1997 than in 1998 (coefficient=0.506, SE=0.114, $p<0.001$) and significantly more were counted in autumn than in other months (coefficient=0.568, SE=0.135, $p<0.001$). In the more detailed analysis, the most parsimonious model grouped the sampling periods into three significantly different levels of abundance (Table 2), with larvae being most (and similarly) abundant in sampling periods that fell during January 1997, late June and July 1997, October–November 1997, and September–December 1998 (Figure 1). Larvae were least abundant in March to early May 1997, July 1997, January–May 1998, and July and August 1998 (Figure 1). When this grouping of sample periods was included, differences between years, seasons, and the periods observed by Randolph became nonsignificant l (chi square=4.13, df =3, $p>0.2$).

For nymphs, numbers counted were significantly higher in those sample periods that included months when nymphs were most abundant in the studies of Randolph (coefficient=1.907, SE=0.281, $p<0.001$), but again this finding only partly explained the between-sampling variation in the present study. In the most parsimonious model, significantly more nymphs were counted on the rodents in winter than in other months (coefficient=2.477, SE=1.027, p=0.016), and in the more detailed analysis, the most parsimonious model grouped the sampling periods into two significantly different levels of seasonal abundance (Table 2). We found no significant difference between years in the numbers of nymphs counted on the rodents ($p>0.5$) and when nymphs were most abundant in May–September and November in both years (Figure 1). Differences between seasons and the months of nymphal abundance observed by Randolph became nonsignificant when this grouping of sample periods was included in the same model (chi square=2.73, df=2, $p>0.25$).

Low numbers of adult female ticks were counted on the rodents. Although no significant differences were found among sample periods in their abundance, the raw data suggested that adult ticks were more abundant in early summer and autumn in both years than at other times (Figure 1).

Based on these findings, scales of a seasonal likelihood that a rodent encountered a larva or nymph (three- and two-point scales for larvae and nymphs, respectively) were included as explanatory variables in the second stage of the

Table 1. Summary data of the rodents captured and the numbers of attached ticks

	Bank voles (mean per rodent)	Wood mice (mean per rodent)	Totals (ratio, vole:mouse)
No. captures	597	1,368	1,965 (1:2)
No. larvae (mean per rodent)	125 (0.21)	368 (0.27)	493 (0.25) (1:3)
No. nymphs (mean per rodent)	57 (0.10)	30 (0.02)	87 (0.04) (2:1)
No. adult ticks (mean per rodent)	19 (0.03)[a]	18 (0.01)	37 (0.02) (1:1)

[a]One bank vole carried 10 adult female ticks.

Table 2. Differences in abundance of larval and nymphal *Ixodes trianguliceps* ticks, counted on rodents of the study, in groups of sample periods deduced from the most parsimonious negative binomial regression models of the variations in tick abundance among sample periods[a]

Months	chi square	df	p value
Larvae			
Month group 2 vs. 1	5.21	1	<0.03
Month group 3 vs. 1	68.34	1	<0.001
Month group 3 vs. 2	6.25	1	<0.025
Nymphs			
Month group 2 vs. 1	53.75	1	<0.001

[a]Rodent ID was included in the models as a random effect. For larvae, month group 1 = March to early May 1997, July and December of 1997, January to late May, and July and August of 1998; month group 2 = February, August and September of 1997, and June of 1998; month group 3 = January, late May, June, October and November of 1997, and September to December 1998. For nymphs, month group 1 = January–April, September, November, and December of both 1997 and 1998; month group 2 = early May to August and October of both 1997 and 1998.

analysis. We found that heavier rodents carried greater numbers of ticks of any stage (coefficients=0.029, 0.099, and 0.170; p=0.033, 0.001, and 0.002, for larvae, nymphs, and adults, respectively; Table 3). Male bank voles carried significantly more larvae than did female bank voles and wood mice (coefficient=0.580, SE=0.279, p=0.037; Table 3). Wood mice of either sex carried significantly more larvae than female bank voles (coefficient=0.462; SE=0.229; p=0.044; Table 3). Male bank voles carried significantly more nymphs than did female bank voles or wood mice of either sex (coefficient=2.394; p=0.004; Table 3). Although some confounding between rodent mass and age category occurred, the latter was not significantly associated with variations in tick infestations (p>0.1 in all models). In models in which the scales of seasonal likelihood were excluded, almost all rodent-level factors remained significant, with the exception of the relationship between the counted numbers of larvae and rodent weight (p<0.334).

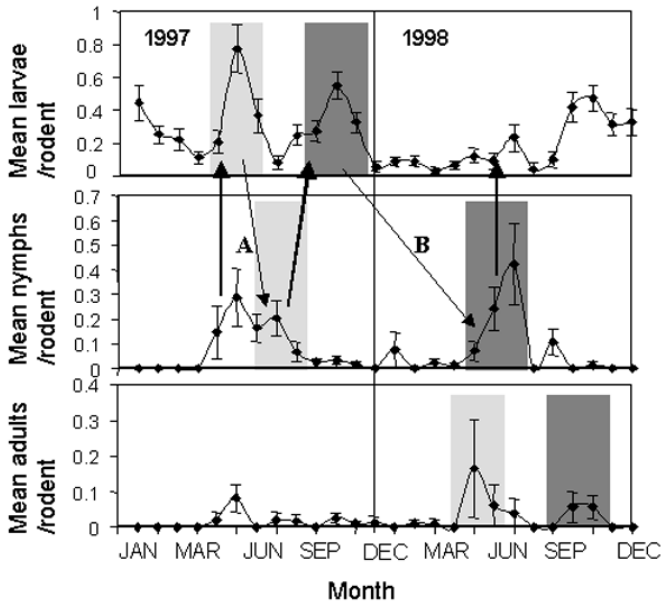

Figure 1. The mean (+/- SE) numbers of larval, nymphal, and adult *Ixodes trianguliceps* ticks counted per rodent at 4-week intervals, 1997–1998. Shaded areas of similar intensity indicate ticks of different instars that may have belonged to the same cohort, according to interstadial development times deduced by Randolph (21). Arrows indicate potential transmission cycles: bold arrows indicate potential transmission from infected nymphs to uninfected larvae by means of rodent infections. Fine arrows indicate potential transstadial transmission from infected engorged larvae to infected host-seeking nymphs. For clarity only one within-year (A) and one between-year (B) cycle involving nymphal and larval ticks are illustrated.

Accounting for the seasonal likelihood of encountering a larva or nymph and rodent weight, sex, and species, a significant, positive relationship existed between the numbers of larvae and nymphs that fed on individual rodents (coefficient=0.373, SE=0.123, p=0.002). No significant relationships existed between the numbers of adult and larval ticks nor between the numbers of adult and nymphal ticks carried by the rodents (p>0.5 for both).

A. phagocytophila Infections in Rodents

Of 1,429 rodent blood samples tested, 527 were collected from bank voles and 902 from wood mice. Of these, 26 (5%) samples from bank voles (11%; 23/201 individual animals) and 7 (0.8%) samples from wood mice (1.8%; 7/390 individual animals) were PCR positive for *A. phagocytophila*. Analysis showed the sequence of bank vole origin (GenBank accession no. AY082656) was 99.9% similar to previously published sequences (e.g., GenBank accession no. AF470701.1); the sole difference was a guanine at base 33 in place of an adenine.

Only blood from rodents captured during the periods June–November 1997, May–August 1998, and December 1998 was

Table 3. Determinants of parasitism of rodents by larval, nymphal, and adult *Ixodes trianguliceps* ticks in the most parsimonious negative binomial regression models

Variable	Coefficient	SE[b]	p value
Larvae			
Rodent body mass (g)	0.029	0.013	0.03
Month (3-point scale)	0.712	0.063	<0.001
Male bank voles vs. wood mice and female bank voles	0.580	0.279	0.04
Wood mice vs. female bank voles	0.462	0.229	0.04
Intercept	–0.702	0.468	
Nymphs			
Rodent body mass (g)	0.097	0.030	0.001
Month (2-point scale)	1.761	0.250	<0.001
Male bank voles vs. wood mice and female bank voles	2.394	0.832	0.004
Intercept	–6.590	0.915	
Adults			
Rodent body mass (g)	0.170	0.055	0.002
Intercept	–3.769	1.326	

[a]Rodent ID included as a random effect.
[b]SE, standard error.

PCR positive (Figure 2). The highest prevalence of infection among bank voles was 30% (3/10 rodents in August 1998). The highest prevalence of infection among wood mice was 7.5% (6/80 rodents in October 1997). Blood from one bank vole was PCR positive in three consecutive sample periods, and blood from another was positive in two consecutive periods. One bank vole had PCR-positive blood on two occasions but had PCR-negative blood in the intervening sample period. Most rodents, however, had PCR-positive blood at only one sample period either because they were not trapped again after being positive (1 wood mouse and 9 bank voles) or because the results were negative at the subsequent sample period (6 wood mice and 14 bank voles). Of all rodents with PCR-positive blood, four bank voles and five wood mice had been captured and had negative results by PCR at more than one subsequent sampling period (a mean of four sample periods for the wood mice and five for the bank voles).

Univariate analyses showed bank voles were significantly more likely to have been PCR positive than wood mice (odds ratio [OR] 8.15, 95% confidence interval [CI] 3.08 to 21.59, p<0.001), and rodents were significantly more likely to be PCR positive if they carried a nymph (OR 5.49, 95% CI 1.62 to 18.54, p=0.006) or carried an adult tick (OR 9.25, 95% CI 2.10 to 40.84, p=0.003). Indices of the seasonal likelihood that rodents encountered a nymphal (as described above) or an adult tick (whether or not adult ticks were observed on any rodent in that sample period) were also significantly and positively associated with the likelihood that rodents were PCR positive (OR 4.9, CI 1.54 to15.86, p=0.007; OR 8.84, CI 2.74 to 28.47, p<0.001 for nymphs and adults, respectively). In the most parsimonious multivariable model, bank voles remained significantly more likely to be PCR positive and rodents were significantly more likely to be PCR positive if they carried a nymph or carried an adult (Table 4), although there was considerable confounding between the latter two factors and the indices of seasonal likelihood that rodents encountered nymphal or adult ticks. Seven (30%) of the 23 bank voles that had PCR-positive blood on the first occasion carried a nymphal or adult tick at time of sampling or at the previous sampling. In comparison, over the whole study, 80 (13%) of 598 captured bank voles and 162 (10%) of 1,698 of all rodents captured carried a nymph or and adult tick. Two PCR-positive bank voles carried a nymph or adult at the time of sampling only, three carried a nymph or adult at the previous sampling only, and two carried a nymph or adult at both samplings. None of the PCR-positive wood mice carried nymphs or adults at either sampling. No significant associations (p>0.1) were found between detected rodent infections and the presence of fleas at the time of sampling or if they also had a history of carrying a flea at the previous sampling. None of the other variables investigated, including interactions, was significantly associated with detected infection in the rodents in any of the models (p>0.1 for all). All significant factors remained so in models in which data from repeat-positive rodents were excluded.

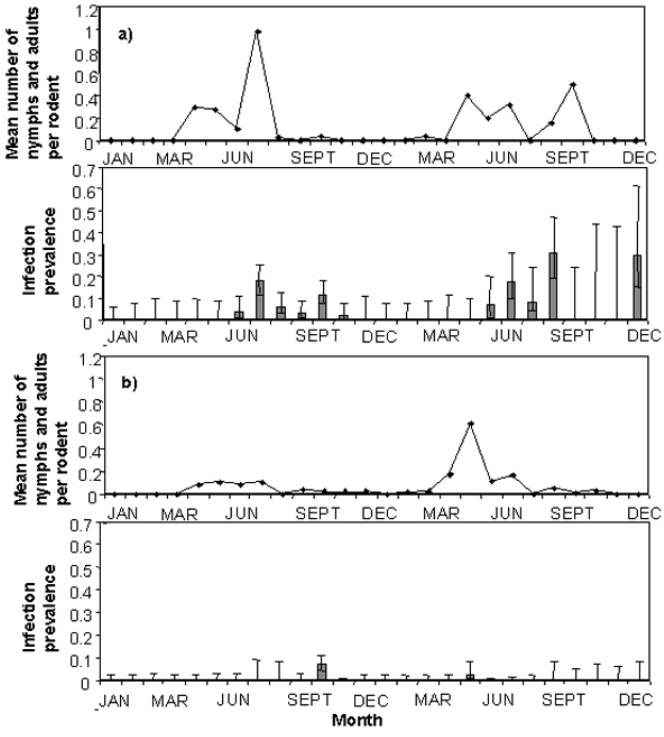

Figure 2. Prevalence of infection of *Anaplasma phagocytophila* (bar graphs +/- exact binomial errors) in blood samples collected from bank voles (graph marked a) and wood mice (graph marked b) compared to the mean monthly numbers of nymphal and adult *Ixodes trianguliceps* ticks counted per rodent at the time blood samples were collected (line graphs), 1997–1998.

A. phagocytophila Infection in *I. trianguliceps* Ticks

Of 59 *I. trianguliceps* ticks tested for *A. phagocytophila* infection, 39 were larvae, 7 were nymphs, and 13 were adult females. One (2.6%) of the larvae and 2 (15.3%) of the adult females tested positive, but none of the nymphs did. The PCR-positive larva was collected from one PCR-negative wood mouse captured in November 1997, whereas the positive adults came from one PCR-positive bank vole and one PCR-negative wood mouse captured in May 1998.

Discussion

This study provides strong evidence that *A. phagocytophila* can be maintained in a system in which woodland rodents are a dominant reservoir host species and further suggests that *I. trianguliceps* is a competent vector. In addition, this study increases our understanding of the ecology of *A. phagocytophila* in a natural system. Detectable rodent infections were highly seasonal: PCR-positive rodents were detected from summer through autumn in both years of the study, but not from January to April in either year. This seasonality in infection prevalence appears to be associated with seasonal increases in the abundance of *I. trianguliceps* nymphs and adults, but not larvae. This finding is consistent with transstadial, but not transovarial, maintenance of *A. phagocytophila* by *I. trianguliceps* as appears to be the case for its other ixodid tick vectors (3,6,12). These findings, together with the detec-

Table 4. Relationships between individual variables and polymerase chain reaction result of rodent blood samples in the most parsimonious, minimal multivariable logistic regression model[a]

Variable	Coefficient (SE)	z	p value	Odds ratio	95% CI
Bank voles vs. wood mice	1.894 (0.468)	4.047	<0.001	6.65	2.66 to 16.64
Carried a nymphal tick	1.239 (0.556)	2.228	0.03	3.45	1.16 to 10.28
Carried an adult tick	2.369 (0.735)	3.224	0.001	10.69	2.53 to 45.09

[a]SE, standard error; CI, confidence interval.

tion of PCR-positive adult ticks, suggest that *I. trianguliceps* is a competent vector of *A. phagocytophila*.

Although individuals of both the common rodent species present in this woodland were PCR positive, bank voles were significantly more likely to be so (approximately eightfold) than wood mice, and positive wood mice were only detected in 1 month in each year. These differences may have been due in part to the greater numbers of nymphs carried by bank voles (approximately fourfold) than by wood mice. Differences in the roles of these two species as hosts for different developmental stages of *I. trianguliceps* have been recorded previously (22,28). In the present study, male bank voles carried a significantly greater proportion of nymphal and larval ticks, and heavier rodents of either species were more likely to carry a tick of any stage. These relationships imply that the lower resistance of reproductively active male bank voles for ticks (including *I. trianguliceps*; [29]) could be an explanatory factor, but other behavioral characteristics (e.g., resident rather than dispersing) (30) may have made them more likely to encounter ticks. Even when interspecific differences in contact rates with nymphal *I. trianguliceps* are allowed for, however, bank voles were significantly more likely to be detected as infected with *A. phagocytophila* than were wood mice. The course of infection in the two species may, therefore, be different. Although the majority of infections appeared to be transiently detectable, as are most infections in white-footed mice (31), 3 of the 22 PCR-positive bank voles were positive for more than one 4-week period, suggesting that *A. phagocytophila* infections may be more persistent in bank voles. If bank vole infections are more persistent, then by sampling every 4 weeks we may have missed proportionally more infections in wood mice than in bank voles.

The seasonal variations in the abundance of larval and nymphal *I. trianguliceps* in this study were very similar to those observed by Randolph (21), with some differences in detail. In both studies, larvae were most abundant from August to December or January, with a shorter period of activity in early summer that varied in amplitude between years. Nymphs were most abundant from May to August with some activity continuing through autumn. Although the numbers of adults were very low in this study, their seasonal appearance also corresponded to the findings in Randolph's study. The similarities of the results of these and other studies (22–24) suggest that the observed variations in tick abundance may represent a more general seasonally repeated pattern of *I. trianguliceps* abundance, driven by the temperature-dependent tick development times deduced by Randolph (21). In this case, any cycles of *I. trianguliceps*–transmitted *A. phagocytophila* infection in the woodland may have comprised two components: a rapid within-year midsummer to early autumn component because of rapid intersstadial development of ticks influenced by higher summer temperatures (Figure 1A), and a longer component from autumn one year to spring/summer the next year because of lower intersstadial development rates influenced by low winter temperatures (Figure 1B).

The relatively short duration of *A. phagocytophila* infections in these rodents may have more general implications for the nature of endemic cycles involving rodents and the occurrence of rodent-derived infected ticks to which humans may be exposed in Europe. First, infected ticks are more likely to have been the most important overwinter reservoir of *A. phagocytophila* than the rodents, particularly as the tick development period over the winter may have been at the limit of the life expectancy of the rodents (16), a factor that may limit overwinter survival of rodent *Borrelia burgdorferi* sensu lato infections in some foci in northern Europe (32). This implication contrasts with the role of some rodent reservoirs such as the dusky-footed wood rat (*Neotoma fuscipes*) in the United States, which can remain PCR-positive for more than 1 year and act as an overwinter reservoir of infection (33,34).

Second, in experimentally infected rodents, efficient *A. phagocytophila* transmission to ticks occurs for only a short period because transmission is inhibited by the onset of acquired host resistance (35), a characteristic shared by tick-borne encephalitis virus (TBEV) (36) but not *B. burgdorferi* s.l. infections in rodents (37). Because of such short periods of infectivity, the occurrence of endemic cycles of TBEV depends on coincident seasonal activities of different *I. ricinus* tick instars, coupled with aggregation of ticks of more than one instar on a small proportion of the rodent population (38). In our study, seasonal activities of larvae, nymphs, and adults were partly coincident, the distribution of ticks among rodents was highly aggregated, and larvae and nymphs co-fed on a small proportion of the population (particularly bank voles), conditions that may have enhanced transmission of short-lived *A. phagocytophila* infections. Such conditions may also promote co-feeding transmission of *A. phagocytophila* (35): the detection of infection in a larval tick collected from a PCR-negative rodent may suggest that this transmission route occurs naturally on rodents. European rodents may, therefore, be important reservoirs of *A. phagocytophila*, but the risk of rodent-derived human infections may be constrained by fac-

tors that also constrain the risk of TBEV infection when endemic cycles are maintained by exophilic *I. ricinus* ticks alone.

In this study, cycles of infection were maintained even though the mean numbers of *I. trianguliceps* per rodent were very low (never >1 for any instar). Therefore, when *I. trianguliceps* and *I. ricinus* ticks are sympatric, *I. trianguliceps*–driven endemic cycles may provide an efficient reservoir from which *I. ricinus* may acquire infections from rodents, thus increasing the risk of rodent-derived human infections. In this respect, rodent-*trianguliceps* cycles may have a similar role in *A. phagocytophila* maintenance in Europe to that of cycles maintained in dusky wood rats and nidicolous *I. spinipalpis* ticks in the western United States where sympatric exophilic *I. pacificus* ticks are the bridge vector transmitting infections to humans and domesticated animals (33). This maintenance system may be particularly important in Great Britain, where woodland rodents carry few nymphal or adult *I. ricinus* (39) and, in the absence of *I. trianguliceps*, rodent *A. phagocytophila* infections may be uncommon (11). Further studies are required to test these hypotheses and investigate the role of rodent-derived *A. phagocytophila* in human infections in Europe.

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Two Epidemiologic Patterns of *Norovirus* Outbreaks: Surveillance in England and Wales, 1992–2000

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In the period 1992–2000, the Public Health Laboratory Service Communicable Disease Surveillance Centre collected standardized epidemiologic data on 1,877 general outbreaks of *Norovirus* (formerly "Norwalk-like virus") infection in England and Wales. Seventy-nine percent of general outbreaks occurred in health-care institutions, i.e., hospitals (40%) and residential-care facilities (39%). When compared with outbreaks in other settings, those in health-care institutions were unique in exhibiting a winter peak ($p<0.0001$); these outbreaks were also associated with significantly higher death rates and prolonged duration but were smaller in size and less likely to be foodborne. These data suggest that *Norovirus* infection has considerable impact on the health service and the vulnerable populations residing in institutions such as hospitals and residential homes. A distinct outbreak pattern in health-care institutions suggests a combination of host, virologic, and environmental factors that mediate these divergent epidemiologic patterns.

Recent population-based studies have shown that Noroviruses ([NVs] formal name: *Norovirus*; formerly "Norwalk-like viruses") are the most commonly identified cause of infectious intestinal diseases in Western European communities (1,2). These viruses account for an estimated 6% and 11% of all infectious intestinal diseases in England and the Netherlands, respectively (1,2) and for an estimated 23 million cases of NV in the United States each year (3). NVs are also the most common cause of outbreaks of infectious intestinal diseases in Western Europe and North America (3–7).

Three factors contribute to the considerable impact of disease caused by NV: a large human reservoir of infection (2,8), a very low infectious dose (9), and the ability to be transmitted by a variety of routes. Person-to-person spread by means of the fecal-oral route or aerosol formation after projectile vomiting is the most commonly recognized mode of transmission (4,10), although foodborne (3,11) and waterborne (12–14) transmission are also well documented.

Gastroenteritis caused by NV is mild and self-limiting in the absence of other factors. Kaplan et al. and others have proposed that NV outbreaks can be recognized on clinical symptoms (short duration and incubation) and epidemiology (high attack rates and high frequency of vomiting) alone (4,15–17). Unlike rotavirus, NVs affect all age groups (2,8) The highest incidence is in children <5 years of age (2,18), but the greatest impact of NV is probably an economic one among the elderly in health-care institutions (4,6,19,20).

We describe the epidemiology of NVs in different outbreak settings. The data we present were collected by routine surveillance of general outbreaks of infectious intestinal diseases in England and Wales from 1992 to 2000 (4,21). Laboratory report surveillance of NV has been shown to be subject to a high degree of underascertainment (8) and age bias (4). Therefore, routine laboratory reporting of cases does not serve as a reliable sample for illness due to NV. For this reason, we describe only outbreak data.

Methods

Since January 1992, the Public Health Laboratory Service Communicable Disease Surveillance Centre has operated a standardized comprehensive surveillance system for general outbreaks of infectious intestinal diseases (see Appendix). The details of how this system operates are described elsewhere (4,21). In 1995 and 1996, the Public Health Laboratory Service instituted an active reporting program for outbreaks of NV through the Electron Microscopy Network. Ten electron microscopy units, representing the principal regional diagnostic centers for viral gastroenteritis in England, reported to the Centre all general outbreaks for which clinical specimens had been submitted. These reports were then integrated into the existing outbreak surveillance system, and standardized epidemiologic data were sought from investigating public health physicians. The public health physicians contacted were asked to return completed questionnaires when investigations were concluded. Data from these questionnaires were entered and stored on an Epi Info 6.0 database (23).

Statistical Analysis

We used the statistical software package STATA 6.0 for these analyses (24). Chi-square tests were used to compare proportions, and the Student t test was used to compare means. Data on persons affected and duration of outbreaks were observed to follow a non-normal distribution. Therefore, a natural log transformation was performed on the persons affected and duration of outbreak data to normalize the distribution of variables and satisfy the normality assumption for the t test (25). A reverse natural log transformation was then performed; results are presented as geometric means.

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Results

Completed outbreak questionnaires were returned for 5,241 general outbreaks occurring from January 1, 1992, to December 31, 2000 (response rate 73%). Laboratory confirmation of NV was recorded for 1,877 (36%) outbreaks (Figure 1). The median number of laboratory-confirmed cases in NV outbreaks was 2 (range 1–36). Another 731 outbreaks (14% of all outbreaks) were suspected of being caused by viral agents; 8 outbreaks were attributed to NV plus other pathogens; these outbreaks were excluded from these analyses.

Settings

Information on setting was available for every NV outbreak (n=1,877). The most common settings were health-care institutions: 754 (40%) outbreaks occurred in hospitals and 724 (39%) in residential-care facilities. Information on the type of unit affected was available for 648 (86%) of 754 hospital outbreaks and 190 (26%) of the 724 in residential-care facilities. NV infection was centered on elderly care and geriatric units in 251 (39%) of 648 hospital outbreaks and 169 (89%) of 190 residential home outbreaks. A total of 147 (7.8%) outbreaks occurred in hotels, 73 (4%) occurred in schools, and 105 (6%) were linked to food outlets (Appendix). Seventy-four outbreaks (3.9%) occurred in other settings such as private homes, holiday camps, and military bases.

Illness and Death

A total of 57,060 people were affected in the 1,877 NV outbreaks. After excluding hospital outbreaks (n=711), we recorded 128 hospitalizations (case-hospitalization rate = 33/10,000 cases) from 52 outbreaks (mean hospitalizations per outbreak 0.19; range 0–38). Forty-three deaths (case-fatality rate 7.5/10,000 cases) occurred in 38 outbreaks (mean deaths per outbreak 0.07; range 0–2); all were associated with outbreaks in hospitals (24 deaths) and residential-care facilities (19 deaths).

Time Trends and Seasonality

Reports of NV outbreaks peaked in 1995 (367 outbreaks) (Figure 1), falling to 139 outbreaks in 1997. Since then, outbreaks have steadily increased; 281 outbreaks were reported in 2000. Since 1995, outbreaks have shown a strong seasonal peak (Figure 1). Outbreaks begin increasing in September and peak in the months of January, February, and March. Outbreaks in hospitals and residential facilities occur more commonly in the 6 months from November to April than the rest of the year (994/421; ratio 2.36) (Figure 2). Outbreaks in other settings display no winter peak (189/205; ratio 0.92). This difference in the seasonality between outbreaks in health-care institutions and those in other settings is significant (χ^2 51.1, $p<0.0001$)

Mode of Transmission

The reported modes of transmission were as follows (Table 1): person to person in 1,599 (85%) outbreaks; foodborne in 93 (5%) outbreaks; foodborne followed by person-to-person spread in 91 (5%) outbreaks; waterborne in 1 outbreak; unknown in 92 (5%) outbreaks.

Person-to-person spread was reported in 716 (95%) of the 754 hospital outbreaks. This figure was a significantly higher proportion than observed in food outlets (22%; 23/105 [χ^2 551.3; $p<0.0001$], hotels (64%; 94/147 [χ^2 175.9; $p<0.0001$], schools (89%; 65/73 [χ^2 27.6; $p<0.0001$]), or residential facilities (91.0%; 658/723 [χ^2 13.9; $p=0.0002$]). Food outlets were the only setting where foodborne transmission predominated (67%; 70/105).

Person-to-person outbreaks occurred more commonly from November to April than in the rest of the year (1,020/514; ratio 1.98). Foodborne outbreaks showed a significantly weaker seasonality (105/73; ratio 1.43) than person-to-person outbreaks (χ^2 3.99; $p=0.05$).

Food Vehicles

Specific vehicles were implicated in 72 (39.1%) of the 184 NV outbreaks reported to be transmitted by food. In 12 of these outbreaks, multiple food vehicles were reported, for a total of 86 implicated items. A wide range of food types were reported as vehicles of infection, including oysters, salad vegetables, poultry, red meat, fruit, soups, desserts, and savory snacks. The evidence implicating these food vehicles included

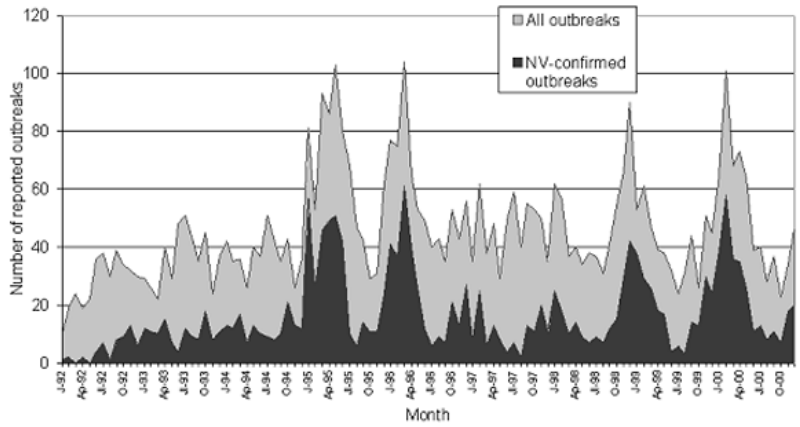

Figure 1. Seasonality of all outbreaks and confirmed *Norovirus* outbreaks, England and Wales, 1992–2000.

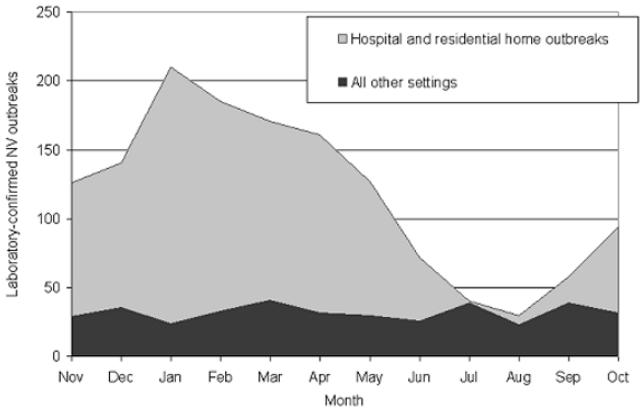

Figure 2. Seasonality of *Norovirus* outbreaks in residential homes and hospitals compared to all other settings, England and Wales, 1992–2000.

cohort studies (55%; 47/86), case-control studies (8%; 7/86), and microbiologic studies (6%; 5/86) (Table 2). Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to confirm viral contamination in oysters in all five outbreaks where microbiologic evidence was reported.

Contributory Factors

Contributory factors were reported in 113 (61%) of the 184 foodborne outbreaks. Infected food handlers were more commonly identified in food-related NV outbreaks (32%; 58/184) than in those caused by other pathogens (9%; 164/1750) (χ^2 80.39; p<0.0001). Contamination by an infected food handler was reported less frequently in outbreaks involving oysters than other foods (oysters 0%, other foods 47%; χ^2 14.69; p<0.0001). Cross-contamination was also reported less frequently in outbreaks involving oysters than other foods (oysters 5%, other foods 17%; χ^2 3.35; p=0.07).

Duration

The median duration of outbreaks was 8 days (range 1–139 days). By setting, data on the duration of outbreaks were right-skewed since some outbreaks persisted for exceptionally long periods. The following results are therefore presented as geometric means. The duration of hospital outbreaks (8.8 days; 95% confidence intervals [CI] 8.4 to 9.3) was greater than those in food outlets (3.3 days; 95% CI 2.8 to 3.8; t = –12.699; p<0.0001) and hotels (4.3 days; 95% CI 3.6 to 5.1; t = –7.025; p<0.0001). However, the duration of hospital outbreaks and those in residential facilities did not differ significantly (8.7 days; 95% CI 8.1 to 9.4; t = –0.321; p=0.7) or schools (8.1 days; 95% CI 6.8 to 9.7; t = –0.879; p=0.4) (Table 3).

Numbers of Persons Affected

The median number of persons affected per outbreak was 21 (range 2–1,200).

Data on the number of people affected in outbreaks were right-skewed since a number of outbreaks were exceptionally large. The following results are therefore presented as geometric means. The number affected in hospital outbreaks (17.5; 95% CI 16.4 to 18.5) was significantly lower than for other settings (geometric means 21.5 to 26.5; Table 3).

Discussion

Examination of the features of NV outbreaks by setting reveals that outbreaks in health-care facilities have a distinctive epidemiologic profile. When compared with outbreaks in other settings, those in health-care institutions were unique in exhibiting a winter peak; they were also associated with higher death rates and prolonged duration but were smaller in size and were less likely to be foodborne. School outbreaks shared some but not all of the features that characterize outbreaks in health-care institutions.

Several epidemiologic and biologic reasons may contribute to the divergent seasonality. The respiratory infections season, which increases activity in health-care institutions, occurs concurrently with the peak in NV outbreaks in these facilities. Greater admission of patients in hospitals increases both the population at risk and the opportunities for NV to be introduced. An increase in transfers of people between residential-care facilities and hospitals also facilitates the movement of viruses between institutions. Populations in health-care facilities differ from the rest of the population in that they require nursing care. Health-care settings are semi-closed environments where patients and residents are subject to person-to-person spread and potentially contaminated environments.

Biologic differences between strains may also result in different clinical patterns. NVs from outbreaks in health-care institutions have less genetic diversity compared with those

Table 1. Primary modes of transmission of *Norovirus* outbreaks, England and Wales, 1992–2000

Setting of outbreak	Foodborne[a]	Person to person[a]	Other/unknown[a]	Total
Hospital	10 (1.3)	716 (95.0)	28 (3.7)	754
Residential facilities	33 (4.5)	658 (91.0)	32 (4.4)	723
School	4 (5.5)	65 (89.0)	4 (5.5)	73
Food outlet	70 (66.7)	23 (21.9)	12 (11.4)	105
Hotel	42 (28.6)	94 (63.9)	11 (7.5)	147
Other	25 (33.8)	43 (58.1)	6 (8.1)	75
Total	184 (9.9)	1,599 (85.2)	93 (5.0)	1,877

[a]No. of outbreaks (% of all outbreaks in setting).

Table 2. Microbiologic and statistical evidence of foods implicated in outbreaks of *Norovirus*, England and Wales, 1992-2000[a]

Implicated food	Microbiologic evidence	Cohort study	Case-control study	Any evidence	Total no. of outbreaks in which food vehicle implicated
Oysters	5 (25%)[b]	9 (45%)	0	14(70%)	20
Poultry	0	6 (67%)	0	6 (67%)	9
Meat	0	3 (60%)	0	3 (60%)	5
Fish	0	3 (50%)	1 (16%)	4 (67%)	6
Salads and vegetables	0	10 (59%)	3 (18%)	13 (76%)	17
Other items	0	16 (55%)	3 (10%)	19 (65%)	29
Total	5 (6%)	47 (55%)	7 (8%)	59 (68%)	86

[a]Percentages represent outbreaks with evidence per total outbreaks where food vehicle was implicated.
[a]In certain outbreaks more than one form of evidence was reported.

from other settings (26) or sporadic cases (7), and certain variants are more commonly found in health-care facilities than in other settings (26). Thus, the strong seasonality in health-care institutions may be the result of complex interaction between host, pathogen, and environment. If and how these factors contribute to the divergent patterns of health-care-associated and community outbreaks are unknown, but we believe that our findings warrant focused investigation in the UK and elsewhere.

The observation that a hospitalization was associated with 1 in every 40 outbreaks and a death with 1 in every 50 outbreaks calls into question the belief that NV gastroenteritis is a trivial disease. Although we have no information about the other health conditions of patients who were hospitalized or died, these figures are generated from laboratory-confirmed outbreaks. Previous estimates generated by Mead et al. (which were derived from Mounts et al.) were based on the assumption that NV causes a certain proportion of gastroenteritis hospitalizations and deaths (11%), an assumption that was not based on diagnostic results (3,27).

Deaths were only reported from outbreaks in health-care institutions. The populations in these institutions differ from those found in other settings by virtue of their greater age or presence of other underlying diseases. While NV infection is not likely the principal cause of death in most cases, this infection might constitute an additional burden on patients already weakened by other conditions and thus become an important contributory factor. In hospital outbreaks, attack rates among staff are similar to those among patients (4,28), suggesting that health status is not related to acquisition of disease but to severity of outcome. Therefore, efforts to control NV infection should be directed towards vulnerable persons who already require nursing care because of illness or injury.

The only settings in which foodborne transmission predominated were food outlets. That setting was the only category in which the purchase or consumption of food was the

Table 3. Outbreak characteristics compared by setting of outbreak, England and Wales, 1992–2000

Setting	Median (days)	N	Geometric mean of duration (days)(95% CI)[a]	t test	p value
Duration of outbreaks					
Hospital	8	679	8.8 (8.4 to 9.3)		
Residential facilities	9	664	8.7 (8.1 to 9.4)	–0.321	0.73
School	8	63	8.1 (6.8 to 9.7)	–0.879	0.40
Food outlet	3	94	3.3 (2.8 to 3.8)	–12.699	<0.0001
Hotel	5	133	4.3 (3.6 to 5.1)	–7.025	<0.0001
Other	4	69	4.3 (3.6 to 5.1)	–8.043	<0.0001
All settings	8	1,702	7.7 (7.5 to 8.0)		
Numbers affected per outbreak					
Hospital	17	751	17.5 (16.4 to 18.5)		
Residential facilities	23	723	21.5 (19.8 to 23.3)	4.895	<0.0001
School	24	73	24.9 (20.5 to 30.3)	3.594	<0.0001
Food outlet	23	104	23.4 (19.8 to 27.6)	3.444	0.001
Hotel	29	147	26.5 (23.0 to 30.6)	5.729	<0.0001
Other	29	74	24.5 (20.2 to 29.7)	3.432	0.001
All settings	21	1,872	20.3 (19.7 to 21.1)		

[a]CI, confidence interval.

main factor linking at-risk populations. In other settings, living, working, or recreational areas were shared by at-risk populations for varying lengths of time, thus increasing the opportunities for person-to-person spread. Even in those instances where foodborne transmission initiated an outbreak within a health-care institution, high levels of person-to-person spread usually followed. Therefore, prolonged levels of contact between persons in semi-closed institutions such as hospitals, residential-care facilities, and schools facilitate person-to-person spread to an extent not seen in other settings, which in turn leads to more prolonged outbreaks. However, schools differ from health-care institutions in terms of the seasonality and duration of NV outbreaks. In this respect, schools are more like hotels, food outlets, and other settings.

The number of affected persons was smaller in hospital outbreaks than in all other settings. This finding may reflect the lack of a universally employed definition of the spatial boundaries of an outbreak. In some hospitals, each unit affected was reported as a separate outbreak, resulting in smaller but more numerous outbreaks. In addition, cases that occur in institutions are more easily recognized as part of an outbreak than cases in open settings or the community. Thus, smaller outbreaks occurring in open settings might not be recognized or reported to investigating agencies.

The peak in recorded outbreaks seen in the winter of 1995–1996 can largely be seen as a consequence of enhanced surveillance through the development of the Public Health Laboratory Service electron microscopy network. However, there are anecdotal reports of an increase in workload in these laboratories, and other countries also recorded an increase in NV activity during the same period (11). The steady increase of reports from 1998 to 2000 may be due to increased awareness, increasing use of the molecular diagnostics RT-PCR and enzyme immunoassays, or a real increase in the occurrence of outbreaks.

Biases in different surveillance systems partly explain the wide variation in estimates of the levels of foodborne transmission in NV outbreaks. The data presented in this report suggest foodborne transmission in 10% of outbreaks in England and Wales. Estimates in Sweden (16%) (6), the Netherlands (17%) (11), and the United States (40%) (3) were all higher; however, figures from these countries are derived from much smaller datasets. In the United States, foodborne outbreaks were more likely to be reported because surveillance may be focused on detecting foodborne outbreaks (3).

The data sources that contribute to a surveillance system are a key factor affecting the estimate of the importance of foodborne transmission. In England and Wales, surveillance is broad-based and collects reports on outbreaks spread by all modes of transmission from a range of public health professionals such as physicians, environmental health officers, and diagnostic laboratories. By contrast, FoodNet, a U.S. network, is designed to detect foodborne infections (29). Since hospitals in England and Wales are in the public sector, they might be expected to readily report outbreaks to the national surveillance scheme. However, by this logic, residential homes (which are privately operated) would not be expected to report outbreaks since they might be under commercial pressures to keep information on infection confidential. The fact that nearly as many outbreak reports came from residential homes as from hospitals in the survey period suggests that reporting predominantly from the public, not the private sector, is not the case. The biases on a passive surveillance system are multiple and cannot be expected to act in only one direction.

The importance of NV as a cause of gastroenteritis outbreaks in U.S. nursing homes has been demonstrated by Green et al. (30), although the role of this virus in hospital settings has not. Aside from bias, other reasons such as variability in infection control practices in different health-care systems could result in a real difference in the importance of foodborne transmission or transmission in health-care facilities. Although NV has been estimated to cause 67% of all such illness caused by identified microbial agents (3), only 5% of public health professionals considered this pathogen to be "one of the three most common pathogens causing foodborne illness in the United States" (31); this lack of awareness probably affects outbreak investigation.

The link between oysters and NV infection is well described (32–35). These filter feeders become contaminated during growth or transport in sewage-contaminated water (33), unlike other food products that become contaminated by an infected food handler or cross-contamination. However, oysters were implicated as the vehicle of infection in <25% of the foodborne outbreaks, and a wide range of other vehicles were also reported. The greatest proportion of these outbreaks was attributed to ready-to-eat foods contaminated by infected food handlers. In the absence of a known zoonotic reservoir for NVs, the main reservoir of infection appears to be humans. Thus, reducing the incidence of foodborne NV infection requires interventions designed to prevent infected persons from contaminating prepared food and sewage from contaminating oyster beds.

These data, which show NV as the causative agent in 36% of outbreaks, support previous reports that NVs are the most common cause of infectious intestinal diseases in industrialized nations (6,11,20,36). NV accounts for a substantial extent of disease and potential economic loss, particularly to the health service where a large proportion of outbreaks occur. Wider consequences include ward closure, delayed discharge, and postponement of operations. Although NVs cause mild symptoms in healthy adults, the consequences of infection in vulnerable populations may be more serious. Considering that the populations of developed countries are aging, ensuring high levels of infection control in institutions caring for vulnerable groups is important.

Conclusions

These analyses demonstrate the value of maintaining standardized outbreak surveillance over an extended period. By examining the epidemiologic characteristics of general out-

breaks of NV by setting, we demonstrated that this pathogen is not merely an extremely common cause of infectious intestinal disease but that its effects vary widely according to the population at risk. Within health-care institutions, NV contributes to substantial illness and is associated with substantial numbers of deaths. The elucidation of a distinct outbreak pattern that is characteristic of health-care institutions suggests that a combination of host, virologic, and environmental factors mediate these divergent epidemiologic patterns. Focused research studies need to be developed to investigate the population as well as the microbiologic and behavioral processes that might explain these observations. In addition, population-based studies incorporating virus typing are required to gain a deeper understanding of the epidemiology of sporadic NV infection in the wider population. Such studies are a prerequisite to the development of firm evidence-based and targeted control strategies.

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Mr. Lopman is an epidemiologist at the Gastrointestinal Diseases Division of the Public Health Laboratory Service Communicable Disease Surveillance Centre. His work focuses on the epidemiology of viral gastroenteritis in health-care settings and coordination of epidemiologic surveillance for the Foodborne Viruses in Europe Consortium.

Appendix. Surveillance and analysis definitions

Outbreak: an incident in which two or more people, thought to have a common exposure, experience a similar illness or proven infection, at least one of them being ill (22).

General outbreak: an outbreak that affects members of more than one household, or residents of an institution (36).

General outbreak of *Norovirus:* a general outbreak in which *Norovirus* is determined to be the causative agent by electron microscopy, RT-PCR, or enzyme immunoassay in one or more affected persons.

Residential facilities: includes residential homes, which provide some assistance in day-to-day living, and nursing homes, which provide care for persons whose infirmity or illness requires nursing care on a regular basis.

Food outlets: commercial food retailers including restaurants, pubs, bars, cafeterias, mobile food vendors, and caterers.

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Epidemic Hand, Foot and Mouth Disease Caused by Human Enterovirus 71, Singapore

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Singapore experienced a large epidemic of hand, foot and mouth disease (HFMD) in 2000. After reviewing HFMD notifications from doctors and child-care centers, we found that the incidence of HFMD rose in September and declined at the end of October. During this period, 3,790 cases were reported. We performed enteroviral cultures on 311 and 157 specimens from 175 HFMD patients and 107 non-HFMD patients, respectively; human enterovirus 71 (HEV71) was the most frequently isolated virus from both groups. Most of the HFMD patients were ≤4 years of age. Three HFMD and two non-HFMD patients died. Specimens from two HFMD and both non-HFMD patients were culture positive for HEV71; a third HFMD patient was possibly associated with the virus. Autopsies performed on all three HFMD and one of the non-HFMD case-patients showed encephalitis, interstitial pneumonitis, and myocarditis. A preparedness plan for severe HFMD outbreaks provided for the prompt, coordinated actions needed to control the epidemic.

Hand, foot and mouth disease (HFMD) is typically a benign and common self-limiting childhood disease, characterized by rapidly ulcerating vesicles in the mouth and lesions, usually vesicular, on the hands and feet (1). Lesions also frequently occur on the buttocks, but other parts of the body are usually not affected (2). HFMD is caused by a few serotypes of enteroviruses, most frequently coxsackie virus A16 (CAV16) and human enterovirus 71 (HEV71). Other viruses associated with the syndrome are coxsackie virus A (CAV) 4, 5, 9, and 10 and coxsackie virus B (CBV) 2 and 5 (1). The first recognized HFMD outbreak in Singapore occurred in 1970; the etiologic agent was unknown (3). Two other outbreaks were reported in 1972 and 1981 and involved 104 and 742 persons, respectively; in both outbreaks, CAV16 was implicated as the cause (4,5).

After epidemics of HFMD in Sarawak, East Malaysia, and the Malaysian Peninsula in 1997 (6–8) and Taiwan in 1998 (9,10), which were associated with complications of encephalitis, myocarditis, and death, a system of surveillance for the disease, based on notifications from child-care centers, was implemented in Singapore in April 1998. Reporting the disease was made legally mandatory on October 1, 2000. Concurrent with the intensified surveillance, an interministry and interhospital HFMD Task Force, composed of representatives from the Ministries of Health, Environment, Education, and Community Development and Sports, as well as virologists and pediatricians, was created in 1998 to formulate a preparedness response plan to monitor and manage severe HFMD outbreaks in Singapore.

At the end of 2000, Singapore experienced its largest known outbreak of HFMD. After media reports in September of HFMD-related deaths in Singaporean children, many patient samples were sent for virologic investigation to the Virology Laboratory of the Department of Pathology, Singapore General Hospital. Because the Virology Laboratory receives all requests for virus culture or enterovirus typing from the entire country, it was the repository of information on virtually all laboratory investigations during the HFMD epidemic.

We describe the epidemiologic, virologic, and pathologic features of this epidemic.

Methods

In this study, we used a case definition for HFMD of fever, accompanied by oral ulcers and a rash, maculopapular or vesicular, on the hands and feet, with or without buttock involvement. We reviewed records of HFMD notifications to the Ministry of the Environment for the incidence and trend of the disease. All children with suspected HFMD reported by preschool centers were examined, and the cases were certified by family physicians. Cases reported by parents or school principals and teachers were excluded unless a medical certificate from a physician verified them. At the same time, Ministry of the Environment nurses conducted active case detection in both preschools and primary schools. All case-patients were identified by a unique national registration identification number, and duplicate reports were eliminated by the computer.

Data obtained from samples received by the Virology Laboratory at Singapore General Hospital for enterovirus isolation during the epidemic were also analyzed. In addition to stool samples, samples included swabs of vesicles, mouth, throat, rectum, and ulcers, and samples from the brain, heart, lung, tonsil, lymph node, spleen, and intestine of those with fatal disease. The samples were added into HeLa, HEp-2, human embryonic lung fibroblasts, and human rhabdomyosarcoma

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cells. The cultures were incubated at 36°C and examined daily for cytopathic effects for 21 to 28 days.

Enteroviruses cultured from the samples were typed by micro-neutralization tests (11) by using Lim Benyesh-Melnick A-H equine antiserum pools (World Health Organization, Statens Serum Institut, Copenhagen, Denmark), equine antiserum pools (Rijksinstituut voor Volksgesondheid en Milieuhygiene, Bilthoven, the Netherlands), rabbit 385JS HEV71-specific polyclonal antiserum (Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia), and rabbit or monkey antisera specific for CAV serotypes (National Institutes of Health, Bethesda, MD). Nonenteroviruses that produced cytopathic effects characteristic of *Cytomegalovirus* (CMV) or herpes simplex virus were identified by immunofluorescence assay as described (12), by using mouse monoclonal antibodies to CMV (Bartels CMV DFA kit, Trinity Biotech plc, Wicklow, Ireland) and herpes simplex virus (MicroTrak HSV1/HSV2 culture identification/typing test, Trinity Biotech plc). When the presence of rhinovirus was suggested from the cytopathic effects, the virus was identified by the acid lability test (13). Autopsies were performed on four patients who died, and tissue samples were subjected to virus cultures.

Results

Cases

The number of notifications of HFMD cases to the Ministry of the Environment increased in early September 2000 (Figure 1). The incidence peaked at 308 cases per day on October 10 and decreased to 10 cases per day by October 28. Hospital and general practice physicians and preschool-center operators reported a total of 3,790 cases during these 2 months.

During the epidemic, 311 samples from 175 clinically diagnosed HFMD case-patients were submitted for virus culture. A total of 138 (78.8%) of these patients were ≤4 years of age, with 12 (6.9%) >10 years of age, the oldest being 71 years old (Table 1). The male-to-female ratio was 1.7:1.

At least one virus was isolated from 147 (47.3%) of the samples collected from 104 (59.4%) HFMD patients, including 2 of 3 who died. Almost all (91.5%) of these patients were ≤5 years of age with the peak incidence at 1 year (Table 1). A 21-year-old woman was the only patient >10 years of age to yield a virus, identified as HEV71, from vesicles on her hands and feet.

HEV71 was the most commonly isolated virus, detected in 76 (73.1%) of 104 case-patients (Table 2). Three of these patients had a second virus isolated concurrently: echovirus (EV) 25, *Rhinovirus*, and CMV. Other enteroviruses were isolated in 24 (23.1%) of samples from case-patients. Five cases of CAV16 were identified, as well as four cases each of CAV6, CAV24, and EV18; three cases of CAV10; and one case each of CAV4, CBV3, CBV4, and CBV5. Four patients (3.8%) tested positive for nonenteroviruses; CMV was isolated from their mouth and from throat swabs.

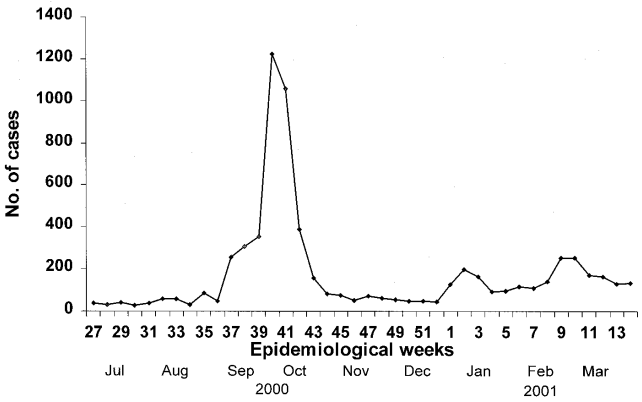

Figure 1. Number of cases of hand, foot and mouth disease reported to the Singapore Ministry of Environment as surveillance for the disease, July 2000–March 2001. Each epidemiologic week begins on Sunday. Mandatory reporting of the disease began on October 1, 2000.

The two patients with fatal HFMD, from whom HEV71 was isolated, were siblings, a 14-month-old girl and her 2-year-old brother. The girl was admitted to the hospital with fever, rashes on the hands and feet, and oral ulcers of 3 days' duration. Progressive hemodynamic instability, oliguria, metabolic acidosis, and hyperkalemia developed; despite intensive care and resuscitative efforts, she died on day 2 after admission. At autopsy, her lungs showed acute pulmonary edema, acute intraalveolar hemorrhage and diffuse alveolar damage associated with interstitial lymphocytic infiltrates, extensive hyaline membrane formation, patchy atelectasis, and focal pneumocyte desquamation and hypertrophy (Figure 2). Samples from her brain tissue showed lymphocytic leptomeningitis with widespread perivascular cuffing by lymphocytes and plasma cells within the cortex and white matter (Figure 3). The pons, in particular, showed evidence of encephalitis, associated with localized perivascular hemorrhage, focal neuronal necrosis, and microglial reaction (Figure 4). Features of myocarditis were observed; the myocardium showed occasional

Table 1. Age distribution of clinical and virus-positive hand, foot and mouth disease patients

Age (yrs)	No. clinical cases (%)	No. virus-positive cases (%)
<1	16 (9.1)	11 (10.6)
1	44 (25.2)	32 (30.8)
2	41 (23.4)	24 (23.1)
3	21 (12.0)	14 (13.5)
4	16 (9.1)	8 (7.7)
5	8 (4.6)	6 (5.8)
6	6 (3.4)	4 (3.9)
7	4 (2.3)	1 (0.9)
8	3 (1.7)	2 (1.9)
9	1 (0.6)	0 (0.0)
10	3 (1.7)	1 (0.9)
>10	12 (6.9)	1 (0.9)
Total	175 (100.0)	104 (100.0)

Table 2. Viruses isolated from HFMD cases during the epidemic[a]

Virus	No. HFMD patients (%)	No. non-HFMD patients (%)
HEV71	76 (73.1)	5 (29.4)
HEV71 only	73	5
HEV71 + EV25	1	0
HEV71 + *Rhinovirus*	1	0
HEV71 + CMV	1	0
CAV4	1 (1.0)	0
CAV6	4 (3.8)	0
CAV10	3 (2.9)	1 (5.9)
CAV16	5 (4.8)	0
CAV24	4 (3.8)	3 (17.6)
CBV3	1 (1.0)	1 (5.9)
CBV4	1 (1.0)	2 (11.8)
CBV5	1 (1.0)	3 (17.6)
EV18	4 (3.8)	0
CMV	4 (3.8)	0
Herpes simplex virus 1	0	2 (11.8)
Total	104 (100.0)	17 (100.0)

[a]HFMD, hand, foot and mouth disease; HEV, human enterovirus; EV, echovirus; CMV, *Cytomegalovirus*; CAV, coxsackie virus A; CBV, coxsackie virus B.

interstitial infiltrates of lymphocytes and plasma cells associated with focal myonecrosis (Figure 5). HEV71 was isolated from samples taken from the brain, tonsils, intestines, stools, and throat and from swabs of the mouth and rectum. Viral cultures of the lung, heart, and spleen were negative.

This patient had two older brothers. The 2-year-old brother showed an almost identical clinical course. After 3 days of fever, rash on the hands and feet, and oral ulcers, he too deteriorated under intensive care and died about 2 hours after his sister, within 24 hours of hospitalization. Autopsy findings were similar, showing evidence of acute interstitial pneumonitis, pulmonary edema, encephalitis (including focal neuronal necrosis of the pons), and myocarditis. HEV71 was isolated from postmortem specimens of the tonsils and intestines. Viral cultures of the brain, heart, lungs, spleen, and lymph nodes showed negative results.

The 5-year-old brother of these patients was also admitted to the hospital at the same time with rashes and mouth ulcers, which in his case did not progress to severe disease. However, because of the rapid deaths of his siblings, intravenous immunoglobulin was administered prophylactically 2 days after hospital admission. After two doses, vomiting and a headache developed. A computed tomographic scan of his head showed normal results, and after due assessment, the headache and vomiting were attributed to the intravenous immunoglobulin, which was then stopped. The symptoms ceased and the boy recovered well. HEV71 was cultured from his throat swab and stool.

Another 2-year-old boy with 5 days of fever, cough, and rash on the hands and feet was diagnosed with HFMD and

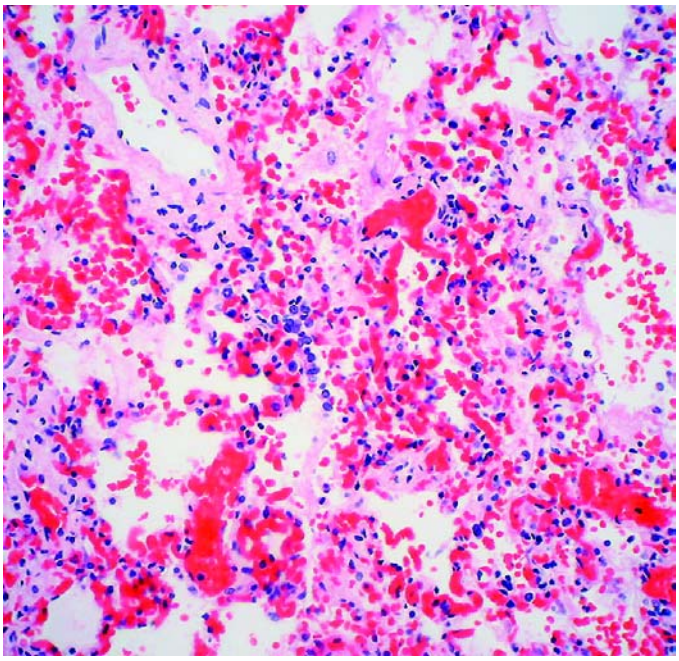

Figure 2. Interstitial pneumonitis in the 14-month-old girl who died of human enterovirus 71 disease. Photomicrograph shows alveolar wall congestion, intra-alveolar hemorrhage, and interstitial lymphocytic infiltrate. (Hematoxylin and eosin stain, original magnification x 200).

died on the same day he was admitted to the hospital. Postmortem examination showed pulmonary edema, interstitial pneumonitis, leptomeningeal infiltrates of lymphocytes and plasma cells, and occasional foci of perivascular lymphoid cuffing within the cerebral cortex. Although the heart contained patchy epicardial lymphoid infiltrates, no evidence of myonecrosis was found. Virus was not isolated from the brain, heart, lungs, intestine, and tracheal swab specimen.

One other complicated HFMD case was seen. Aseptic meningitis manifested by headache and terminal neck stiffness developed in an 8-year-old girl with characteristic symptoms of HFMD. HEV71 was isolated from her oral ulcers, but her cerebrospinal fluid (CSF) was negative for viruses and bacteria. After she was treated for her symptoms, the patient was

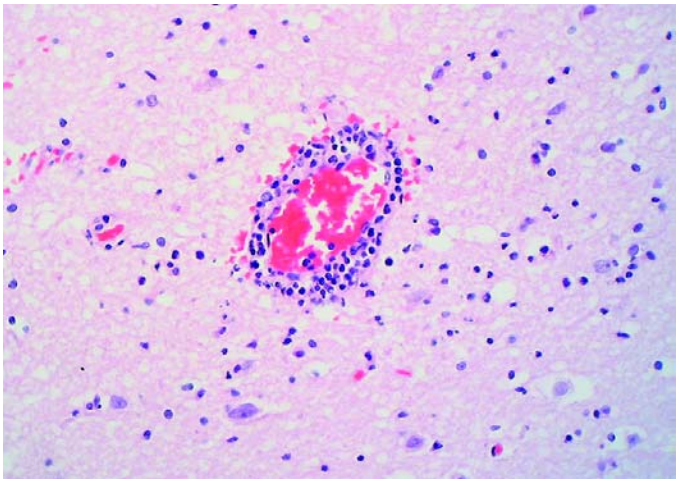

Figure 3. Perivascular cuffing in the brain. (Hematoxylin and eosin stain, original magnification x 200).

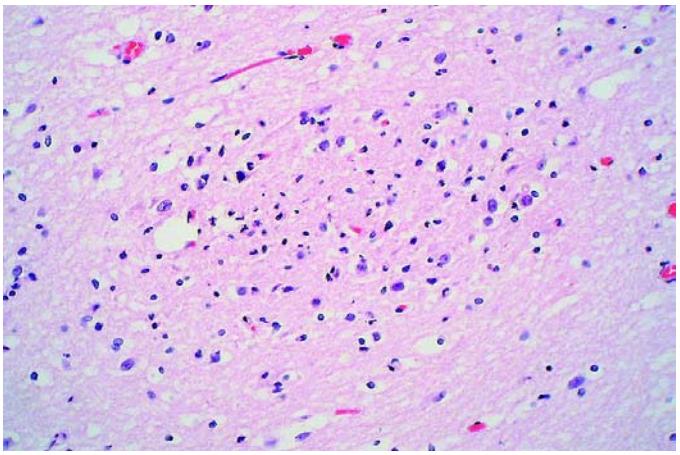

Figure 4. Section of brain showing a focus of necrosis. (Hematoxylin and eosin stain, original magnification x 200).

discharged from the hospital after 4 days. No patient in this epidemic showed acute flaccid paralysis.

Over the same period, 107 other patients who did not have HFMD were investigated for enteroviral infection. HEV71 was isolated from 5 of 17 culture-positive patients (Table 2). Of the five HEV71 patients, three had a nonspecific febrile illness without rash. The other two patients died. One was a 1-year-old boy with a 3-day history of fever, vomiting, diarrhea, and increasing restlessness; he died the day after being admitted to the hospital. Tests for bacteria, *Dengue virus*, *Rotavirus*, and *Plasmodium* spp. were negative; however, HEV71 was isolated from his throat and rectal swabs. Cardiac enzyme levels were raised, and a clinical diagnosis of myocarditis was made. No autopsy was performed. The other patient who died was a 19-year-old man who had a fever and headache for 3 days, associated with slurred speech and an episode of generalized tonic-clonic seizure. He died about 16 hours after hospital admission in spite of maximum resuscitative efforts. Postmortem investigation found meningoencephalitis involving the cerebral cortex and pons. The latter also showed focal liquefactive necrosis. His lungs showed marked intraalveolar hemorrhage and edema, and enlarged pneumocytes with intense nuclear smudging consistent with acute interstitial pneumonitis. The myocardium did not show notable inflammation or necrosis. The culture of brain tissue showed HEV71, but cultures of his heart, lungs, and intestines were negative for viruses.

Non-HEV71 enteroviruses were cultured from patients with hemorrhagic conjunctivitis (CAV24), aseptic meningitis (CBV4, CBV5), neonatal pyrexia (CBV3, CBV4), gastroenteritis (CAV24), sudden infant death (CAV24), and pharyngitis (CAV10) (Table 2). Two patients in whom herpangina was diagnosed had herpes simplex virus type 1 cultured from their oral swabs.

Specimens

The majority of specimens received from HFMD case-patients included those from stool, vesicles, and mouth and throat swabs (Table 3), for which the HEV71 culture-positivity rate was 44.3%, 43.6%, 25.0%, and 32.0% respectively. For the non-HFMD patients, stool and CSF specimens were most frequently submitted; 5.4% of the stool specimens and none of the CSF specimens were HEV71 positive. Non-HEV71 viruses, however, were cultured from five CSF specimens; one yielded CBV3, two yielded CBV4, and two yielded CBV5.

Discussion

The HFMD epidemic of 2000 is remarkable for differing from previous outbreaks in Singapore in three ways: the size of the epidemic, the causative virus, and the deaths associated with the epidemic. In these aspects, this outbreak is similar to those that occurred in Malaysia and Taiwan in recent years (6,7,9,10).

Cases in previous Singaporean outbreaks had numbered in the hundreds (3–5), contrasting with the approximately 4,000 cases reported in September and October 2000. The large number could in part have been the result of the HFMD surveillance initiated in 1998. In addition, physicians, parents, and child-care givers had a heightened awareness of the disease as a result of media publicity over the local HFMD-related deaths in September. Parents and caregivers sought medical attention for many children, including for those with mild illness. Another contributing factor was the compulsory reporting of the disease beginning on October 1, 2000.

HEV71 was isolated from 73.1% of the virus-positive HFMD patients and was the most probable cause of the epidemic, unlike the earlier documented outbreaks in Singapore

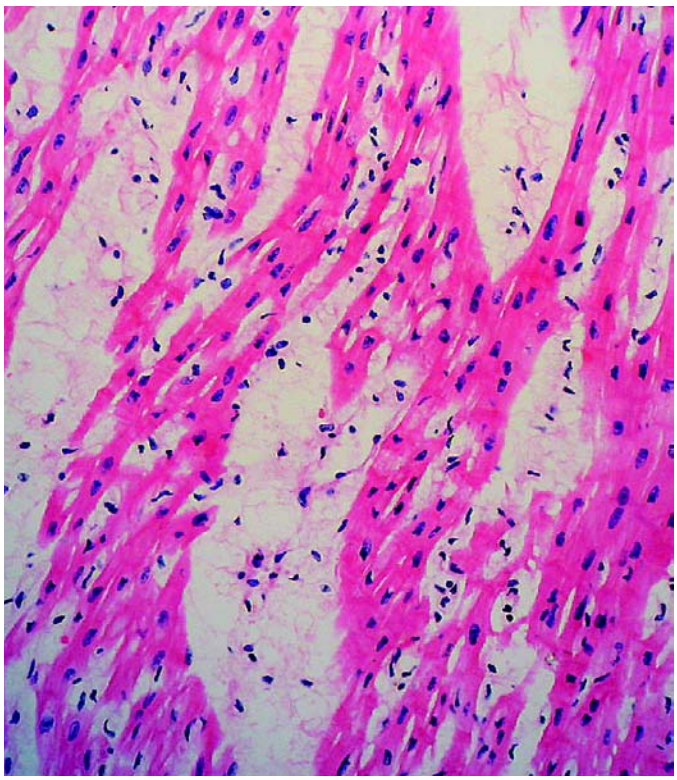

Figure 5. Tissue section of heart showing lymphocytic infiltrate, interstitial edema, and myocardial necrosis. (Hematoxylin and eosin stain, original magnification x 200).

Table 3. Virus yield by specimen type[a]

	No. specimens					
	HFMD patients			Non-HFMD patients		
Specimen type	Culture +	HEV 71 +	No. tested	Culture +	HEV 71 +	No. tested
Stool	58	39[b]	88	11	4	74
Rectal swab	6	5	8	1	1	3
Vesicle	31	27	62	0	0	3
Oral swab	23	15	60	2	0	6
Throat swab	19	16[c]	50	2	1	9
Nasal aspirate	3	3[d]	6	1	1	1
Saliva	1	1[d]	3	0	0	0
Tonsil	2	2	2	0	0	0
Intestine and contents	2	2	5	1	0	6
Brain	1	1	3	1	1	7
Cerebrospinal fluid	0	0	2	5	0	32
Ulcer	1	1	2	0	0	0
Conjunctiva	0	0	1	1	0	1
Blood	0	0	2	0	0	0
Heart	0	0	4	0	0	12
Tracheal swab	0	0	4	0	0	1
Lymph node	0	0	1	0	0	0
Spleen	0	0	2	0	0	0
Lung	0	0	4	0	0	1
Nasal swab	0	0	2	0	0	0
BAL	0	0	0	0	0	1
Total	147	112	311	25	8	157

[a]HFMD, hand, foot and mouth disease; HEV, human enterovirus; +, positive; EV, echovirus; CMV, *Cytomegalovirus*; BAL, broncho-alveolar lavage.
[b]Dual isolation of HEV71 and EV25 from one specimen.
[c]Dual isolation of HEV71 and *Rhinovirus* from one specimen.
[d]Dual isolation of HEV71 and CMV from one specimen. Both double-virus positive specimens were received from the same patient.

(4,5), which were attributed to CAV16. Other viruses cultured in smaller numbers included CAV16, CAV4, CAV10, and CBV5, known etiologic agents of HFMD, as well as CAV6, CAV24, CBV3, CBV4, and EV18, cocirculating enteroviruses that may have caused at least some cases of HFMD. CAV6 was isolated from the vesicles of two patients and CAV24 from the vesicles of one patient. Some of these non-HEV71 enteroviruses could have played an indirect role in the HEV71 epidemic. Indeed, the possibility of HEV71 interacting with other enteroviruses in a previous HEV71 epidemic has been raised (14).

Among the patients with suspected enteroviral infection but without the classic symptoms of HFMD, the most frequently isolated virus was still HEV71. These cases represented the extremes of the clinical spectrum of HEV71, including nonspecific febrile illness in three patients and death from myocarditis and encephalitis in two patients. The other clinical presentations of the non-HFMD patients included aseptic meningitis, herpangina, and Guillain-Barré syndrome, conditions that could also be caused by HEV71. However, the viruses isolated from these patients were CBV4, CBV5, and herpes simplex virus 1. Of the total of 81 patients with culture evidence of HEV71 infection, most (93.8%) showed illness consistent with HFMD.

Until this epidemic occurred, no deaths had been associated with HFMD in Singapore, although HEV71-related deaths from encephalitis (15–17), pulmonary edema, and hemorrhage (8,18) have occurred elsewhere since the virus was first isolated in 1969 (15). In this epidemic in Singapore, the case-fatality rate among all reported HFMD case-patients was 0.08%, which is similar to the rate of 0.06% experienced in the 1998 Taiwanese outbreak (9).

Four deaths (two HFMD and two non-HFMD cases) were associated with HEV71. All occurred rapidly despite intensive care, within a day of the patient's hospital admission, and after an average of 3.4 days of illness. The circumstances of these deaths were reminiscent of recent HEV71 deaths in the region (10,18–20). Of these four case-patients, three were autopsied, including a pair of siblings with HFMD and a patient with non-HFMD encephalitis. Their postmortem findings were sim-

ilar, with HEV71 isolated from the brains of two case-patients and from the tonsils and intestines of the third. Whether HEV71 caused the death of the patient with myocarditis who was not autopsied is less clear since HEV71 was isolated from nonsterile sites (the throat and rectum), although the illness and epidemiology suggest the possibility.

During the epidemic, a fifth death occurred involving a boy with HFMD on whom an autopsy was conducted. No virus was cultured from him, possibly because of the advanced postmortem degradation of his tissues. However, HEV71 was likely also to have been the cause of death on the basis of the similarity of his clinical and postmortem findings to those of the siblings who died, as well as the epidemiologic links to age, time, and place.

Like other fatal HEV71 cases reported elsewhere (7,16,20,21), the primary pathologic changes found at autopsy of four case-patients in this study were in the brain, including the brainstem, which showed extensive inflammatory cell infiltrate and focal necrosis. In addition, pneumonitis was found in all the case-patients and myocarditis in two. In the Malaysian and Taiwanese outbreaks (7,18,20), however, no significant inflammation was found in the lungs of patients with fatal cases. Notably, the myocardium of 10 Malaysian patients was described as normal (7), whereas autopsy reports of 2 patients from the Taiwanese outbreak described mild myocarditis in 1 (20) and no myocarditis in the other (18).

HEV71 was cultured from the brain specimens of two of our autopsied case-patients, but viral cultures of the lung and heart were negative. Similarly, no HEV71 was isolated from 34 CSF samples studied, notwithstanding the diagnosis of aseptic meningitis. Besides encephalitis and death, other complications (such as aseptic meningitis and acute flaccid paralysis) have also been reported in other HFMD outbreaks (7,9). However, other than the three fatal cases and one case of aseptic meningitis, all HFMD cases in the Singapore epidemic were uncomplicated, despite the large number of patients.

Most HFMD patients were very young children ($\leq$4 years of age) with the peak incidence at 1 year, a finding consistent with other HFMD outbreaks (7,9,22,23). Male patients outnumbered female patients by 1.7 to 1. This predominance has been observed in other enteroviral infections in which the male-to-female ratio ranges from 1.5:1 to 2.5:1 (24). The reason for this finding is not clear but may suggest a susceptibility at the host genetic level. That two siblings died of HEV71 disease, which has a low case-fatality rate, further strengthens this suspicion. Further studies are warranted on the possible role of host genetic factors in the pathogenesis of HEV71 disease.

Since 1997, HEV71 outbreaks have occurred in Sarawak (6,7), the Malaysian Peninsula (8), Taiwan (9), Singapore (25), and Australia (26). To account for this wave of HEV71 outbreaks in the region, we suspected the presence of a susceptible population as a plausible explanation; however, HEV71 had appeared previously in Singapore in 1984 (27). The virus disappeared for a time, resurfacing initially in small numbers of patients in 1997–1999 (25); the number of infections then jumped in 2000. Because the same group of children with the highest incidence of infection in 2000 would have had been exposed to HEV71 since 1997, why a large outbreak did not occur earlier is unclear. Likewise, the large HEV71 outbreak in Taiwan (9) also took place when most of the population had apparent immunity. We suspect that changes in viral factors, including virulence and tropism, are possible factors in these occurrences.

The genetic sequences of the complete VP1 gene of HEV71 isolates from the four patients who died and three of the patients who did not, obtained during the Singapore outbreak in 2000, were compared in a recent study involving 66 HEV71 strains isolated between 1999 and 2001 from Malaysia, Singapore, and Western Australia (28). That study showed that the Singapore 2000 strains, like those isolated in 2000 in Sarawak, Malaysia, belong to genogroup B4, whereas the Singapore 1998 and Western Australia 1999 strains (from nonfatal case-patients) and Malaysian 1997 strains (including fatal case-patients) belong to the closely related B3 genogroup. The strains from the Taiwanese outbreak of 1998 were found to be in the more distantly related C2 genogroup. The viruses that caused fatalities in outbreaks in Malaysia (1997), Taiwan (1998), and Singapore (2000) were thus not genetically similar, at least in the VP1 region. These viruses did not belong to the same genogroup, which would have explained the similar characteristics of the outbreaks. Furthermore, although the same study suggests that a substitution of alanine with valine at position 170 of the VP1 region of genogroup C2 (lineage 1) strains may be associated with increased neurovirulence, no similar virulence-related mutation in the same genomic region was found for genogroups B3 and B4, to which the Malaysian and Singaporean fatal strains belong. No evidence exists from the deduced VP1 amino acid sequences of these two genogroups to link specific amino acid residues with the severity of illness or death. These observations indicate that the genetic determinants for virulence are still unclear.

Coinfection with a second virus has been suggested as yet another possible pathogenetic factor (6,9), and this theory is supported by the concomitant isolation of a subgenus B adenovirus with an enterovirus from three persons who died during a HFMD outbreak in Sarawak (6). Among the Singaporean patients with HEV71 infection, three had a second virus isolated concurrently. However, the presence of dual viruses did not result in severe disease, although a child with HEV71 and CAV16 coinfection died in Singapore in 1997 (29).

We considered whether any unusual medications, treatments, or dietary exposures contributed to the deaths. No evidence from the fatalities in Singapore suggests this possibility. Conversely, we reviewed whether any particular therapeutic modality improved clinical outcome, but found that this idea cannot be argued conclusively because the HFMD patient with aseptic meningitis recovered well with symptomatic treatment. The child who survived his two younger siblings had no complications from HFMD and was not cared for differently from

his siblings before hospitalization. He was given prophylactic intravenous immunoglobulin solely because his siblings died. Whether this treatment, which was not administered to the patients who died, helped prevent severe disease in him is uncertain.

Considering that transmission of enteroviruses is mainly fecal-oral and through the respiratory route (to some extent) (22), we note that spread of the viruses is prevalent in child-care centers. To break the chain of transmission during the epidemic, the HFMD Task Force coordinated a swift, nationwide closure of preschool centers on October 1, 2000, reopening them on October 16, 2000, only when the HFMD reports recorded a declining trend, and no additional severe cases and deaths associated with the disease were reported. Other measures included repeated public health education through the mass media on observance of good personal hygiene, cleaning and disinfection of premises and articles both at home and at preschool centers, and keeping children away from crowds. These interventions may have played a role in bringing the epidemic under control by the end of October, although the outbreak may have also run its natural course by that time.

In September and October 2000, HEV71 caused the largest HFMD epidemic recorded to date in Singapore, an epidemic that involved mainly young children ≤4 years of age. Five deaths occurred, and HEV71 was isolated from four case-patients. Autopsies of four case-patients showed encephalitis, interstitial pneumonitis, and myocarditis. Virulence determinants of HEV71 and the precipitating factors for the epidemic itself unfortunately remain unknown. Based on our experiences during this epidemic, we found that an HFMD epidemic preparedness plan was useful in providing the framework for prompt actions to monitor the situation, identify the causative agent, interrupt virus transmission, and communicate with and solicit the cooperation of the media, parents, physicians, and preschool center personnel.

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Texas Lifestyle Limits Transmission of Dengue Virus

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Urban dengue is common in most countries of the Americas, but has been rare in the United States for more than half a century. In 1999 we investigated an outbreak of the disease that affected Nuevo Laredo, Tamaulipas, Mexico, and Laredo, Texas, United States, contiguous cities that straddle the international border. The incidence of recent cases, indicated by immunoglobulin M antibody serosurvey, was higher in Nuevo Laredo, although the vector, *Aedes aegypti*, was more abundant in Laredo. Environmental factors that affect contact with mosquitoes, such as air-conditioning and human behavior, appear to account for this paradox. We conclude that the low prevalence of dengue in the United States is primarily due to economic, rather than climatic, factors.

Outbreaks of mosquito-borne infection are commonly assumed to occur wherever competent vectors and a suitable climate exist, and that "global warming"—climate change caused by human activities—will cause these diseases to move to higher altitudes and latitudes. In many parts of the world, however, such diseases have become uncommon, despite an abundance of vectors and an ideal climate.

Denguelike illness was first noted in the New World as a major outbreak in Philadelphia in 1780 (1), and similar episodes occurred in the United States for more than 150 years. In 1922, the disease struck many major cities in the southern states, including an estimated 500,000 cases in Texas. Another widespread outbreak occurred in 1947–48 (2). In the past 50 years, however, autochthonous cases have been rare, despite an abundance of *Aedes aegypti* in the southeastern United States, and the arrival of millions of travelers from neighboring countries where the disease is endemic. From 1980 to 1999, only 64 locally acquired cases were confirmed in Texas, whereas 62,514 suspected cases were recorded in three adjoining Mexican states—Coahuila, Nuevo León, and Tamaulipas. In the same period, immigration authorities reported $\leq$ 70 million personal crossings from these states into Texas in a single year (3). Thus, the international border separates a dengue-endemic region from one in which the disease is rare.

Laredo, Texas, United States (population 200,000), and Nuevo Laredo, Taumalipas, Mexico (population 289,000), are essentially a single city (locally known as "los dos Laredos") divided by a small river, the Rio Grande (Figure). The rapid growth of this metropolitan area—70% in the past decade—is mainly due to massive cross-border traffic across three multilane bridges (Laredo Chamber of Commerce. Laredo, Texas; available from: URL: http://www.laredochamber.com/contact-information.htm). In the summer of 1999, toward the end of a local dengue outbreak, we conducted a seroepidemiologic survey to examine factors affecting dengue transmission on both sides of the border.

Methods

Households were selected by a modified version of the cluster survey of the World Health Organization Expanded Program on Immunization (4). First, we mapped the population of each census block in Laredo and in a major portion of Nuevo Laredo (Sector 1). In each city, 30 clusters were chosen from these census blocks by using a selection probability proportional to population. Four city blocks were randomly chosen from each of these clusters, and individual houses in one or more of those blocks were selected at random (where block maps were available) or systematically from a randomly chosen starting point. Blocks were sampled until 7–12 households had been enrolled from each cluster.

Binational teams, each composed of an epidemiologist, a nurse, and an entomologist, conducted the surveys. A blood sample was obtained by fingerstick from a randomly selected resident (ages 18–65).[1] A short questionnaire solicited general household information (number of inhabitants, type of construction, proximity to neighboring houses, number of bedrooms, presence and type of air-cooling system, and the presence and quality of window screens). Demographic data and travel histories of the blood donors were also recorded. Yards and patios were searched for *Ae. aegypti* breeding sites.

Serum samples were tested for anti-dengue immunoglobulin M (IgM) by IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA), and for anti-dengue IgG by

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[1]The investigation plan was reviewed by the Human Subjects Coordinator at the National Center for Infectious Diseases, Centers for Disease Control and Prevention, and determined to be a public health response that did not require further human subjects review. Blood samples were taken only from consenting adults.

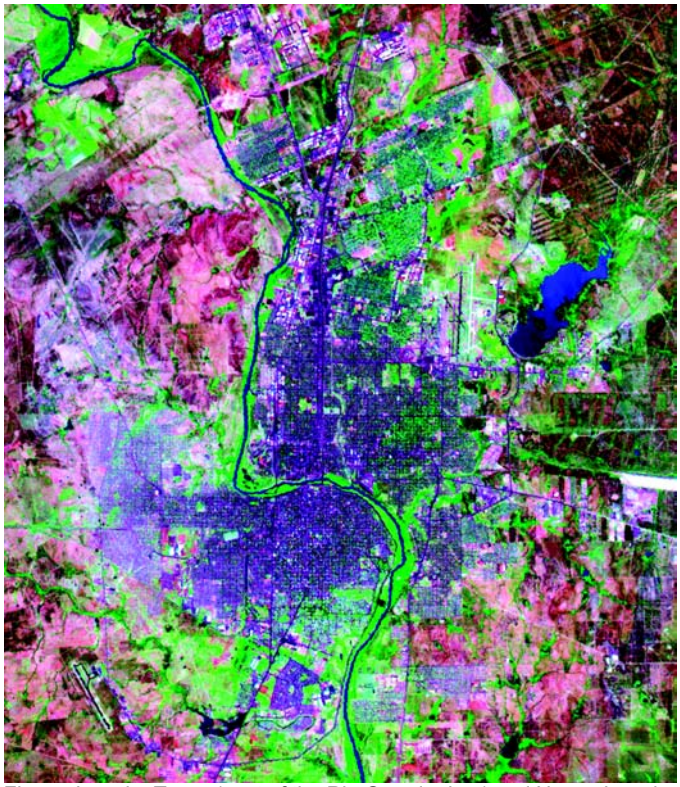

Figure. Laredo, Texas (east of the Rio Grande river) and Nuevo Laredo, Taumalipas (west of the river). Blue, water; green, vegetation; blue-violet, roads and buildings; paler blue-violet areas, low-income neighborhoods; pink, land with little or no vegetation. (National Aeronautics and Space Administration (NASA)/U.S. Geological Survey LANDSAT 7 image [TM bands 7, 4, and 3]; courtesy of NASA.)

IgG-ELISA and mixed dengue antigens (5,6). Data were analyzed with SAS v.6.12 (SAS Institute, Inc., Cary, NC) and SAS-callable SUDAAN (Research Triangle Institute, Research Triangle Park, NC) software. Risk factors for IgM and IgG seropositivity were assessed by multivariable weighted logistic regression, accounting for stratification by country, clustering within each city, and different numbers of surveys per cluster. Backward selection of variables was used to create the final models. Variables were retained if statistically significant ($p<0.05$).

Results

Surveys were completed in 622 households (309 in Laredo, 313 in Nuevo Laredo), and 516 persons (228 in Laredo, 288 in Nuevo Laredo) provided blood samples. IgM seropositivity (Table 1) was lower in Laredo (1.3%; 95% confidence interval [CI] 0 to 3%) than in Nuevo Laredo (16%; CI 12% to 20%). IgG seropositivity (Table 1) was also lower in Laredo (23%; CI 17% to 28% vs. 48%; CI 41% to 55%). Conversely, mosquito-infested containers were more abundant on the Texas side of the border: the Breteau Index (the number of infested containers per 100 houses) was 91 in Laredo versus 37 in Nuevo Laredo. Eighty-two percent of homes in Laredo had central or room air-conditioning versus 24% in Nuevo Laredo. In Laredo, evaporative coolers (a low-technology air-conditioning device that cools and humidifies air by drawing it from outdoors through a continually wetted screen) were less prevalent, a greater proportion of houses had intact screens, the average distance between houses was greater, and fewer persons lived in each house (Table 2).

Table 1. Seroprevalence of anti-dengue immunoglobulin G (IgG) and IgM antibodies in Nuevo Laredo, Mexico, and Laredo, Texas, United States

	Nuevo Laredo	Laredo
Households	313	309
Serum samples	288	228
IgM prevalence	16% (12 to 20)[a]	1.3% (0 to 3)[a]
IgG prevalence	48% (41 to 55)[a]	23% (17 to 28)[a]

[a]Weighted point estimate and (95% confidence interval).

Univariate analysis indicated a significant association between IgM seropositivity and five variables: absence of air-conditioning, fewer room air-conditioning units, the presence of an evaporative cooler, no travel outside the Laredo/Nuevo Laredo area, and shorter distances to neighboring houses (Table 3). IgG seropositivity was significantly associated with absence of central air-conditioning, fewer room air-conditioning units, smaller plot size, and a shorter distance to neighboring houses (Table 4).

On multivariate analysis, backward selection of variables yielded two that remained significantly associated with IgM seropositivity: absence of air-conditioning (odds ratio [OR] 2.6; CI 1.2 to 5.6) and no history of travel beyond Laredo/Nuevo Laredo in the previous 3 months (OR 2.0; CI 1.0 to 4.0). IgG seropositivity was associated with absence of air-conditioning (OR 2.4; CI 1.5 to 4.0), a history of crossing the border during the previous 3 months (OR 1.8; CI 1.1 to 2.8), and a greater number of occupants per household (OR 1.1; CI 1.0 to 1.2). By using the calculated prevalence ratio of 2.6 as an estimate of the relative risk of dengue in houses without air-conditioning, the proportion of dengue infections attributable to lack of air-conditioning in Nuevo Laredo was 55%, i.e., 55% of cases of dengue in Nuevo Laredo would not have occurred if all households in Nuevo Laredo had air-conditioning.

Table 2. Selected housing and travel characteristics in Nuevo Laredo and Laredo residents

Characteristic	Nuevo Laredo	Laredo
Central air-conditioning	2%	36%
Room air conditioner	23%	52%
Evaporative cooler	29%	17%
Screens on windows	54%	78%
Intact window screens	36%	60%
Mean no. occupants/residence	4.5	3.8
Mean distance to neighbors (m)	3.0	4.5
No travel outside Laredo/Nuevo Laredo area	70%	63%
Crossed border within 3 months of survey	52%	43%

Table 3. Risk factors associated with anti-dengue immunoglobulin M (IgM) seropositivity in Nuevo Laredo/Laredo residents, by univariate analysis

Risk factor	Prevalence ratio	Mean value for IgM seropositive residents	Mean value for IgM seronegative residents	(95% confidence interval) or p value
No air-conditioning	2.6	—	—	(1.3 to 5.2)
Evaporative cooler	2	—	—	(1.2 to 3.3)
No travel beyond Laredo/Nuevo Laredo area	1.9	—	—	(1.0 to 3.5)
No. room air conditioners	—	0.3	0.6	0.05
Distance to neighbor (m)	—	2.4	3.8	0.003

Discussion

Given the proximity of the two cities, the difference in transmission rates cannot be attributed to climate. Moreover, the mean daily temperature for August, the peak month of transmission, was 32.2°C (mean maximum 40.0°C; mean minimum 24.4°C), several degrees higher than the mean for the hottest months on Caribbean islands where dengue is common. Indeed, summer temperatures throughout the range of *Ae. aegypti* in the southern United States are hotter than in many tropical regions where the disease is endemic.

Despite mosquito control campaigns on both sides of the border, *Ae. aegypti* infestation rates in Laredo were remarkably high. The Breteau Index was on a par with that observed during major dengue epidemics in Puerto Rico (CDC, unpub. data). The House Index (the percentage of houses with at least one infested container) was 37%, seven times higher than the level (5%) equated with a "high risk" of dengue transmission by the World Health Organization (7). Thus, vector populations cannot account for the low rate of transmission on the Texas side of the border.

Ae. aegypti is closely associated with human habitation and readily enters buildings to feed and to rest during periods of inactivity (8). In this context, casual observation supported the association of lack of air-conditioning with dengue transmission. In Laredo, most shops, restaurants and other public places are air conditioned and have closed windows and self-closing doors, as do houses in residential areas, even in low income neighborhoods. By contrast, in Nuevo Laredo, many shops, bars, and restaurants are open to the street, and the windows and doors of houses are left open, particularly in the daytime. Thus, there is less opportunity for mosquito/human contact in Laredo than in the Mexican city.

More than 85% of all buildings in Texas are fully air conditioned (American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., Atlanta, GA; available from: URL: http://www.ashrae.org/). Indeed, air-conditioning is ubiquitous in many parts of the United States. To maximize heating/cooling efficiency, windows are usually fully glazed and are often kept permanently closed. Thus, most people spend much of their daily life sequestered in sealed buildings. Even if infected mosquitoes gain entry to such buildings, the artificially dry atmosphere lowers their survival rate, and the cool temperature extends the extrinsic incubation period, reducing the likelihood of transmission. Presumably, when denied access to humans, mosquitoes must seek other hosts. In Puerto Rico and Thailand, some *Ae. aegypti* feed on dogs, even when humans are readily accessible (9,10). In Laredo, we observed that large dogs were housed in outdoor kennels at many homes. Whether these animals are an important blood source for the species would be an interesting topic for future research.

The dollar cost of electricity is similar in Laredo and Nuevo Laredo, but income, as indicated by per capita gross domestic product, is much higher in Texas than in Taumalipas (Table 5). The proportional cost of maintaining air-conditioning for an entire dengue season is therefore much higher for the average family in Mexico and is unaffordable for the majority. Thus, the ultimate determinant of dengue prevalence in this setting is socioeconomic rather than environmental.

Conclusion

It has frequently been stated that dengue, malaria, and other mosquito-borne diseases will become common in the United States as a result of global warming (11–14). Such predictions often refer to vectorial capacity, a simple model that incorporates the population density, biting frequency, and daily survival probability of the vector, and the extrinsic incubation period of the pathogen (15,16). Although the vectorial capacity model has proved useful for interpreting entomo-epidemiologic data, particularly for transmission of malaria (17),

Table 4. Risk factors associated with anti-dengue IgG seropositivity in Nuevo Laredo/Laredo residents, by univariate analysis

Factor	Prevalence ratio	Mean value for IgG seropositive residents	Mean value for IgG seronegative residents	(95% confidence interval) or p value
No air-conditioning	1.65	—	—	(1.27 to 2.15)
No. occupants	—	4.7	4.3	0.05
Lot size (m^2)	—	377	395	0.03
No. room air conditioners	—	0.4	0.7	0.002
Distance to neighbor (m)	—	3.3	3.9	0.03

Table 5. Estimated cost (US dollars) of air-conditioning a house in Texas vs. Taumalipas, Mexico

	Texas	Taumalipas
Cost per kilowatt hour (kWh)	0.06119	0.04863
Cost of 25,000 kWh	1,530	1,216
Per capita GDP[a]	34,288	5,014
% of per capita GDP	4.5	24.2

[a]GDP, gross domestic product.

it does not incorporate factors like air-conditioning, use of vaporative coolers, and the behavior of mosquitoes and humans. If the current warming trend in world climates continues, air-conditioning may become even more prevalent in the United States, in which case, the probability of dengue transmission is likely to decrease. If the economy of Mexico continues to grow, the use of air-conditioners may gain momentum south of the border.

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Viral Gastroenteritis Outbreaks in Europe, 1995–2000

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To gain understanding of surveillance and epidemiology of viral gastroenteritis outbreaks in Europe, we compiled data from 10 surveillance systems in the Foodborne Viruses in Europe network. Established surveillance systems found *Norovirus* to be responsible for >85% (N=3,714) of all nonbacterial outbreaks of gastroenteritis reported from 1995 to 2000. However, the absolute number and population-based rates of viral gastroenteritis outbreaks differed markedly among European surveillance systems. A wide range of estimates of the importance of foodborne transmission were also found. We review these differences within the context of the sources of outbreak surveillance information, clinical definitions, and structures of the outbreak surveillance systems.

Viral pathogens are the most common cause of gastroenteritis in industrialized countries (1,2). Mead et al. have estimated that of the 38.6 million annual cases of gastroenteritis in the United States, 30.8 million (80%) are the result of viral infections (3). Enteric viral pathogens include *Rotovirus A*, *Astrovirus*, adenovirus, and *Sapovirus*, but most viral gastroenteritis infections are caused by *Norovirus* (formerly Norwalk-like viruses) (1–3). The use of molecular diagnostics including reverse-transcriptase polymerase chain reaction (RT-PCR) and antigen detecting enzyme immunoassays (EIA) (4–20) have changed researchers' understanding of the epidemiology of human *Caliciviridae* (including *Norovirus* and *Sapovirus*) (21). For example, using RT-PCR assays, Pang et al. showed that caliciviruses were as common a cause of infection as rotaviruses among children <2 years of age (22).

In addition, many reports have established the importance of noroviruses as a cause of outbreaks of food- and waterborne illness (23–28), though estimates of the proportion of infection spread by these modes vary widely: from 14% in England and Wales (29) to <40% in the United States (7). While person-to-person transmission is probably the mode of infection of most cases, food- and waterborne infections may be of particular importance since these outbreaks have the potential to involve large numbers of people and wide geographic areas and, perhaps, to introduce new variants to an area (30).

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A research network to study foodborne viruses in Europe was recently funded by the European Union. Through this project, the participant institutes have networked their virologic and epidemiologic surveillance in order to detect transnational outbreaks, elucidate transmission routes, and make international comparisons of the epidemiology of viral gastroenteritis. We chose to study outbreaks rather than community cases because viral gastroenteritis is a very common infection (1); therefore, enumeration of epidemics (or outbreaks) may be more practical and useful since individual cases are poorly reported (31). International comparisons of surveillance data are difficult because criteria for effective surveillance customarily varies across borders (32).

The objective of this survey was to capture information on the structure of outbreak surveillance in each country (including sources of data and definitions employed) and to gain estimates of the frequency of outbreaks, as well as to compare the setting of outbreaks, the importance of foodborne transmission, and the use of characterization techniques. We present surveillance data from viral gastroenteritis outbreaks from 1995 to 2000 collected by participant European countries. These data provide baseline information for future harmonization and comparison efforts.

Methods

A questionnaire was sent by e-mail to the project leaders of the 13 participant institutions (from 10 countries) in the Foodborne Viruses in Europe group. The questionnaire, administered in English, was developed and completed in collaboration with research and medical virologists and epidemiologists working in viral gastroenteritis surveillance. General information on surveillance systems (including sources of data, estimate of national population under surveillance, definition of a viral gastroenteritis outbreak, and number of such outbreaks investigated) was collected for the period 1995–2000. More detailed epidemiologic data (setting, mode of

[1]On behalf of the Foodborne Viruses in Europe group, which includes: the Netherlands: M. Koopmans, H. Vennena, Y. van Duynhoven, and M.A. Widdowson; Finland: K.-H. von Bonsdorff, L. Maunula; Denmark: B. Böttiger, K. Mølbak, F.X. Hanon; Sweden: L. Svensson, K.-O. Hedlund, Maria Thorvag, Juan Carrique-Mas; United Kingdom: D. Brown, M. Reacher, J. Green, B. Lopman; Germany: E. Schreier, H. Gelderblom, Andrea Ammon; Spain: A. Sanchez-Faquier, G. Hernández-Pezzi, A. Bosch, J. Buesa; France: F. LeGuyader, P. Pothier, E. Kohli; Italy: F. Ruggeri, D. DeMedici; and Slovenia: M. Poljsak-Prijatelj, A. Hocevar-Grom.

transmission, and implicated food vehicles) were collected from outbreaks that occurred in 2000. Contributors were sent a summary report and asked to confirm that the data presented accurately represented their surveillance.

Results

Data Sources of Surveillance Systems

One completed survey questionnaire was received from all 10 countries. A range of sources contributed data on viral gastroenteritis outbreaks for European surveillance systems (Table 1), including diagnostic reference laboratories, local public health staff, food inspectorates, and physicians. We derive our data from routine surveillance except for Germany, where systematic national surveillance was not operational during the survey period. German data were collected from laboratories that performed RT-PCR diagnostics in the surveyed period. The same applies to the Netherlands, Finland, and Sweden, although the collaborating centers in these countries run the sole reference laboratory service.

Outbreak Definition and Geographic Coverage of Surveillance Systems

All surveillance systems reported data collected on outbreaks from the whole population of their respective countries except for Italy, where a small geographically convenient sample of approximately 1% of the population was covered by surveillance (Table 2). Both the criteria and the use of outbreak definitions differed among the surveillance systems (Table 2). Some systems collected information only on incidents that met a specific definition; other systems collected information on all incidents and then applied definitions retrospectively for analysis. Some surveillance systems required laboratory confirmation to attribute an outbreak to an enteric viral pathogen. Among systems requiring laboratory confirmation, a range of stringency existed from at least one positive sample (England and Wales) to half of all stools positive for virus (Finland and the Netherlands).

Outbreaks Investigated

Outbreak reports were available from the entire surveyed period (1995–2000) from a few countries: England and Wales, Slovenia, Spain, the Netherlands, and Sweden. The overall numbers of outbreaks investigated ranged from 2 in Italy to 1,643 in England and Wales (Table 3).

National outbreak reporting rates for each country were calculated by dividing annual outbreaks by national population (Figure 1). Rates in Sweden (9–22 outbreaks/million in population) were markedly higher than in any other country. In most countries, approximately 3–7 outbreaks per million population were ascertained annually. Since 1997, outbreak reporting rates have been increasing in most countries.

Completeness of Basic Epidemiologic Data

Participants were asked how many of the outbreaks reports from the year 2000 included details on first date of onset, last date of onset, number of persons ill, number of persons hospitalized, number of related deaths, and setting of the outbreak. Completeness of these data differed substantially between countries: none of the data were available from Sweden, whereas data were almost 100% complete for all categories in England and Wales, Denmark, and Slovenia (Figure 2).

Setting of Outbreaks

The settings where reported outbreaks occurred differed substantially by country (Figure 3). In England and Wales, Spain, and the Netherlands, most reported outbreaks occurred in hospitals and residential homes (78%, 64%, and 66%,

Table 1. Sources of information of viral gastroenteritis surveillance systems in the Foodborne Viruses in Europe network

	Sources of outbreak data				
Country	Diagnostic microbiology laboratory	Food safety inspectorate	Physician/ patient reports	Local/regional public health authority	Type of outbreaks reported
Denmark		Yes	Yes	Yes	Food/waterborne
France	Yes			Yes	Food/waterborne
England and Wales				Yes	All
Italy	Yes				All
Finland	Yes[a]				All
Sweden	Yes				All
Germany	Yes			Yes[b]	All
Slovenia	Yes			Yes	All
Spain	Yes			Yes	All
the Netherlands[c]	Yes	Yes	Yes	Yes	All[d]

[a]Participant is sole laboratory performing viral testing, and coordination is conducted at National Public Health Laboratory and National Food Administration.
[b]*Norovirus* became a reported disease in January 2000. From 1997 to 2000, reports from local health departments were collected unsystematically.
[c]Dutch national data were collected from three systems: notification system, food safety inspectorate, and laboratory-based system (from diagnostic microbiology laboratories, local/regional public health authorities, physician/patient reports, and other institutions in which outbreaks occurred).
[d]Foodborne only for systems 1 and 2.

Table 2. National coverage and use of clinical definitions for viral gastroenteritis by European surveillance systems

Country	National coverage %[a]	Definition of viral gastroenteritis outbreak[b]	Laboratory confirmation required	Outbreak definition applied	
				As entry criteria in database	Retrospectively for analysis
Denmark	100	Kaplan's, shellfish		Always	Always
England and Wales	100	General	Yes	Always	Never
Finland	100	Clinical	Yes	Always	Never
France	100	Clinical, shellfish		Always	Never
Germany					
Italy	1	Clinical		Always	Always
Slovenia	100	Clinical		Sometimes	Sometimes
Spain	100	General	Yes	Always	Sometimes
Sweden	100	Kaplan's, clinical	Yes	Always	Sometimes
Netherlands	100	System 1: clinical System 2: Kaplan's System 3: clinical	Yes Yes	Sometimes	Always

[a]Refers to geographic coverage by surveillance, not completeness of reporting.
[b]Kaplan's criteria for recognition of *Norovirus* outbreaks (33); clinical, clinical criteria (different from Kaplan's) specifying that cases must be clustered in time and place; general, general definition used for all outbreaks of gastroenteritis with laboratory confirmation required to attribute outbreak to viral pathogen; shellfish, specific criteria used for identifying shellfish outbreaks.

respectively), whereas in Denmark, 13 (76%) of 17 reported outbreaks occurred in food outlets. In Denmark, surveillance is done by the Food Safety Inspectorate, which collects reports of suspected foodborne outbreaks only. The Inspectorate is not informed of person-to-person spread outbreaks, which are more commonly seen in residential institutions and hospitals.

In Slovenia, the majority of reported outbreaks occurred in day-care centers (10/14; 71%), and in France, most reported outbreaks occurred in private houses (7/9; 78%). In France, reporting was recommended only for large outbreaks or if oysters, an item commonly consumed in French households, were the suspected vehicle of infection.

Food and Water as Sources of Outbreaks

Among countries conducting broad-based outbreak surveillance, the following proportions of viral gastroenteritis outbreaks were reported to be associated with food- or waterborne transmission: Finland (24%), the Netherlands (17%), Slovenia (14%), Spain (7%), and England and Wales (7%) (Table 4). Very rarely was laboratory evidence (detection of the same organism in the vehicle and stool specimens) or statistical evidence (case-control or cohort) available that demonstrated the association of the vehicle with illness. During the survey period, Danish and French surveillance almost exclusively focused on outbreaks transmitted through food and water. Therefore, estimates of the proportion of food and water

Table 3. Reported outbreaks of viral gastroenteritis, European surveillance, 1995–2000

	1995	1996	1997	1998	1999	2000			Total
						All viral organisms	*Rotavirus*[a]	*Norovirus*[a]	1995–2000
Denmark				9	11	17	0	17 (100)	37
England and Wales	392	352	151	219	239	290	13 (4)	273 (96)	1,643
Finland			5	27	35	58	1 (2)	56 (97)	125
France	4	9	7	8	19	28	1 (14)[b]	5 (71)[b]	43
Germany			1	53	145	227	0	227(100)	426
Italy	0	0	0	0	0	2	0	2 (100)	2
Slovenia	8	6	8	4	5	14	8 (57)	6 (43)	45
Spain	37	24	25	29	66	55	6 (43)[c]	8 (57)[c]	236
Sweden	81	130	130	130[d]	190[d]	195[d]		190 (97)	856
the Netherlands	25	69	54	36	58	59	5 (13)[e]	32 (84)[e]	301

[a]Number of outbreaks attributed to organism (percentage of year 2000 outbreaks).
[b]Based on seven laboratory-confirmed viral outbreaks.
[c]Based on 14 laboratory-confirmed viral outbreaks.
[d]Approximate figures.
[e]Based on 38 laboratory-confirmed viral outbreaks.

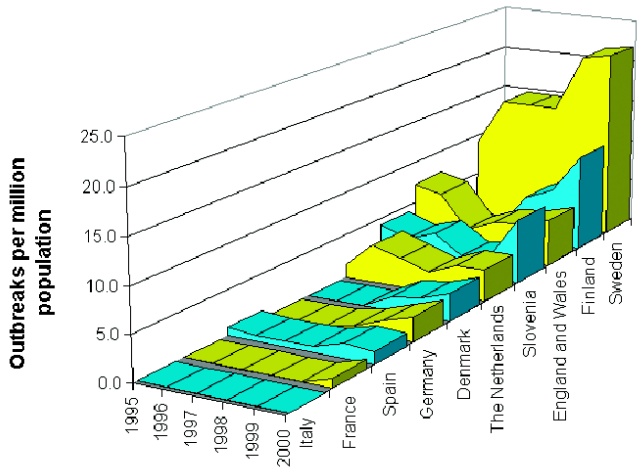

Figure 1. Viral gastroenteritis outbreak rates, European surveillance, 1995–2000. Rates based on year 2000 national population estimates.

transmission from these countries cannot be compared to the general estimates in other countries.

Molecular Characterization Techniques

Different molecular techniques were used by participating institutes to characterize virus from outbreaks in 2000. Reverse line blot was used in the Netherlands and Spain, and the heteroduplex mobility assay was used in England and Wales. Sequence analysis was performed in England and Wales, Finland, France, Italy, Germany, Spain, and the Netherlands; EIA were used in England and Wales, and a microplate hybridization technique was used in Finland.

Discussion

Viral gastroenteritis infection, typically a self-limiting condition of short duration in humans, is extremely common and associated with relatively few deaths. Surveillance of outbreaks of this infection, rather than individual cases, may be more appropriate. In our review of the surveillance for this infection in Europe, we found variations in the organizations conducting surveillance, the surveillance definition of a viral gastroenteritis outbreak, the populations under surveillance, and the completeness of descriptive and analytical epidemiologic and diagnostic information.

Researchers comparing surveillance information at an international level should consider the outputs of surveillance, as well as the influence of methodology and structure of surveillance on these outputs. Surveillance for viral gastroenteritis in Europe is poorly developed; systems vary in their sources of data, definitions, and use of diagnostic techniques. These differences are reflected in the wide range of numbers of outbreaks, population-based rates, and epidemiologic patterns observed across Europe. Nonetheless, our comparison of this surveillance data was an informative exercise because international epidemiologic databases of viral gastroenteritis infections have not been developed. In many of the countries included in the Foodborne Viruses in Europe network, viral gastroenteritis has not been considered a priority, and these countries do not have a well-developed surveillance system. This inventory of surveillance data will aid in the development of a more consistent and complete surveillance across Europe.

These data clearly show that both the absolute number and the population-based rates of viral gastroenteritis outbreaks differ substantially between European surveillance systems. From 1995 to 2000, 1,643 outbreaks of viral gastroenteritis were investigated by the Public Health Laboratory Service in England and Wales, but the outbreak rates (number of outbreaks/population) were highest in Sweden for every surveyed year. Some variation in these figures occurred because a number of the surveillance systems required laboratory confirmation while others did not (Table 2). However, the criteria suggested by Kaplan et al. to recognize an outbreak of viral etiology is widely used and is generally accepted as an effective clinical tool in the absence of diagnostic information (33). Interestingly, surveillance systems with the most stringent outbreak criteria, including laboratory confirmation of outbreaks (England and Wales, Finland, and Sweden) ascertained the most outbreaks, likely because surveillance in these countries is more developed and integrated better with reporting bodies.

However, even the surveillance systems with the highest figures greatly underascertain viral gastroenteritis. A study of infectious intestinal disease in England and Wales estimated that only 1/300–1,500 cases of *Norovirus* gastroenteritis are reported to national surveillance (34). For a case to be ascer-

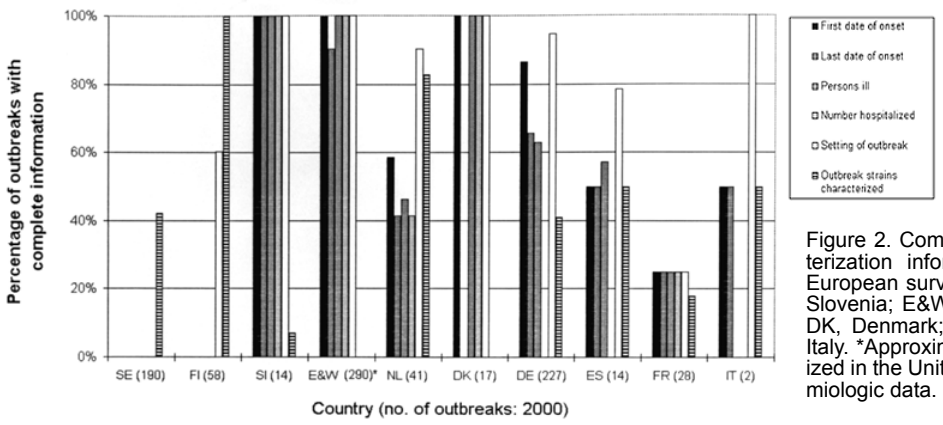

Figure 2. Completeness of epidemiologic and viral characterization information on viral gastroenteritis outbreaks, European surveillance, 2000. SE, Sweden; FI, Finland; SI, Slovenia; E&W, England and Wales; NL, the Netherlands; DK, Denmark; DE, Germany; ES, Spain; FR, France; IT, Italy. *Approximately 500 outbreaks strains were characterized in the United Kingdom, but typing is not linked to epidemiologic data.

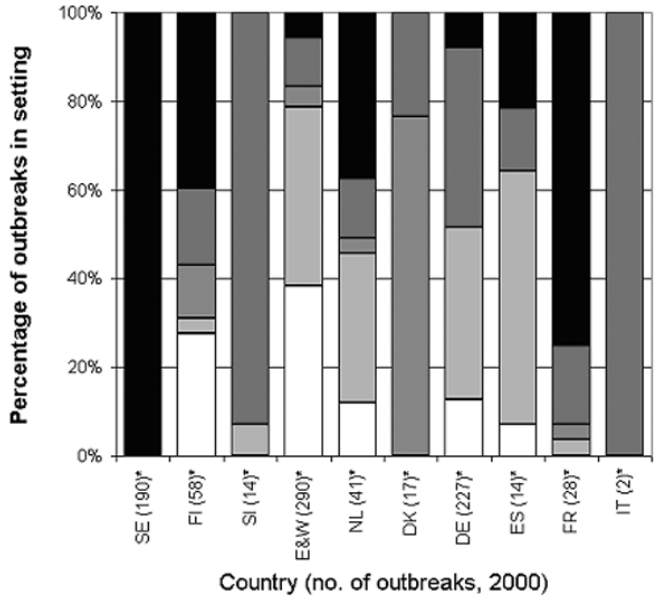

Figure 3. Setting of viral gastroenteritis outbreaks, European surveillance, 2000. SE, Sweden; FI, Finland; SI, Slovenia; E&W, England and Wales; NL, the Netherlands; DK, Denmark; DE, Germany; ES, Spain; FR, France; IT, Italy. *Includes restaurants, cafes, public bars, mobile vendors, canteens, and catered events.

tained by national surveillance, patients must be examined by their primary-care doctor, a specimen must be taken and submitted for laboratory testing, the test must be positive (the amount of false negatives will depend on the diagnostic technique), and the surveillance unit must be notified. Ascertaining outbreaks requires an additional step in which investigators must recognize epidemiologic links between cases. While this chain of events will differ from country to country, the principle of underascertainment affects all surveillance. However, outbreak recognition and investigation will, through case finding, lead to better ascertainment of persons affected in outbreaks.

Although most surveillance systems may be designed for national coverage, reports were incomplete to a varying degree. Ascertained outbreaks varied geographically and were incomplete, as demonstrated by the large variation in reported outbreaks (Table 3).

This survey found that the great majority of European viral outbreaks could be attributed to *Norovirus*. In Denmark, England and Wales, Finland, France, and Sweden, >95% of nonbacterial outbreaks were attributed to noroviruses as were 84% of outbreaks in the Netherlands. The relative number of infections from noroviruses was lower in Slovenia (43%) and Spain (57%), although these estimates are based on a small number of outbreaks (n=14 for both). These figures are consistent with previous reports that *Norovirus* could be detected in 91% of all nonbacterial infectious intestinal disease outbreaks in the Netherlands (9) and 89% of such outbreaks in Sweden (35). Similarly, Fankhauser et al. found *Norovirus* responsible for 96% of nonbacterial outbreaks in the United States (7).

Estimates of the importance of foodborne transmission also varied widely in this survey. Foods were implicated as the vehicle of transmission in 16 (94%) of 17 outbreaks in Denmark and 28 (100%) of 28 outbreaks in France because surveillance systems in these countries were designed to detect foodborne disease. In countries with more general outbreak data, estimates of foodborne transmission were lower: 7 (17%) of 41 in the Netherlands, 14 (24%) of 58 in Finland, and 20 (7%) of 290 in England and Wales, although laboratory and statistical evidence of association with food or water was scant.

The settings of outbreaks also reflected the proportion of reported outbreaks that were ascertained to be foodborne. For example, in Denmark, 75% of all reported outbreaks were set in food outlets. In Spain, the Netherlands, and England and Wales, most reported outbreaks occurred in residential homes and hospitals, with only a small fraction occurring in food outlets.

In Finland, the National Public Health Laboratory is the only facility in the country testing for *Norovirus* and, therefore, is aware of all such investigations. Most other surveil-

Table 4. Foodborne transmission and supporting evidence of implicated food vehicles, European surveillance, 2000

			Evidence	
Country	Total outbreaks	Food/waterborne outbreaks (%)	Laboratory[a]	Statistical[b]
Denmark	17	16 (94)	1	0
England and Wales	290	20 (7)	1	4
Finland	58	14 (24)	0	0
France	28	28 (100)	2	1
Germany	227			
Italy	2	0		
Slovenia	14	2 (14)	0	0
Spain	14	1 (7)	0	1
Sweden	190			
the Netherlands	41	7 (17)	0	2

[a]Same organism found in stool specimen and food vehicle.
[b]Statistically significant result from cohort or case-control study.

lance systems receive data on outbreaks from a number of sources including local public health authorities, other diagnostic laboratories, and physicians. Surveillance in Denmark is anomalous in that only outbreaks from the national food inspection service are reported, which, in conjunction with the special mention of shellfish in the definition of an outbreak, explains the preponderance of food-related outbreaks in Danish surveillance. Such diversity in data sources and definitions may also explain the differences in estimates among other countries, including those external to the Foodborne Viruses in Europe network. Based on data from 90 outbreaks, Fankhauser et al. estimated that 47% of *Norovirus* outbreaks in the United States were spread by food (7). This estimate, derived from local and state health department reports, may be affected by reporting bias or may truly reflect different epidemiologic patterns of viral gastroenteritis outbreaks compared to those seen in European countries. Factors that might affect the relative amount of foodborne transmission of *Norovirus* are the virologic quality of food, food-handling guidelines, and infection control practice in health-care settings (36).

DNA sequencing of PCR amplicons, used to characterize outbreak strains by laboratories in England and Wales, Finland, France, Italy, Germany, Spain, and the Netherlands, yields the most virologic information, although this technique is a labor-intensive procedure. The heteroduplex mobility assay (37), as well as an EIA based on one of the *Norovirus* genotypes (Grimsby virus), was used by the laboratory in England and Wales. Reverse line blot (38) was used in the Netherlands and Spain in 2000 and has since been adopted by a number of the other collaborating institutes to characterize *Norovirus*. The use of the heteroduplex mobility assay (37), reverse line blot (38), and sequencing to characterize virus has demonstrated the considerable and dynamic genetic diversity of human *Caliciviridae* (39). The use of such techniques by a wider group may demonstrate important differences in molecular epidemiology between countries and may detect the introduction of a novel strain to an area (40) by tracking and linking outbreaks over wide geographic areas.

In the retrospective survey presented here, determining whether differences in frequency, setting, and importance of foodborne transmission are real or artifacts caused by international variation in surveillance system design was difficult. Timely collection of information for case-control or cohort studies and development of tests for virus detection in food are needed to advance understanding of the extent of foodborne transmission of *Norovirus*. Success of the Foodborne Viruses in Europe network will depend on the ability to compare both virologic and epidemiologic data. Protocols for harmonizing the characterization of noroviruses and an outbreak questionnaire with a minimum dataset have been defined. While data collection will be harmonized, information will be obtained from an international group whose range of perspectives will yield different interpretations of epidemiologic events. The timely feedback of surveillance data to participants is an essential step in the cycle of continued improvement of a surveillance system (41) that we have made possible through this European Union–funded network. In addition to describing the current state of viral gastroenteritis surveillance in Europe, this report will act as a baseline to interpret prospective outcomes of the Foodborne Viruses in Europe network.

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The U.S.-Mexico Border Infectious Disease Surveillance Project: Establishing Bi-national Border Surveillance

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In 1997, the Centers for Disease Control and Prevention, the Mexican Secretariat of Health, and border health officials began the development of the Border Infectious Disease Surveillance (BIDS) project, a surveillance system for infectious diseases along the U.S.-Mexico border. During a 3-year period, a binational team implemented an active, sentinel surveillance system for hepatitis and febrile exanthems at 13 clinical sites. The network developed surveillance protocols, trained nine surveillance coordinators, established serologic testing at four Mexican border laboratories, and created agreements for data sharing and notification of selected diseases and outbreaks. BIDS facilitated investigations of dengue fever in Texas-Tamaulipas and measles in California–Baja California. BIDS demonstrates that a binational effort with local, state, and federal participation can create a regional surveillance system that crosses an international border. Reducing administrative, infrastructure, and political barriers to cross-border public health collaboration will enhance the effectiveness of disease prevention projects such as BIDS.

The 2,000-mile U.S.-Mexico border is one of the world's busiest international boundaries. An estimated 320 million people cross the northbound border legally every year (1). The U.S.-Mexico border is a unique region where the geopolitical boundary does not inhibit social and economic interactions nor the transmission of infectious diseases among residents on each side of the border. Some border cities (such as El Paso and Ciudad Juarez) are separated by a short distance and serve as one large metropolitan area for the local community (Figure 1). From an epidemiologic perspective, the border population must be considered as one, rather than different populations on two sides of a border; pathogens do not recognize the geopolitical boundaries established by human beings. The border region has a population of approximately 11 million people (2), many of whom cross the border daily to work, shop, attend school, seek medical care, or visit family and friends (3,4). The border population also includes persons who pass transiently through the region and others who come the area to work in maquilas, the border factories. The region has experienced tremendous population growth. During 1993–1997, the U.S. border population grew by 1.8% annually, more than double the national U.S. average of 0.8%, while the Mexican border population has grown by 4.3% per year, almost three times the national Mexican annual growth rate of 1.6% (2,5). Population growth has been spurred by increased economic opportunities after the North American Free Trade Agreement was implemented in 1994. Currently, an estimated 3,300 maquilas, employing >1 million workers, are located along the border (6,7). The proliferation of border factories has generated a wave of internal migration of persons from other regions of Mexico and Central America toward the border (8).

From Mexico's perspective, the border encompasses some of the country's most economically prosperous states. In con-

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Figure 1. The Rio Grande River separates the border between Ciudad Juarez, Chihuahua, Mexico, and El Paso, Texas, USA.

trast, the U.S. border region is among the poorest areas in the United States, with >30% of families living at or below the poverty level (8). Along the Texas border, an estimated 350,000 or more people live in 1,450 unincorporated areas known as colonias, which lack adequate sanitation infrastructure (8).

The large population movement, limited public health infrastructure, and poor environmental conditions contribute to increased incidence of certain infectious diseases (8–11) Analysis of data from the U.S. National Notifiable Diseases Surveillance System for 1990 through 1998 showed increased risks for certain foodborne, waterborne, and vaccine-preventable diseases in U.S. counties within 100 kilometers of the border, compared with nonborder states. These data show a two- to fourfold greater incidence of hepatitis A, measles, rubella, shigellosis, and rabies and an eightfold greater incidence of brucellosis in border counties than in nonborder states (11). Studies have identified the importance of cross-border movement in the transmission of various diseases, including hepatitis A (12,13), tuberculosis (14–18), shigellosis (19), syphilis (20), *Mycobacterium bovis* infection (21), and brucellosis (22,23).

Despite the high prevalence of infectious diseases and increasing movement of people across the borders, no surveillance system had been established to assess the border population as a geographic unit. Gaining an accurate picture of public health needs was limited by the following factors. First, the surveillance case definitions used for public health reporting in Mexico and the United States are different. Also, laboratory confirmation is often unavailable in the Mexican border states, and therefore reported cases of infectious diseases are defined primarily by clinical findings. In contrast, for the many notifiable diseases in the United States, laboratory confirmation is required, and U.S. surveillance is heavily based on laboratory reporting. This system likely underestimates the true incidence rates. In the past, the two countries have exchanged limited border surveillance data. However, these differences diminish the usefulness of national surveillance data for developing a comprehensive, regional understanding of infectious disease epidemiology in the border areas. A consistent binational perspective is essential to effectively control and prevent the transmission of infectious diseases that move easily through the geopolitical boundary.

The Border Infectious Disease Surveillance (BIDS) project was designed to bridge this surveillance gap by forming partnerships among institutions in both countries serving the region and bringing together each country's complementary experiences in syndromic and laboratory-based surveillance. This report describes the establishment of a binational surveillance system for hepatitis and febrile exanthems along the U.S.-Mexico border.

Project Mandate

In June 1997, the United States–Mexico Border Health Association and the U.S. Council of State and Territorial Epidemiologists passed resolutions to support surveillance for infectious diseases and emerging infectious diseases along the U.S.-Mexico border (24,25). The Centers for Disease Control and Prevention (CDC) and the Mexican Secretariat of Health spearheaded efforts to initiate the project and formalized an agreement to establish BIDS through a memorandum of cooperation in epidemiology. A binational team of local, state, and federal epidemiologists, laboratory scientists, and public health officials met to organize and define project objectives. Decisions were made by consensus among the participants.

Site Selection

The team selected four sister city groups that had previously collaborated on binational projects (Figure 2). Local and state health departments identified one or more clinical facilities in each city. The U.S. institutions are four primary-care clinics and three tertiary care hospitals. The Mexican sites comprise two general hospitals and four primary-care clinics. The primary-care institutions service 10,000–20,000 acute-care visits per site annually, while the hospitals service 23,000–51,000 acute-care visits per site annually.

Surveillance Strategy

After extensive discussions about local and national disease priorities, the team agreed on active sentinel surveillance for hepatitis and febrile exanthem syndromes. A standard protocol of laboratory testing is performed for specimens from patients who fulfill the clinical entry criteria (Table 1). Patients with acute hepatitis are tested at local laboratories for hepatitis A, B, and C. Depending on the initial results, specimens are tested at CDC for hepatitis D and E, and confirmatory testing for hepatitis C is performed. Patients with febrile exanthems are tested locally for measles and rubella. If these tests are negative, the specimens are tested at a state or national reference laboratory for rickettsiae, ehrlichiae, and, in selected areas, *Dengue virus* (DENV).

Many diseases selected for BIDS surveillance have well-defined preventive strategies, including vaccination. In Mexico, measles is targeted for elimination, and rubella vaccination was incorporated into the national vaccination program in 1998 (26). In the United States., rubella outbreaks have

Figure 2. Border Infectious Disease Surveillance project sentinel sites in sister cities along U.S.-Mexico border: Tiajuana–San Diego, Nogales-Nogales, Las Cruces–Ciudad Juarez–El Paso, and Reynosa-McAllen. The new cities are Mexicali-Imperial (the sister city pair near Tijuana–San Diego) and Brownsville (near McAllen).

occurred among Hispanic immigrants who were not previously immunized (27–30). The south Texas border also represents an important zone for transmission of DENV (31–33) and typhus (34–36). In the United States, routine vaccination for hepatitis A was recently recommended for children in most of the U.S.-Mexico border region (37). Since laboratory confirmation was not readily available in Mexico, most acute hepatitis was assumed to be acute hepatitis A; however, the extent of hepatitis B and hepatitis C infection was unknown. Syndromic surveillance facilitated the monitoring of diseases targeted for elimination as well as identification of emerging infectious diseases, such as hepatitis E and ehrlichliosis, which have not been previously well studied along the border. The selection of these surveillance conditions was influenced by the need to establish laboratory infrastructure in Mexico; the hepatitis and febrile exanthem testing protocols involve serologic assays with similar equipment and assay techniques. From the practical perspective, sentinel surveillance enabled clinics in several border cities to participate at a reasonable cost. Active surveillance was selected to enhance existing passive surveillance activities in both countries, and, rather than creating a parallel structure, all activities were integrated into state and national reporting systems.

Organizational Structure, Personnel, and Training

The group formed an executive committee and three subcommittees (epidemiology, laboratory, and communications). Nine sentinel site surveillance coordinators, who report to a local health department epidemiologist and a state-based epidemiologist, were hired and trained. The sentinel site coordinators are responsible for interviewing patients, completing data entry, and handling logistical issues such as specimen shipping and tracking. Considerable training was provided for border laboratory personnel. Only one of the Mexican border laboratories had experience performing serologic testing for hepatitis viruses, and none were experienced with testing for measles or rubella viruses. Laboratory scientists from Instituto de Diagnóstico y Referencias Epidemiológicas, Mexico City, received training in testing methods for rickettsiae and ehrlichiae.

Communications

Improving binational communication systems was critical to project success. In addition to language and cultural barriers, a large gap exists in communications infrastructure between the United States and Mexico. Many Mexican local and state health departments use combined telephone and fax lines and have no Internet access; regularly scheduled conference calls are a principal mechanism for communication. Borderwide meetings are held annually and sister city groups have regional meetings. A binational team of epidemiologists, laboratory scientists, and a representative from the El Paso Field Office of the Pan American Health Organization conducts site visits and evaluations.

Logistics

The movement of equipment, supplies, specimens, and financial resources between the two countries has been difficult and labor-intensive. Logistical issues include the challenges of moving laboratory equipment and supplies into Mexico and specimens across the border into the United States. Several U.S. and Mexican agencies regulate these cross-border movements. Although regulations are established at a federal level, they are often subject to local interpretation. As a result, the BIDS participants work closely with local agencies in their state.

Information-Sharing Protocols and Binational Cases

In the past, sharing of surveillance data has been constrained due to differences in political systems, limited forums in which to share information, and poor comparability of the data. Two protocols were developed to improve information sharing. The first describes the flow of information process in each country. Although the Mexican health sector is currently undergoing decentralization, officials from the federal Mexican Secretariat of Health continue to play the major role in reviewing, analyzing, and approving data before information is shared with U.S. counterparts and CDC. In contrast, U.S. local and state data can be shared without federal approval.

Table 1. Entry criteria for active sentinel surveillance

Hepatitis	Illness with jaundice or dark urine, or illness ≥6 days without jaundice and ≥3 of the following: abdominal pain, acholic stools, nausea or vomiting, fever, anorexia
Febrile exanthem	Fever and nonvesicular rash, or an illness >3 days with fever but no rash, cough, and diarrhea

The second protocol establishes conditions for urgent notification of sister city sites of outbreaks and cases of selected diseases, such as measles. Urgent notification also occurs for cases of binational public health importance, such as a case of hepatitis A in a person who works as a food handler on either side of the border. In the routine disease surveillance systems of both the United States and Mexico, a reportable condition diagnosed at a medical facility on one side of the border, in a patient who lives on the opposite side of the border, may not be included in routine disease reporting, and this information is not usually provided to the neighboring health officials. Data about these binational cases have not been traditionally captured in either country's reporting system. Therefore, binational case definitions were developed for BIDS participants (Table 2).

Table 2. Case criteria for a binational case of hepatitis or a febrile exanthem

Binational case (at least one of the following):
Person with hepatitis or febrile exanthem who traveled or lived in neighboring country during incubation period for suspected or confirmed disease.
Person with hepatitis or febrile exanthem who had contact with persons who traveled or lived in neighboring country during incubation period for suspected or confirmed disease.
Case for which binational cooperation is needed for case investigation, case management, or both.

Outcomes

The project timeline is shown in Table 3. Data collection began in late 1999, and data are currently being analyzed. As of mid-2002, the network had identified 867 persons with hepatitis and hepatitis syndrome (369 in the United States and 498 in Mexico) and 421 persons with a febrile exanthem syndrome (243 in the United States and 178 in Mexico). The project has enhanced local reporting of these conditions. In 2000, BIDS surveillance data from the sentinel site in Tijuana, Baja California, identified 300% more cases of laboratory-confirmed hepatitis A than would have been expected. All hepatitis A cases identified at the San Diego site were binational and were reported to the sister city health department in Tijuana.

The BIDS network has prompted valuable data exchange as well. In 2000, California and Baja California shared important information about measles in California and rubella in both states. The Baja California Health Department used BIDS surveillance data to conduct epidemiologic follow-up and targeted vaccination as part of their efforts to eliminate measles and reduce rubella. In 1999, the Mexican Secretariat of Health, CDC, and health officials from Texas and Tamaulipas conducted the first binational investigation of an outbreak of dengue fever (38).

Future Plans

Consolidation, evaluation, and expansion of BIDS will take place in 2002–2003. The project, which began at 9 sites, has expanded to 13 sites. Continuing efforts will focus on incorporating six new clinical sites and three new border cities (Figure 2). With sufficient expansion, BIDS may be able to calculate population-based incidence rates for selected infectious diseases in some border areas. However, BIDS will continue to deal with the complexities of conducting surveillance among mobile populations and obtaining accurate denominator information for the border region.

Although activities focused initially on hepatitis and febrile exanthems, the project is sufficiently flexible to incorporate other syndromes and diseases, including *West Nile virus* and infectious agents that could be used in bioterrorism events. BIDS continues to improve communications systems and support mechanisms for information exchange.

Conclusions

The BIDS project demonstrates that the development of a binational regional surveillance system for one of the world's busiest geographic boundaries is feasible. Success is highly dependent on extensive U.S. and Mexican local and state involvement; maintaining a balance among the competing priorities of this diverse group of participants continues to be one of the project's greatest challenges. A high level of participation among the group is an essential ingredient in creating a binational, locally-relevant agenda and enhancing long-term project sustainability. The BIDS surveillance system has required flexibility to incorporate local and state reporting requirements, while maintaining sufficient standardization of case definitions, data, and laboratory testing procedures. Dedicated coordination at the federal level of both countries has been essential. Maintaining federal political commitment and funding for the 3-year development and implementation period has been and continues to be critical.

BIDS promotes communication and cooperation through border-wide meetings and the binational subcommittees. The border sister city meetings provide a forum for exchange of ideas and discussion of issues of binational importance, thus strengthening cross-border relationships among counterpart epidemiologists and laboratory staff. Many logistical problems require local solutions best handled by the sister cities working together. Effective problem solving requires coordination and optimal communication among participants. However, this level of effectiveness will not be achieved in the border region until the infrastructure barriers are overcome, including substantial improvements in access to telephones, fax machines, computers, the Internet, and satellite teleconferencing.

BIDS continues to face major obstacles in the movement of equipment, supplies, specimens, and financial resources

Table 3. Timeline for implementation of Border Infectious Disease Surveillance project

1997	Mandate and objectives, site selection
1998	Binational planning, surveillance protocol with case definitions and data collection instruments
1999	Laboratory protocols and infrastructure; epidemiology training
2000–2001	Pilot data collection
2002–2003	Evaluation, consolidation, site expansion

across borders. These activities are cumbersome and time consuming for all project participants. Accords between the United States and Mexico should be developed to promote cooperation in public health and facilitate sharing of human and other resources and the moving of laboratory specimens across the border; these agreements would substantially enhance border health activities and benefit both countries. The states of Arizona and Sonora cooperated successfully in establishing a shared border health facility in Nogales, Sonora. This state-based model could be replicated in other areas of the border and reinforced with federal policies. To further enhance federal support, we suggest that a joint border field station be established by CDC, the Mexican General Directorate of Epidemiology, and the Instituto de Diagnóstico y Referencias Epidemiológicas.

The mandates from the United States–Mexico Border Health Association and the Council of State and Territorial Epidemiologists served as an initiation point for project activities but did not anticipate the need for additional federal-level agreements for data exchange. The BIDS group has drafted guidelines for conducting a binational outbreak investigation and will be implementing these as opportunities arise. Ongoing data collection will enable better characterization of binational cases. However, formal agreements at high levels of government are needed to authorize and endorse the timely binational exchange of epidemiologic and laboratory information about important infectious disease outbreaks and cases that occur along the border at BIDS sites and sites that are not currently part of the BIDS network.

Political changes at the local, state, and federal levels in Mexico frequently lead to changes in public health personnel, resulting in an ongoing need to train and incorporate new personnel into the project; this reality has highlighted the importance of institutionalizing any binational project through high-level formal agreements between the two countries. Weathering political changes is a challenge to infrastructure-building projects, like BIDS, which require several years of investment until tangible results, such as data are available.

To achieve the goal of building border epidemiology and laboratory capacity, BIDS established a system that built on existing strengths in syndromic and laboratory surveillance. The enhancement of border laboratory infrastructure at the Mexican sites was a major benefit. Additional support is essential, including stable funding for laboratory supplies and training courses, ranging from laboratory techniques to preventive maintenance of laboratory equipment; implementation of standardized quality control and quality assurance guidelines, such as a voluntary blinded proficiency testing program for selected tests; and a telephone consultation service with a toll-free telephone number to help staff address the problems.

Syndromic surveillance for hepatitis and febrile exanthems will allow us to estimate the magnitude of public health problems along the border, for example, acute hepatitis B and hepatitis C; determine the geographic distribution of diseases, such as typhus, ehrlichiosis, and dengue; detect outbreaks; evaluate control measures, such as immunization efforts to prevent measles, rubella, and hepatitis A and the reduction of breeding sites for mosquitoes that transmit dengue; and monitor emerging infections, such as hepatitis E, and generate hypotheses about these diseases that can be further studied. Systematic collection of surveillance data on binational cases will also better define the contribution of mobile populations to disease transmission.

As a model for true binational cooperation along the border, BIDS is a starting point from which a comprehensive infrastructure can be developed to accurately assess the health status of border residents and other migrants who come through the area. Ultimately, data provided by BIDS will be useful in the development of more effective prevention and control strategies for infectious diseases in this unique region.

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Chagas Disease in a Domestic Transmission Cycle in Southern Texas, USA

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After three dogs died from acute Chagas cardiomyopathy at one location, an investigation was conducted of the home, garage, and grounds of the owner. A serologic study was conducted on stray dogs, and an ecologic niche model was developed to predict areas where the vector *Triatoma gerstaeckeri* might be expected.

The Study

Chagas disease is caused by the parasitic protozoan *Trypanosoma cruzi* and affects an estimated 12 million persons throughout South and Central America and Mexico (1,2). In the United States, the disease exists almost exclusively as a zoonosis; only five autochthonous insect-borne cases have been reported in humans (3). The distribution of Chagas disease in the United States includes approximately the southern half of the country. Twelve species of triatomines are known to occur in the United States, the most important being *Triatoma sanguisuga* in the eastern United States, *Triatoma gerstaeckeri* in the region of Texas and New Mexico, and *Triatoma rubida* and *Triatoma protracta* in Arizona and California (4,5).

In the small community of San Benito, Texas (Figure 1), after three pet dogs died from Chagas cardiomyopathy, personnel from the Texas Department of Health, the Cameron County Health Department, Environmental Health Division, and the Centers for Disease Control and Prevention (CDC) inspected the owner's home, garage, and grounds for potential triatomine insect vectors (Figure 2). Blood was drawn from four dogs and two persons residing on the property and tested for antibodies to *T. cruzi*. A second site approximately 2 miles away was also inspected and blood drawn from three dogs, one of which had been diagnosed as positive for *T. cruzi* by the original veterinarian. A follow-up serologic survey was conducted to determine the percentage of the stray dogs in Cameron County that would test positive for Chagas disease antibodies. Once a week, samples from stray dogs were shipped to CDC for testing. Each sample was issued an identification number; and information on the animal's location, sex, age, health condition, and size was recorded. Serum specimens were tested for anti-*T. cruzi* antibodies by indirect immunofluorescence (IIF) (6,7).

Figure 1. San Benito, Texas, where three dogs died of Chagas disease.

Ecologic niches and potential geographic distributions were modeled by using the Genetic Algorithm for Rule-set Prediction (GARP) (8–10). In general, the procedure focuses on modeling ecologic niches, the conjunction of ecologic conditions within which a species is able to maintain populations without immigration. Specifically, GARP relates ecologic characteristics of known occurrence points to those of points randomly sampled from the rest of the study region, seeking to develop a series of decision rules that best summarizes those factors associated with the species' presence. Recently, this method has been used to study the distribution of species complex members and vector-reservoir relationships with respect to Chagas disease (11,12).

Figure 2. View of location of the three dogs that died of Chagas disease.

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Inspection of the residence where the three dogs lived indicated a substantial infestation with the triatomine species *T. gerstaeckeri* (Figure 3). Triatomines were collected under cement slabs of a backyard patio adjacent to the house and from a garage located approximately 75 feet from the home (Figure 2). Of 31 live triatomines collected, including adults of both sexes and immature stages (i.e., two fifth-instar nymphs), 24 contained *T. cruzi*-like parasites in their hindgut (Figure 4). Cultures were established from triatomine urine collected from insects that were fed in the laboratory and placed in 1.5-mL microcentrifuge tubes. Approximately 50 µL of clear urine was injected into Novy, Nicolle, & MacNeal culture medium (13). The cultures were positive for parasites confirmed to be *T. cruzi*, on the basis of morphologic criteria. Inspection of the second residence failed to indicate a bug infestation; however, the pet owner recalled frequently observing both rats (*Rattus* spp.) and opossums (*Didelphis virginiana*) on the premises. At the first site, three of the four dogs tested positive for *T. cruzi*, with titers ranging from 1:128 to 1:256. Neither of the two persons had positive antibody titers against *T. cruzi*. At the second site, only the previously diagnosed dog tested positive, with a titer of 1:256. The other two dogs tested negative, as did the pet owner. Serum samples from stray dogs from Cameron County, Texas, were tested for anti–*T. cruzi* antibodies. Of 375 dogs tested, 28 (7.5%) were positive by IIF, with titers ranging from 1:32 to 1:512. The sensitivity of this test in humans is 98.8% (pers. comm., Patricia P. Wilkins, Division of Parasitic Diseases, CDC). Because of the low specificity of serologic tests for distinguishing *T. cruzi* from *Leishmania* spp., all positive samples were tested for antibodies to *L. donovani*. A low level of cross-reactivity was observed in 17 of the 28 samples. In each case, however, the titer was 1–2 dilutions less than the titer to *T. cruzi*, indicating a primary response to *T. cruzi* rather than to *Leishmania* spp. Ecologic niche models for *T. gerstaeckeri* were developed by using GARP, based on published and unpublished collection records from Mexico and the southwestern United States. The model predicted a distribution for this species that extends from central Mexico, through central Texas, the Texas panhandle, into northern Texas and southeastern New Mexico (Figure 5).

Figure 3. *Triatoma gerstaeckeri* collected at the locality.

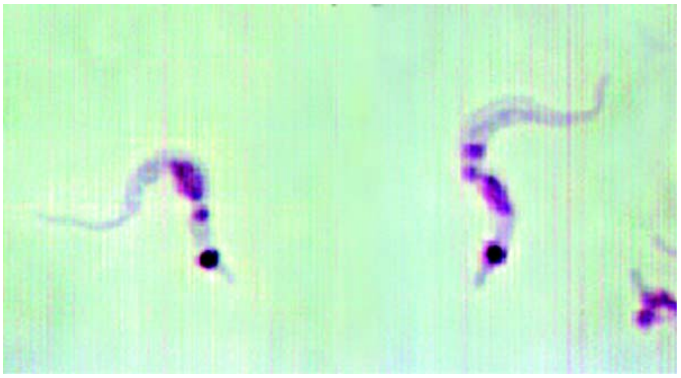

Figure 4. *Trypanosoma cruzi* parasites in hindgut of a field-collected triatomine bugs.

Conclusions

Triatoma gerstaeckeri is considered a sylvatic species, most frequently associated with pack rat (*Neotoma* spp.) burrows (4). Although individual triatomine insects occasionally invade domestic dwellings throughout the southwestern United States and Mexico (4,5,14), this species has not been reported to colonize these habitats. In this investigation, colonization appears to have occurred, based on the observation of large numbers of bugs, including ones in immature stages. In the Chagas disease–endemic regions of South and Central America, the primary risk for insect transmission to humans is related to the efficiency with which local vector species can invade and colonize homes, resulting in a domestic transmission cycle for what is otherwise exclusively a zoonotic disease in the southern United States. In disease-endemic countries, higher house infestation rates generally result in a higher risk of transmission. At the first site in south Texas, six dogs either died or tested positive for *T. cruzi,* and 24 of 31 bugs contained

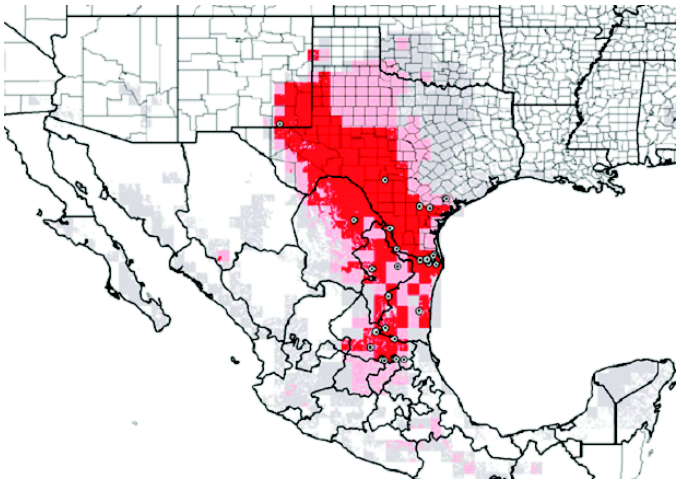

Figure 5. Genetic Algorithm for Rule-set Prediction-generated ecologic niche model, predicting distribution of *Triatoma gerstaeckeri*. Small circles show actual collection sites. Area in dark red is where high certainty exists for the specific niche of the species. The area in light red is the zone of moderate certainty, and the area in gray is for low certainty.

hindgut trypanosomes. These observations demonstrate the existence of a domestic transmission cycle for an insect species that is typically considered a zoonotic vector. Whether this observation represents an isolated case or actually occurs more frequently but remains unrecognized, indicating an emerging public health problem, remains to be determined. The serologic results in stray dogs are very similar to those reported in previous studies from the region, suggesting that the disease is stably maintained in this reservoir host (15,16). The distributional predictions based on GARP models indicate a potentially broad distribution for this species and suggest additional areas of risk beyond those previously reported (14), should this problem become of greater public health concern.

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Shiga Toxin–Producing *Escherichia coli* Infections Associated with Hemolytic Uremic Syndrome, Italy, 1988–2000

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The mean annual incidence of hemolytic uremic syndrome in persons ≤15 years of age in Italy from 1988 to 2000 was 0.28 per 100,000 population. Laboratory investigations showed that Shiga toxin–producing *Escherichia coli* (STEC) infection occurred in 73.1% of patients. STEC O157 was the most common serotype, but a considerable number of cases were from infections by non-O157 STEC.

Hemolytic uremic syndrome (HUS) is the most severe and specific clinical manifestation of infections with Shiga toxin (*Stx*)–producing *Escherichia coli* (STEC), especially *E. coli* O157:H7 and other enterohemorrhagic serotypes; the incidence of HUS represents a robust index of the total incidence of these infections in a population (1). In Italy, HUS notification is not yet mandatory, but a nationwide surveillance system was established in 1988 and has been followed on a voluntary basis with the collaboration of the Italian Society for Pediatric Nephrology. Since then, the HUS surveillance system has been maintained to monitor the incidence of the disease, describe those affected, identify the STEC serotypes associated with HUS, and investigate possible risk factors associated with STEC infection.

The Study

The surveillance system was based on all pediatric nephrology centers that perform dialytic treatment in Italy. HUS cases were defined as patients ≤15 years of age with evidence of renal failure, intravascular hemolysis, and thrombocytopenia (platelet count <100,000/mm^3) (2). From May 1988 to December 2000, a total of 342 HUS cases were reported to the surveillance system. Twenty-four cases were part of two outbreaks that occurred in northern Italy in 1992 (3) and 1993 (4), which were associated with *E. coli* O111 and *E. coli* O157 infections, respectively. A third cluster of three cases associated with *E. coli* O26 infection occurred in spring 1997 in Naples, in southern Italy. No outbreak of diarrhea was detected around these clusters, and no food or environmental source of infection was identified. The mean incidence per year was 0.28/100,000 population in patients ≤14 years of age, with a range of 0.13/100,000 population in 1991 to 0.46/100,000 population in 2000. Since 1994, a steady increase of incidence has been observed (chi square; p=0.003). We observed the highest incidence (>0.4/100,000; children ≤14 years of age) in the northern part of the country; the lowest incidence was reported in the insular regions, Sicily and Sardinia. The incidence was higher in children <5 years of age (mean=0.75 cases/100,000 population; range 0.33–1.10).

The incidence observed in this study is the lowest among those reported in Europe for the same period (5–10). This finding, together with the rare occurrence of outbreaks (3,4), suggests that STEC infections are relatively uncommon in Italy. In northern regions, where most cattle farming takes place, the average annual incidence was similar to that of central European countries. The very low incidence observed in most regions of southern Italy and in the islands suggests that the Mediterranean basin is an area with a low incidence of STEC infection (10,11).

Fifty-one percent of the 342 patients were boys (age range 1 month–14 years; median age 23 months). Of 11 (3.2%) patients who died, 6 were in the acute phase of HUS. Among the 274 patients for whom the clinical information was recorded, prodromal bloody diarrhea was reported in 48%, nonbloody diarrhea in 30%, and no diarrhea in 22%. In 21% of cases, diarrhea was reported in other household members before onset of HUS in the child. In addition, diarrhea among schoolmates was reported in 7.3% of cases. These findings suggest that person-to-person transmission may play a major role in the epidemiology of sporadic HUS cases, as described in other studies (5,12).

Immediately after diagnosis of HUS, stool samples were collected from patients and household contacts when possible. Specimens were examined for the presence of free fecal *Stx* by the Vero cell assay and streaked onto MacConkey agar for STEC isolation. Serum samples were collected after diagnosis of HUS and tested for antibodies to the lipopolysaccharide (LPS) of five major STEC serogroups (O157, O26, O103, O111, and O145) by enzyme-linked immunosorbent assay as described (3,13).

Clinical specimens were collected from 249 case-patients. Stools were obtained from 228 patients and at least one serum

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specimen from 235 patients. The combined use of microbiologic and serologic techniques provided evidence of STEC infection in 182 (73.1%) of 249 cases examined (Table), a percentage similar to those reported in similar studies (5,7–9,14,15).

We documented both microbiologic and serologic evidence of STEC infection in a considerable proportion of patients who did not have diarrhea (52.4%), although less frequently than in patients with bloody diarrhea (79.3%; chi square p=0.001) and nonbloody diarrhea (74.6%; p=0.01). A similar proportion of patients with STEC infection without diarrhea has also been described in other European studies (7–9), suggesting that Shiga toxins are able to translocate across the intestinal mucosa and reach the target endothelial cells even if the STEC infection does not result in overt diarrhea.

Despite the use of different diagnostic methods, 26.9% of patients had no evidence of STEC infection. Some patients may have had an infection caused by a STEC strain belonging to a serogroup that was not included in the LPS panel used in this study. In addition, some patients with STEC infection apparently do not have a serologic response (7).

Stool examination provided evidence of STEC infection in 67 (29.4%) of 228 patients examined. Free fecal *Stx* was identified in stool samples from 63 (28.0%) of 225 patients examined. Most positive samples contained *Stx*2 alone (47 samples) or in combination with *Stx*1 (11 samples). Nineteen STEC strains were isolated from 18 patients (7.9%); these strains were from serogroups O157 (6 strains), O111 (4 strains), O26 (2 strains), O55 (2 strains), O86, O113, O118, O120, and undetermined O (one strain each). The proportion of STEC recovery from stools, similar to that reported in other studies (7,14), may be low because of the interval between onset of diarrhea and stool collection (median 8 days), antimicrobial therapy administered to many patients before and after the development of HUS (data not shown), or the freezing, storing, or shipping of the specimens.

Antibodies to LPS were detected in the sera of 144 (61.3%) of 235 patients examined; in 115, this presence was the only evidence of STEC infection. Antibodies were detected to O157 (72 cases), O26 (31 cases), O111 (15 cases), O145 (15 cases), and O103 (8 cases). Three patients had antibodies to two serogroups: O157 and O26, O26 and O145, and O26 and O103.

Serotyping of isolated STEC strains and detection of serogroup-specific LPS antibodies showed evidence of infection with STEC O157 in 74 patients. Thirty-four patients had infection with STEC O26, 17 with STEC O111, 16 with O145, and 9 with O103. As shown in other studies, *E. coli* O157 was the most common STEC serogroup associated with HUS. However, >50% of STEC-positive cases had evidence of infection with non-O157 STEC. This proportion is higher than that reported in studies conducted in the United States (14), France (7), United Kingdom (6), Belgium (8), and the Netherlands (15) and suggests that the circulation of STEC O157 in Italy is lower than in other countries.

The distribution of the four most frequent STEC serogroups associated with HUS cases by year is shown in the Figure. Infections with non-O157 serogroups, in particular those with *E. coli* O26, increased over time and since 1996 have outnumbered those with STEC O157. Infections with STEC O111 were frequent from 1990 to 1993, after which they became rare. This observation emphasizes that the incidence of non-O157 serotypes may vary over time.

The frequency of STEC O157 infection increased with age; non-O157 infections were more common in younger children. This finding is in agreement with the observation that non-O157 serotypes were the leading cause of HUS in young children in Germany (9) and could reflect differences in the epidemiology or in the pathogenic mechanism of these infections with respect to those sustained by STEC O157.

For 115 STEC-positive HUS cases, we obtained stool specimens from 333 household contacts and 119 household contacts of 36 STEC-negative HUS cases. Laboratory evidence of STEC infection was found in 10 (3.3%) and in 1 (0.8%) of household contacts of STEC-positive and STEC-negative HUS cases, respectively. Two household contacts were infected with STEC strains belonging to a serogroup different from that associated with the HUS case. One of 11 STEC-positive household contacts reported diarrhea. Our findings underscore the potential for person-to-person transmission of STEC infection.

Conclusions

The results of this 13-year surveillance indicate that the overall incidence of HUS in Italy is lower than in other European countries, although a small but steady increase has been

Table. Evidence of STEC infection in 249 Italian children with HUS, shown by presence of prodromal diarrhea[a]

	Patients with symptoms no. positive/no. examined (%)				
Evidence of STEC infection	Bloody diarrhea	Nonbloody diarrhea	No diarrhea	No information	Total
STEC isolation	9/88 (10.2)	4/63 (6.3)	4/39 (10.2)	1/38 (2.6)	18/228 (7.9)
Free fecal *Stx*	23/86 (26.7)	21/63 (33.3)	10/38 (26.3)	9/38 (23.7)	63/225 (28.0)
Antibodies to LPS	61/88 (69.3)	39/63 (61.9)	14/41 (34.1)	30/43 (69.8)	144/235 (61.3)
Any	73/92 (79.3)	50/67 (74.6)	22/42 (52.4)	37/48 (77.1)	182/249 (73.1)

[a]STEC, Shiga toxin–producing *Escherichia coli*; HUS, hemolytic uremic syndrome; *Stx*, Shiga toxin; LPS, lipopolysaccharide.

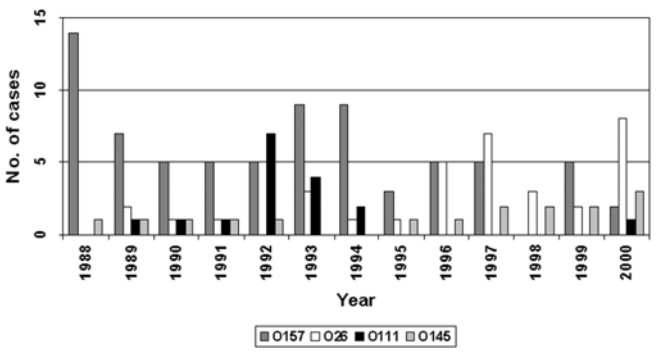

Figure. Distribution of hemolytic uremic syndrome cases associated with Shiga toxin–producing *Escherichia coli* O157, 026, O111, and O145, by year.

observed since 1994. As in most countries, STEC O157 represented the most common serotype, but a considerable proportion of cases was due to infections by non-O157 STEC, particularly O26, increasing over the last 5 years. Surveillance of HUS can provide useful information about the trend of STEC infection in the general population and represents an important means to identify STEC serotypes that are highly pathogenic to humans and may emerge as public health threats.

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Foot and Mouth Disease and Cryptosporidiosis: Possible Interaction between Two Emerging Infectious Diseases

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During 2001, a large outbreak of foot and mouth disease occurred in the United Kingdom, during which approximately 2,030 confirmed cases of the disease were reported, >6 million animals were slaughtered, and strict restrictions on access to the countryside were imposed. We report a dramatic decline in the reported incidence of human cryptosporidiosis in northwest England during weeks 13–38 in 2001, compared with the previous 11 years. This decline coincided with the period of foot and mouth restrictions. No similar reduction occurred in the other 26 weeks of the year. We also noted a substantial decline in the proportion of human infections caused by the bovine strain (genotype 2) of *Cryptosporidium parvum* during weeks 13–38 in that year but not during the other weeks.

Cryptosporidiosis is an acute diarrheal disease caused by a protozoan parasite *Cryptosporidium parvum* (1). Although the disease is self-limiting in most instances, in certain immunocompromised patients the infection can be very severe and potentially fatal (2). This disease is now the most common parasitic cause of human diarrheal disease in the United Kingdom; over the last 10 years, the northwest region of England has regularly reported more cases than any other region in the country (3). Cryptosporidiosis was originally thought to be a zoonosis, but epidemiologic studies (4) and the description of two *C. parvum* genotypes, one of which was only found in humans (genotype 1 or H), has highlighted the importance of person-to-person transmission (5). Nevertheless, zoonotic transmission is still an important route of infection, though the proportion of human *Cryptosporidium* infections originating from animals is still unknown.

During 2001, a major outbreak of foot and mouth disease occurred in the United Kingdom. Coincident with this outbreak, we noted a dramatic decline in reports of *Cryptosporidium* infection in the northwest region. We describe the change in epidemiology of reported cryptosporidiosis during that year and discuss the hypothesis that this reduction may have resulted from public health measures introduced to control the foot and mouth disease epidemic.

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Foot and Mouth Disease Epidemic in 2001

During 2001, the United Kingdom experienced its largest recorded outbreak of foot and mouth disease. The first case was identified on February 19 in sows awaiting slaughter at an abattoir in Essex County, in the south of England. The epidemic reached its peak on March 30 when 61 new cases were identified. Although most cases had occurred by the end of April, the final case was not identified until September 30, for a total of 2,030 confirmed cases (Figure 1) (6). A case was defined as infection in one or more animals on a single premises.

To control the epidemic, 4,196,580 animals were slaughtered (7). Animals were slaughtered if they were on infected premises, on farms neighboring infected premises, on premises with animals that had direct contact with infected animals, and if any infection was suspected. In addition, 2,048,769 animals were slaughtered under the livestock welfare (disposal) scheme as movement of animals was otherwise banned. In total, 6,245,349 animals were slaughtered. Officials disposed of approximately 600,000 tons of carcasses: approximately 130,000 by rendering; 95,000 in licensed commercial landfill sites; and 61,000 at four mass burial sites (8). Burial of carcasses occurred on >900 farms and burning on >950 farms. Approximately 100,000 tons of pyre ash were transferred to landfills. Specific details on the timing of slaughter are not available, although because most slaughtering occurred in response to, and soon after, the diagnosis of new cases, the slaughter curve would be expected to follow the epidemic curve (Figure 1).

In addition, widespread bans were set on the movement of animals and human access to the countryside was restricted. The first restriction order in the United Kingdom was issued on February 21, covering parts of Essex and Kent in southern England. The first restriction order in the northwest region was issued 6 days later. Thereafter, new orders were issued as new

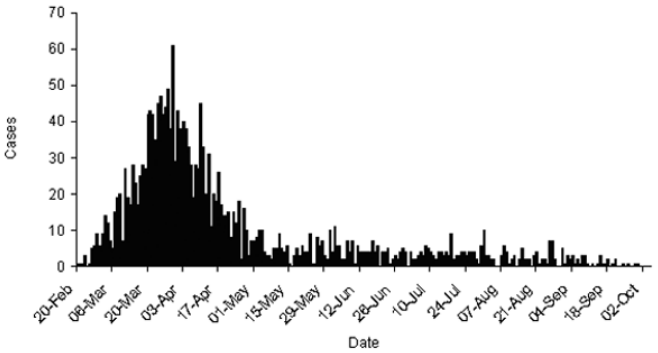

Figure 1. Epidemic curve of the foot-and-mouth disease epidemic, United Kingdom, 2001.

cases were identified. Initially, most counties in or near infected areas imposed strict and widespread bans on access to the countryside, although the central government subsequently persuaded most counties to relax their most rigorous bans except in areas of confirmed disease activity. Beginning in early June, public rights of way started to be reopened; by July 27, an estimated 85% of public rights of way were open again. However, even after restrictions were lifted, fewer persons visited the countryside during the summer of 2001. More details, including maps of the distribution of cases, can be obtained from the Department of the Environment, Food and Rural Affairs (available from: URL: www.defra.gov.uk/footandmouth).

In general, restrictions were lifted when local areas had been free of infection for 6–8 weeks. The final case of foot and mouth disease diagnosed, on September 30, was in the northwest region of England. The last restrictions were lifted on November 19.

Human Cryptosporidiosis

Data used in this study came from routine reports to the Communicable Disease Surveillance Centre–North West (CDSC–NW) and from isolate submissions to the Public Health Laboratory Service (PHLS) Cryptosporidium Reference Unit. These reports and isolates come from both National Health Service and PHLS microbiology laboratories in the region. Case-patients were defined as persons who visited a physician's office or hospital because of diarrhea and provided a stool sample that tested positive for *Cryptosporidium*. All laboratories in the northwest region report their positive stool sample results to CDSC–NW, usually electronically.

The area covered by the northwest region is home to 6.6 million people, 65% of whom live in the large urban areas of Liverpool and Manchester. The central and southern parts of the region have both cattle and sheep farming. The northern part of the region covers the southern English Lake District in the county of Cumbria, where the main industries are tourism and sheep farming.

Figure 2, which shows the number of reports for 1991–2001, depicts cumulative totals by week for each year, providing the total number of cases in each year up to and including the week indicated. The key observation is the virtually flat slope of the curve for weeks 17 to 32, indicating that very few cases occurred during that time.

Defining the period when foot and mouth disease controls were in place across the region was difficult because controls were imposed at different times at each locality, reflecting the progress of the epidemic. The index case was identified in the last week of February (week 9), but controls were not widespread until a few weeks later. We chose to designate week 13 (week beginning March 26, 2001) as the first week in which any controls would likely have an impact on laboratory reports of cryptosporidiosis. The incubation period for cryptosporidiosis is long (1–2 weeks), and often delays occur in submitting samples to the laboratory and the subsequent reporting of

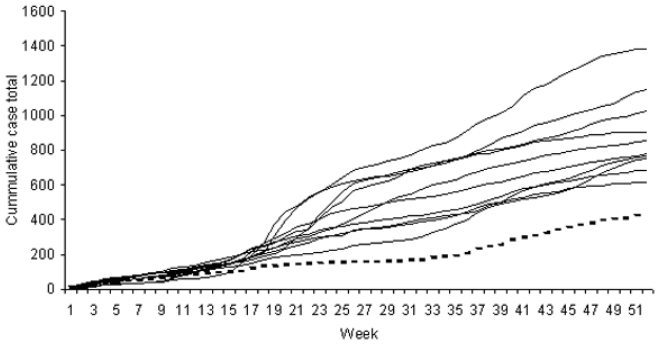

Figure 2. Cumulative reports of cryptosporidiosis, northwest region of England, 1990–2001. Broken line indicates data for 2001; other lines indicate data for 1990–2000.

cases by the laboratories. From our past experience of outbreak investigations, we know that the time from the causative event to detectable changes in laboratory reporting is 3–4 weeks (9). We then arbitrarily deemed the 26 weeks after week 13 to be the period when controls were in place. Reported cases for weeks 13–38 in 2001 were then compared with the same weekly period for the previous 11 years. We made this same comparison for the remaining 26 weeks of each year (weeks 1–12 and 39–52).

Reports during weeks 13–38 were substantially lower than in previous years, though not for the other weeks in the year (Figure 2) (Table 1). To determine the strength of this reduction, we calculated a t-test value as follows:

$$t_{n-1} = \frac{y - \bar{x}}{s\sqrt{1 + \frac{1}{n}}}$$

, where y is the value for 2001, n=number, s=standard deviation, and $\bar{x}$=mean of the previous 10 years. The number of reports during weeks 13–38 was significantly lower in 2001 compared with reports during those weeks in the previous 10 years (t_9= –1.993, p=0.039), but the number of

Table 1. Total reported cases of human cryptosporidiosis in time periods, northwest England, 1990–2001

Yr	Total cases for England and Wales	Cases reported in northwest		
		Total for yr	Wks 13–38	Other weeks in yr
1991	5,165	768	372	396
1992	5,211	1,151	708	443
1993	4,832	850	577	273
1994	4,432	683	387	296
1995	5,691	750	367	383
1996	3,660	612	353	259
1997	4,321	1,023	701	322
1998	3,745	777	517	260
1999	4,759	903	722	181
2000	5,799	1,382	872	510
Mean for 1991–2000	4,762	890	558	332
2001	3,681	428	159	269

reports for the other weeks of 2001 was not significantly lower (t_9 = –0.384, p=0.355).

During 2001, 428 cryptosporidiosis cases were reported during the year compared with 1,382 cases in 2000 (69.0% decline). For the period from weeks 13–38, 159 cases were reported compared with 872 reports in 2000 (81.8% decline). Throughout England and Wales, a 36.5% reduction in cases for 2001 was reported compared with 2000 (from 5,799 to 3,681) (10).

Strains of *Cryptosporidium* from those infected in the northwest region are generally sent to the Cryptosporidium Reference Laboratory at Swansea Public Health laboratory for typing. However, laboratories use varied criteria to decide which strains to send for typing, and not all laboratories send strains. Strains were genotyped by using polymerase chain reaction and restriction fragment length polymorphism analysis of a region of the *Cryptosporidium* oocyst wall protein gene (11). The results of typing for 2000 and 2001, the only years with typing data available, are shown in Table 2. A significant decline in the proportion of strains due to the bovine genotype (compared to all others) occurred in weeks 13–38 in 2001 compared with weeks 13–38 in 2000 (chi square=20.01, p=0.000008) but did not occur during the other 26 weeks (chi square=3.68, p=0.06).

Conclusions

Most of what is known about the epidemiology of *Cryptosporidium* infections comes from outbreak investigations that have generally highlighted drinking water and recreational contact with water as major sources (12). Despite the considerable interest in *Cryptosporidium* in both the United States and the United Kingdom in recent years, very little is known about the epidemiology of sporadic infection with this organism.

The conclusion that the decline in cases was related to the outbreak of foot and mouth disease is warranted, as the decrease in expected reports was almost coincident with the introduction of control measures (after an appropriate lag to account for incubation period and reporting delay). The mechanism for this decline is unclear. The decline is likely to be real and not due to reduced efficiency of the surveillance system because reports of infection with *Campylobacter*, the most commonly reported enteric pathogen, showed no similar reduction in 2001 compared with reports in 2000.

Table 2. Distribution of genotypes of *Cryptosporidium parvum*, United Kingdom, 2000 and 2001

		C. parvum genotype			
Wks	Yr	1 (% human)	2 (% bovine)	Other (%)	Total
13–38	2000	185 (29)	440 (69)	9 (2)	634
	2001	59 (42)	70 (50)	12 (8)	141
1–12 and 39–52	2000	171 (62)	96 (35)	10 (4)	277
	2001	148 (70)	56 (27)	7 (3)	211

Following several outbreaks of disease linked to a single water supply system (13), the local water utility has implemented a number of control measures; these measures likely also had an effect. However, the control measures imposed on a single water supply could not explain the decline seen throughout the region or elsewhere in the United Kingdom.

Another explanation may be the decline in animal population as a result of the slaughter policy. However, marked reductions in cases were seen, even in those areas where there were relatively few animals slaughtered. For example, in the three northern health-authority areas where most cases occurred, 299 cases of cryptosporidiosis occurred during weeks 13–38 in 2000 whereas in 2001, 44 cases occurred (an 85% reduction). In Cheshire, where relatively few cases were identified, the respective figures were 35%, 12%, and 66%. In our view, the most likely explanation for the decline in cases of cryptosporidiosis was the removal of access to the countryside, which prevented humans from coming into contact with farm and wild animals and their excrement.

The surveillance data presented support previous evidence that zoonotic transmission is a major route of infection in this region (14). However, caution must be used when extrapolating this experience to the rest of the United Kingdom. The fact that one third of cryptosporidia detected in England are of human only (genotype 1) type has been described (14). Also, the relative distribution of genotypes 1 and 2 in England varies from region to region with the northwest region having the highest proportion of type 2 (bovine) strains detected. Consequently, the high proportion of infections being suggested as zoonotic in this report would not necessarily apply elsewhere.

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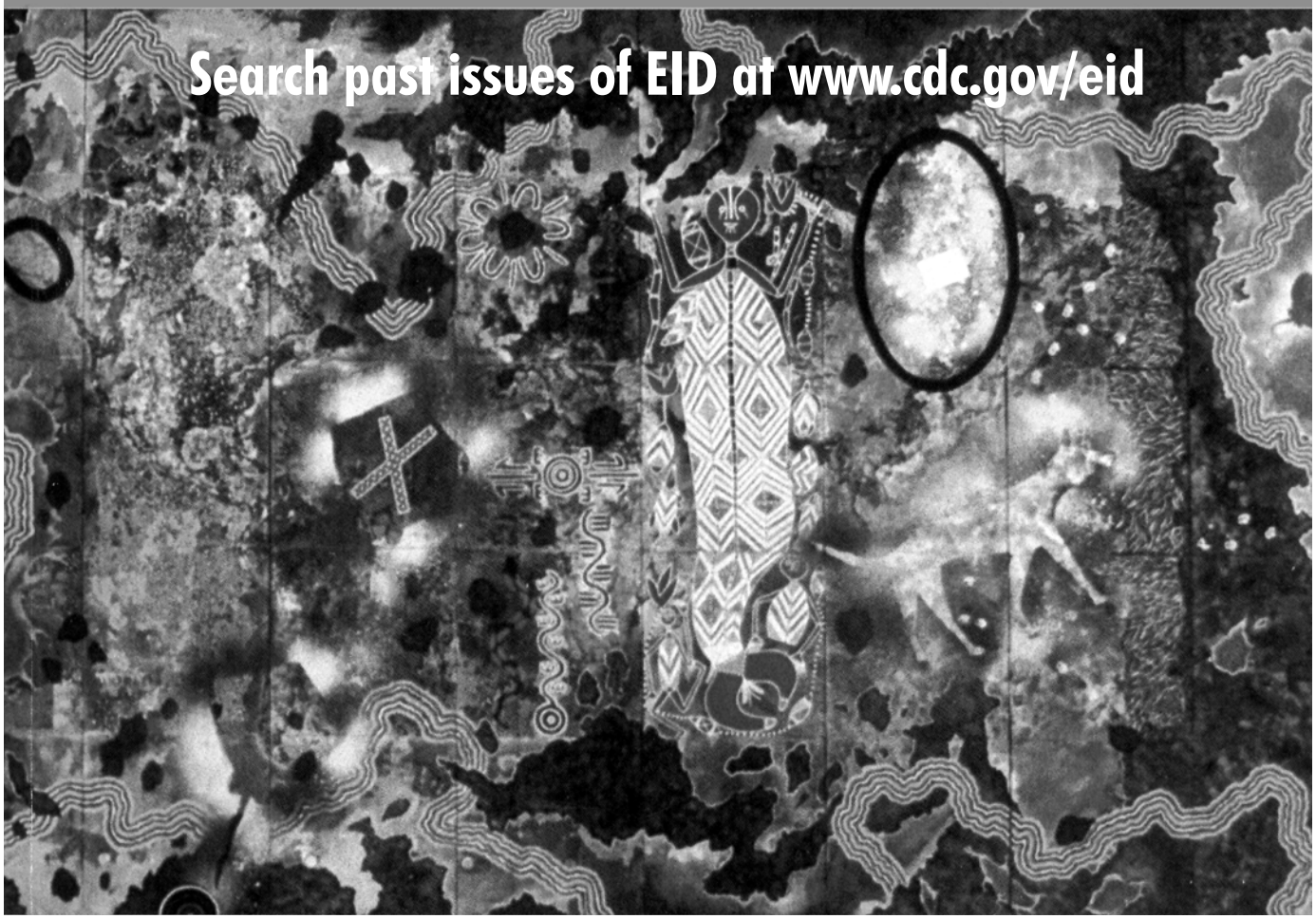

Enteropathogenic *Escherichia coli* O157 Strains from Brazil

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We describe two serogroup O157 *Escherichia coli* strains from Brazilian infants with diarrhea. A variety of assays indicate that these strains belong to the enteropathogenic, not the enterohemorrhagic, pathotype. These strains possess a novel *bfpA* allele encoding the type IV pilin characteristic of typical enteropathogenic *E. coli* strains. Our results emphasize the pitfalls of classifying pathogenic *E. coli* by serogroup.

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is the serotype most commonly associated with hemorrhagic colitis and the hemolytic uremic syndrome (1). EHEC strains share with enteropathogenic *E. coli* (EPEC), a leading cause of infant diarrhea in developing countries, the ability to induce the attaching and effacing effect on host cells. This property is specified by a pathogenicity island that includes the *eae* gene encoding the outer membrane adhesin intimin. EPEC are defined by this attaching and effacing phenotype or, at a molecular level, by the presence of the *eae* gene and the absence of the genes for Shiga toxins (2). Typical EPEC strains have a large plasmid that encodes bundle-forming pili and the localized adherence phenotype, while atypical strains lack these properties. EHEC differ from EPEC in that they produce Shiga toxins but not bundle-forming pili.

Here we describe two strains of the O157 serogroup identified as part of an ongoing epidemiologic survey of pathogenic *E. coli* in Brazil (3). Strain SC373/2 was isolated from a 9-month-old infant in Joinville, Santa Catarina, in 1997, and RN587/1, from a 7-month-old infant in Natal, Rio Grande do Norte, in 1998. Both patients had acute diarrhea of 7 days' duration, accompanied by vomiting, fever, and moderate dehydration. These strains were the only enteropathogenic bacteria isolated from the patients' stools. Rotavirus and cryptosporidia were not detected.

We used a combination of phenotypic assays and DNA sequencing to further characterize these isolates. DNA probe testing was performed by established methods (4). A fragment of the *eae* gene was amplified by polymerase chain reaction (PCR) and sequenced as described (5). Serotyping was performed at the *E. coli* Reference Laboratory (University Park, PA). Tests of localized adherence, autoaggregation, and attaching and effacing were performed as described (6,7). The *bfpA* gene was amplified by PCR and sequenced as described (8). Multilocus sequence typing was performed as described (9).

We found a number of indications that these O157 strains are not EHEC but unexpectedly fall within the EPEC pathotype. First, both strains tested probe negative for the Shiga toxin (*stx*) genes characteristic of EHEC. Second, both strains carried an *eae* (intimin) gene, identified by sequencing as an α allele characteristic of the group of strains known as EPEC 1, rather than the γ allele characteristic of O157:H7 EHEC (C.L. Tarr and T.S. Whittam, unpub. data). As expected of both EPEC and EHEC, both strains are capable of attaching and effacing as demonstrated by using the fluorescence actin staining test. *E. coli* strains that are eae^+ and *stx*– are by definition, EPEC (2). Third, both strains lacked the H7 flagellar antigen, although they were motile. The H antigens were difficult to type; RN587/1 reacts weakly with H8 antiserum. Fourth, both strains rapidly fermented sorbitol, a phenotype lacking in most O157:H7 strains. Fifth, the nucleotide sequences of part of the coding regions of 14 chromosomal loci encoding proteins with housekeeping functions indicated a close relationship of these strains with the EPEC 1 clonal group. The DNA sequences of the two O157 strains were identical over a total of 2,240 codons. Comparison of the multilocus sequence data to the reference strains K-12, EDL-933 (O157:H7; EHEC 1), and E2348/69 (O127:H6; EPEC 1) indicated that the EPEC O157 isolates were most closely related to E2348/69. The percentage of nucleotide difference in the total of 6,720 bp was 0.72 ± 0.10 (E2348/69), 2.66 ± 0.20 (K-12), and 2.95 ± 0.21 (EDL-933). Phylogenetic analysis with these sequences from a diverse collection of strains, including EPEC, EHEC, and other pathotypes, likewise shows that RN587/1 and SC373/2 are much more closely related to EPEC 1 than to EHEC O157 strains (Figure 1). Both strains also tested probe positive for the EPEC adherence factor plasmid, which is associated with localized adherence.

Finally, both strains carried the *bfpA* gene encoding bundlin, the structural subunit of the bundle-forming pilus expressed by typical EPEC strains but not by EHEC. Both strains are also capable of autoaggregation and localized adherence, the two phenotypes associated with expression of bundle-forming pili. The *bfpA* gene isolated from these strains has a unique sequence (GenBank accession number AF474407). We call this version of *bfpA* the β6 allele. When compared to the α and β *bfpA* alleles described previously (8), the β6 *bfpA* allele maps between the β1 allele and a cluster of the β2–5 alleles (Figure 2). The predicted β6 bundlin amino acid sequence maps between the β1 and β2 and the β3–5 pilin proteins.

Our findings emphasize the fact that *E. coli* with the O157 O antigen are not always EHEC but may belong to other pathotypes. Scotland et al. described Shiga toxin–negative O157:H8 *E. coli* strains isolated from children with diarrhea

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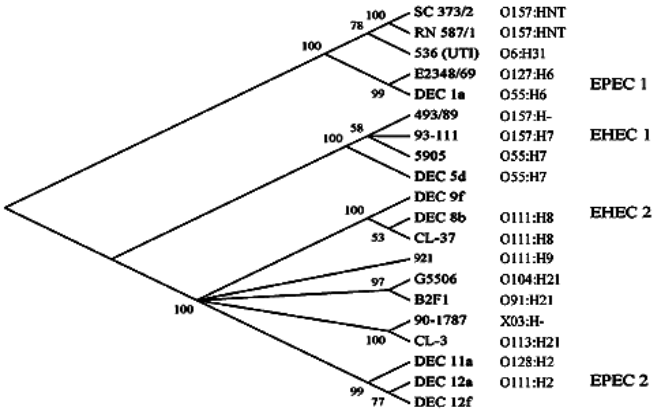

Figure 1. Clonal phylogeny of *Escherichia coli* strains of various pathotypes inferred from distances at synonymous sites in sequences of 13 concatenated loci (*mutS* was not included) by using the neighbor-joining algorithm. This consensus tree has numbers at each node, representing the percentage of bootstrapped trees in which the node was observed. SC373/2 and RN587/1 are the two O157 strains from Brazil. The other pathogenic strains included in the figure are described in Reid et al. (9). The serotype of each strain (when available) appears to the right of its designation. HNT denotes a nontypeable flagellar antigen.

(10). These strains were positive for attaching and effacing and localized adherence phenotypes and are therefore EPEC, although, unlike the strains we describe, they were negative for the adherence factor probe. EPEC strains of the O157:H45 serotype have been noted as agents of diarrhea in a large outbreak in Japan and isolated cases in Germany, Japan, and Thailand (11–13). These previously described O157 EPEC strains were not tested for bundlin alleles, and their phylogenetic relationships to other pathogenic *E. coli* have not been reported. However, the results of several studies have indicated that O157 strains lacking the H7 flagellar antigen and *stx* genes, which were not necessarily EPEC, are clonally distinct from O157:H7 strains and often from each other (14–16). Such findings suggest that recombination events causing the O antigen gene cluster to specify the O157 antigen have occurred in multiple *E. coli* lineages, including an EPEC 1 strain of an unknown serotype that is the progenitor of the strains described here. The results described in this study also highlight the pitfalls of classifying pathogenic *E. coli* by serogroup.

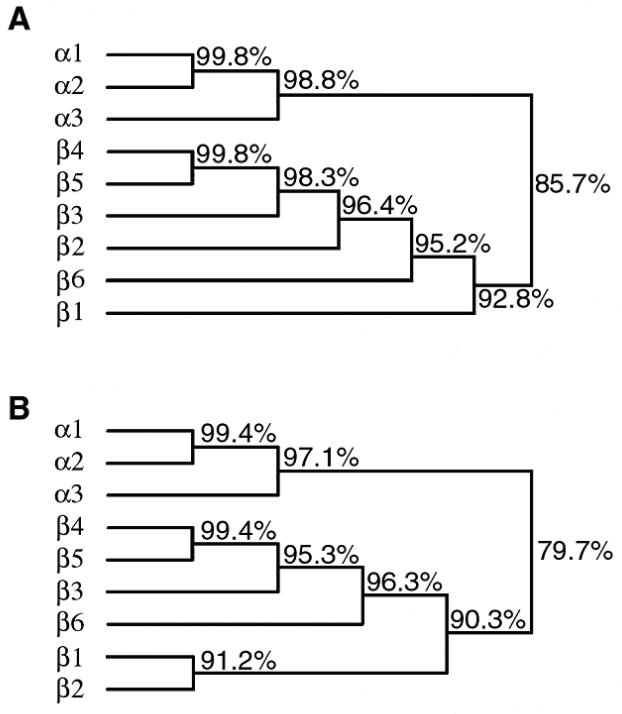

Figure 2. Dendrograms depicting the relationships among *bfpA* alleles (A) and predicted bundlin proteins (B). Percentage identities are indicated.

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Transfusion-Associated Babesiosis after Heart Transplant

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We describe a 54-year-old spleen-intact man with transfusion-associated *Babesia microti* infection after a heart transplant. Adult respiratory distress syndrome developed in the patient, and he required mechanical ventilation. Our experiences with this patient suggest that babesiosis should be considered in the differential diagnosis of transplant patients who have fever and hemolytic anemia.

Babesiosis is a tick-borne protozoan illness caused by infection of erythrocytes with various species in the genus *Babesia*. In the United States, *Babesia microti* is the agent most commonly reported to cause human infection (1). More recently, the MO1-type, WA1-type, and CA1-type *Babesia* species have been identified as causing clinical disease in the United States (2–5). *Babesia* infection can also be acquired by blood transfusion (2,6,7). More than 40 cases of transfusion-transmitted *B. microti* infection have been reported in the United States (R. Cable and B. Herwaldt, unpub. data). *B. microti* infection is often asymptomatic (8) but may cause a malaria-like illness characterized by fever and hemolytic anemia. Babesiosis can also be associated with severe complications that include renal failure (9,10), disseminated intravascular coagulation (9), and adult respiratory distress syndrome (1,9,10). The risk of developing this clinical infection is increased for elderly, asplenic, or immunosuppressed patients (11). Here we describe a case of *B. microti* infection in a 54-year-old spleen-intact man acquired by blood transfusion after cardiac transplantation.

Case Report of Blood Recipient

The patient, a 54-year-old resident of New Jersey, had a medical history of coronary artery disease and atrial fibrillation. Approximately 18 months before his transplant, a "bull's-eye" rash developed, and the patient was empirically treated for Lyme disease. He did not recall a tick bite.

On August 19, 2000, he had an anterior wall myocardial infarction and was hospitalized in his hometown. He required intubation, placement of a coronary stent and an intra-aortic balloon pump, use of intravenous cardiac inotropes, and transfusion of 2 U of packed red blood cells (PRBC). When fever developed, he was empirically treated with vancomycin, ciprofloxacin, and metronidazole without improvement. No source of infection was found. On September 6, he was transferred to a New York City medical facility for placement of a left ventricular assist device. *Staphylococcus epidermidis* was isolated from two sets of blood cultures. He was then treated with vancomycin and trimethoprim-sulfamethoxazole for 4 weeks.

On September 26, the patient received an orthotopic cardiac transplant. He received 32 U of irradiated leukocyte-reduced PRBC, 23 U of fresh frozen plasma (FFP), 6 U of irradiated platelets, and 4 U of cryoprecipitate during his 5.5-week hospital stay. On October 16, he was discharged on an immunosuppression regimen of cyclosporine, mycophenolate, and prednisone.

On November 5, the patient became febrile. He was evaluated by his cardiologist 3 days later. He had fever, chills, diaphoresis, headache, and sore throat. Blood and throat cultures were obtained, as well as an endomyocardial biopsy specimen. No source of infection or evidence of cardiac rejection was found.

On November 9, he was hospitalized again for evaluation of continued fever. On admission, his temperature was 38.4×C. His surgical wound had healed well. Laboratory tests showed a leukocyte count of 4.3 x 10^3/mm^3 (83% neutrophils, 9% lymphocytes, 8% monocytes), hemoglobin concentration of 11.4 g/dL, and a platelet count of 54,000/mm^3. On November 10, the staff of the hospital parasitology laboratory identified intraerythrocytic ring forms and tetrads consistent with *B. microti* infection on a peripheral blood smear; these results were confirmed by the Centers for Disease Control and Prevention (CDC). The parasitemia level was 1.6%. Indirect fluorescent antibody (IFA) testing (12) of his serum at CDC showed that his IFA titers had risen from $\leq$1:8 (i.e., negative) pretransplant to 1:1024 posttransplant (Table). Nested polymerase chain reaction (PCR) analysis of blood, using primers described previously (13), was positive for *B. microti* DNA. Results of pre- and posttransplant serologic testing for antibodies to *Borrelia burgdorferi*, *Ehrlichia chaffeensis*, and the agent of human granulocytic ehrlichiosis (14), performed at the New York State Department of Health, were negative (Table).

On November 10, therapy with quinine (650 mg orally, 3 times a day) and clindamycin (400 mg intravenously, 4 times a day) was begun. His course was complicated by worsening parasitemia (maximum documented level was 3.1% on November 13), hemolytic anemia (hemoglobin concentration decreased from 11.4 g/dL on November 9 to a minimum value of 7.4 g/dL on November 15), acute renal failure (creatinine

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Table. Results of serologic testing, polymerase chain reaction analyses, and hamster injection for specimens from the case-patient with babesiosis and the implicated blood donor[a]

Date of specimen	Timing of specimen	Test	Result of testing
Case-patient			
September 21, 2000	Pretransplant	*Babesia microti* IFA	≤1:8[b]
November 14, 2000	Posttransplant	*B. microti* IFA	1:1024
November 14, 2000	Posttransplant	*B. microti* PCR	Positive
September 21, 2000	Pretransplant	Agent of HGE, IgM[c]	Negative
November 14, 000	Posttransplant	Agent of HGE, IgM	Negative
September 21, 2000	Pretransplant	Agent of HGE, IgG	Negative
November 14, 2000	Posttransplant	Agent of HGE, IgG	Negative
September 21, 2000	Pretransplant	*Ehrlichia chaffeensis* IFA	Negative
November 14, 2000	Posttransplant	*E. chaffeensis* IFA	Negative
September 21, 2000	Pretransplant	Lyme ELFA	Negative
November 14,2000	Posttransplant	Lyme ELFA	Negative
Implicated blood donor (tested approximately 5 months after donation)			
February 28, 2001	Postdonation	*Babesia microti* IFA	1:256
February 28, 2001	Postdonation	*B. microti* PCR	Negative
February 28, 2001	Postdonation	Hamster inoculation[d]	Negative

[a]IFA, indirect fluorescent antibody; PCR, polymerase chain reaction; HGE, human granulocytic ehrlichiosis; Ig, immunoglobulin; ELFA, enzyme-linked fluorescence assay.
[b]*Babesia microti* IFA= 1:8=negative.
[c]IgM and IgG immunoblots for HGE.
[d]Two hamsters were each inoculated intraperitoneally on April 3 with 0.75 mL of blood obtained on February 28. Giemsa-stained smears were made from hamster blood obtained twice weekly, for 8 weeks, by tail snip. The hamsters did not become demonstrably parasitemic.

value rose from 1.4 mg/dL on November 9 to a maximum level of 3.3 mg/dL on November 15), and adult respiratory distress syndrome requiring mechanical ventilation.

On November 14, his therapy for babesiosis was changed to azithromycin (500 mg intravenously, once a day) and atovaquone (750 mg orally, 2 times a day) because of severe tinnitus, increased level of parasitemia (3.1% on November 13), and ongoing fever (maximum of 38.6°C). On November 21, he was afebrile and had a negative blood smear. He was discharged on November 25. Therapy with azithromycin (250 mg orally, once a day) and atovaquone (750 mg orally, 2 times a day) was continued for a total of 2 weeks on these drugs. The patient remained well as of August 2001.

Investigation of Blood Donors

We traced the donors of the 32 PRBC units transfused during the patient's hospitalization in New York City. For two donors, the transfused units had associated frozen components (i.e., FFP) available that could be retrieved; for six donors, PRBC units from subsequent donations were available for testing. The blood products from these eight donors were negative for antibodies to *B. microti* by IFA testing.

The other 24 donors submitted blood for testing. On March 12, 2001, the New York State Department of Health laboratory reported that one donor had an IFA titer of 1:256, which was confirmed by CDC (Table). The other 23 donors had negative IFA titers. The blood specimen from the implicated donor had been obtained on February 28, 2001, 5 months after the original blood donation on September 22, 2000. The PRBC unit was transfused on October 1, 2000, and the patient first reported fever on November 5, 2000. Thus, the incubation period for the case of babesiosis was estimated to be 35 days. CDC performed additional diagnostic testing on the implicated donor's blood. PCR analysis was negative for *B. microti* DNA, and parasitemia did not develop in hamsters injected with his blood (Table). The donor was thus implicated on the basis of serologic rather than parasitologic data. Given that he was not treated for babesiosis, the negative results of the parasitologic testing suggest that the donor's infection cleared spontaneously. However, he could have had low-level or intermittent parasitemia not detected by the parasitologic testing.

After the donor was implicated, other components from the index donation were investigated. No other patients received blood components from the implicated donation. The only other component identified was one unused unit of FFP, which already had been destroyed. The donor's only previous donation was 1 year before the index donation. The lone recipient from that donation remained asymptomatic but was not tested for evidence of *B. microti* infection.

Investigation of Implicated Donor

The implicated donor, a 45-year-old spleen-intact man from Westchester County, New York, had been asymptomatic the year before his blood donation. In 2000, he had visited New Haven County, Connecticut, in May; Long Island (Jones Beach), New York, in July and August; and Narragansett,

Rhode Island, in July. He worked outdoors and gardened as a hobby. He did not recall a tick bite.

Investigation of Heart Donor

The heart donor was a 54-year-old woman who died after a cerebrovascular accident. She had lived and worked in New York City and had had no symptoms consistent with babesiosis before the stroke. While hospitalized, she had remained afebrile with a normal complete blood count and renal function. No serum from her was available for *B. microti* IFA testing.

Conclusions

We conclude that our patient, who lived in New Jersey, acquired babesiosis through transfusion of PRBC in New York City after a cardiac transplant. The evidence consistent with this conclusion includes the following: he did not recall a tick bite (most patients with babesiosis do not [9–11]); he did not spend much time outdoors; he was seronegative by *B. microti* IFA 5 days before his heart transplant and markedly seropositive when babesiosis was diagnosed 45 days after his transplant; he received blood from a donor with a *B. microti* IFA titer of 1:256; and he became symptomatic during the typical two 8-week incubation periods for transfusion-transmitted *B. microti* infection (7).

Other possible modes of transmission seem much less likely. We doubt he became infected through a blood transfusion in New Jersey; he was seronegative by *B. microti* IFA 4.5 weeks after the New Jersey transfusions. We also doubt that he acquired his infection while recuperating after his heart transplant; his limited outdoor activities made tick exposure unlikely.

More than 40 cases of transfusion-transmitted babesiosis have been reported in the United States. Most infections have been acquired through PRBC transfusions, but cases have also resulted from transfusion of frozen-deglycerolized red cell units (15,16) and platelet units (6,7) (the latter have residual red cells). Two previous cases of *B. microti* infection have been reported in solid-organ transplant patients, although no cases acquired through transplantation per se have been reported (17,18).

We initially treated our patient with quinine and clindamycin but later changed the regimen to atovaquone and azithromycin. A recent clinical trial demonstrated that the combination of atovaquone and azithromycin is as effective as quinine and clindamycin for the treatment of babesiosis in patients without life-threatening disease (such patients were excluded from this trial) (19). In our patient, who was critically ill, immunosuppressed, and a solid-organ transplant recipient, the combination of atovaquone and azithromycin was effective therapy.

Babesiosis should be considered in the differential diagnosis of transplant patients who have fever and hemolytic anemia, and blood transfusion should be considered as one of the possible means of acquisition of infection. Solid-organ transplant recipients may receive many blood products, which necessitates an extensive investigation to implicate a donor.

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Household Contamination with *Salmonella enterica*[1]

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Household contamination with *Salmonella enterica* increases when occupational exposure exists (cattle farms with known salmonellosis in cattle, a salmonella research laboratory, or a veterinary clinic experiencing an outbreak of salmonellosis). Fifteen of 55 (27.2%) vacuum cleaner bags from households with occupational exposure to *S. enterica* were positive versus 1 of 24 (4.2% without known exposure. Use of a carpet cleaner and several cleaners/disinfectants reduced, but failed to eliminate, *S. enterica* from artificially contaminated carpet.

Although most cases of nontyphoid salmonellosis in humans are foodborne, a significant number appear to be acquired from households contaminated with *Salmonella enterica* (1–3). Sources and sites of contamination include house members with clinical disease, pets with sub-clinical infection, contaminated items brought into the home, toilet bowls, carpet, floors, refrigerators, and kitchen sinks and counter tops (1–6). Culture of vacuum cleaner bag contents has been used as a tool to screen households for contamination with *S. enterica* (1–3). The purpose of the present study was to determine the frequency of contamination with *S. enterica,* as indicated by culture of vacuum cleaner bag contents, in homes in which the residents had differing levels of occupational exposure.

The contents of vacuum cleaner bags (N=79 bags), collected from household vacuum cleaners, were cultured from five groups: 1) occupants had no known exposure to livestock or *S. enterica* in the workplace (n=12), 2) one or more occupants had direct contact with livestock with no known recent salmonellosis cases (n=12), 3) one or more occupants had direct contact with cattle salmonellosis cases associated with the serovar Typhimurium (n=26), 4) occupants were exposed to cats involved in a veterinary clinic outbreak of feline salmonellosis associated with the serovar Typhimurium (n=16), and 5) one or more occupants were laboratory or field workers engaged in research on *S. enterica* (n=13).

Vacuum cleaner bags were stored at room temperature and cultured for *S. enterica* within one week of collection. The contents of each bag were cultured in duplicate. Twenty-five g of bag contents was added to 225 mL buffered peptone water (BPW, Remel Inc., Lenexa, KS), and incubated overnight at 37°C. Preenriched samples were mixed, and 1 mL of BPW was transferred to 9 mL of tetrathionate broth (Tet, Remel Inc.), incubated overnight at 37°C, and then 100 µL of Tet was transferred to 10 mL Rappaport-Vassillladis broth (R10, Difco, Detroit, MI). The Tet tubes were incubated an additional 24 h with the R10 tubes at 37°C, and then plated onto brilliant green agar containing sulfadiazine (BGS, Difco, Detroit, MI). BGS plates were incubated for 48 h at 37°C , examined at 24 h and 48 h, and suspect colonies were biochemically screened. All *S. enterica* isolates were serotyped by the National Veterinary Services Laboratory, Ames, Iowa.

Salmonella organisms from all groups were found in household vacuum cleaner bags, except those from homes in which occupants had no contact with livestock or exposure to *S. enterica* (Table 1) in the workplace. *S. enterica* serovar Dublin was found in 1 of 12 (8.3%) vacuum bags collected from households with direct contact with livestock having no known recent cases of salmonellosis. Eight of 26 (30.8%) vacuum bags from households with occupants who had contact with *Salmonella*-infected cattle were positive. One of the positive vacuum bag samples came from a home in which an infant developed salmonellosis concurrent with an outbreak on the dairy farm where his father was employed. From households where occupants were exposed to an outbreak of feline salmonellosis, 3 of 16 (18.8%) of bags were positive, and from households of personnel engaged in field and laboratory-based research on *S. enterica*, 4 of 13 (30.8%) bags were positive. All *S. enterica* isolates from households with known occupational exposure belonged to the serovar Typhimurium; as might be expected, given that all known contact exposures were with this serovar.

Since vacuum cleaners are primarily used to clean floors, the floors were likely the primary site of household contamination in this study. To ascertain the best way to remove *S. enterica* from carpeted floors (to advise affected persons), we began a study to identify a means of decontaminating carpet that was artificially contaminated with *S. enterica*. In this experiment, nine carpet segments (40 cm x 80 cm) were attached to separate sections of plywood. Each carpet segment was subdivided into four quadrants and a 15-cm x 25-cm rectangle was marked in each quadrant with indelible ink. Five serovars of *S. enterica* (Typhimurium, Dublin, Infantis, Heidelberg, and Newport), chosen for their resistance to the antibotics ampicillin, chloramphenicol, and streptomycin were mixed in fresh bovine feces (106 cfu/g). Approximately 500 g of this feces was evenly distributed onto each carpet segment by vigorous rubbing with a sponge mop. Feces-coated carpet segments were allowed to dry overnight at room temperature. Pretreatment samples were collected from the upper right and lower left quadrant of each segment by wiping a sponge (Specisponge, Nasco International Inc., Fort Atkinson, WI), satu-

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[1]This study was performed at the Field Disease Investigation Unit, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-7060, USA.

Table 1. *Salmonella enterica* culture results from the contents of household vacuum cleaner bags collected from homes with five different exposure categories

Exposure category	No. positive (%)	No. cultured	Serotypes isolated
No contact with livestock or animal salmonellosis	0 (0)	12	
Contact with livestock, no known salmonellosis	1 (8.3)	12	Dublin
Contact with livestock with salmonellosis	8 (30.8)	26	Typhimurium
Contact with veterinary clinic with many cases of cat salmonellosis	3 (18.8)	16	Typhimurium
Employment in laboratory engaged in research on *S. enterica*	4 (30.8)	13	Typhimurium
Total	16 (20.3)	79	

rated with BPW, over the surface of the carpet (10 times in one direction and 10 times perpendicular to the initial direction). The sponge was placed into a Whirl-pak bag (Nasco International Inc.) containing 25 mL BPW and mixed by using a Stomacher laboratory blender for 60 s. Serial dilutions of the BPW/carpet content suspension were spread onto MacConkey agar plates (Remel Inc.) containing ampicillin (256 µg/mL), chloramphenicol (8 µg/mL), and streptomycin (32 µg/mL) (MacACS) and incubated at 37°C overnight. Non–lactose-fermenting colonies on MacACS were counted and a subset assayed biochemically and serologically for *S. enterica*. One carpet segment was used as a control (not cleaned) and the remaining eight segments were cleaned, until free of visible soiling, with a commercial wet-vacuum carpet cleaning system along with the proprietary carpet-cleaning agent. After cleaning, two carpet segments were treated, by using the wet vacuum system, with each of; chlorhexidine (Virosan Bio-Ceutic, Boehringer Ingelheim Vetmedica Inc., St Joseph, MO, 8 oz/gal), a quaternary ammonium disinfectant (Lysol all purpose cleaner, Reckitt Benckiser Inc., Wayne, NJ, 8 oz/gal) and a phenolic disinfectant (LpH Ag, STERIS Corp., St. Louis, MO, 0.5 oz/gal). Two cleaned segments were not treated with a disinfectant. Carpet segments were allowed to dry overnight at room temperature, after which sponge samples were collected from the upper left and lower right quadrants and cultured as described.

The carpet cleaning/sanitizing experiment produced contamination levels in excess of what likely occurs naturally in carpet; however, this level was necessary to allow a measure of reduction of *S. enterica* by the selected treatments. None of the treatments was successful in eliminating *S. enterica* from carpet (Table 2). Though the differences between treatments were not significant, perhaps owing to the small sample size, carpet cleaner followed by a phenolic disinfectant resulted in the largest reduction, whereas carpet cleaner followed by chlorhexidine resulted in no observable decrease.

This study confirmed the findings of others (1–3) that culture of vacuum cleaner bags is an efficient screening tool for household *S. enterica* contamination. Historically, some human salmonellosis cases have been attributed to direct contact with infected animals (7,8), while the potential for indirect contact in the home is typically not considered in public health case investigations and preventive efforts. Occupational exposure to *S. enterica* poses a potential risk to family members through inadvertent contamination of the home. When the three occupationally exposed groups were combined, Typhimurium was found in 27.2% of households. More and varied types of samples per household would likely have yielded a higher percentage of salmonella-positive homes. The vacuum bag samples in the current study were not quantitatively assayed, and some or all of the positive samples may have been contaminated at very low concentrations. However, in one household with a positive vacuum bag sample, salmonellosis developed in a family member, concurrent with this disease in cattle on the farm where another family member worked, suggesting that household exposure to *S. enterica* can be sufficient to cause an infection. The infective dose of *S. enterica*, especially for children, is not necessarily high (9–11), and circumstantial evidence exists for the acquisition of clinical infections from the household environment (1–3,12). For persons living in at-risk households, the risk of salmonellosis from household contamination could conceivably far out-

Table 2. Log_{10} CFU/mL *Salmonella enterica* (standard error) on contaminated carpet segments cleaned with a commercial carpet cleaner followed by different sanitizers

		Cleaned by a commercial carpet cleaner followed by			
	Control	No sanitizer	Chlorhexidine[a]	Phenolic disinfectant[b]	Quaternary ammonium[c]
No. carpet segments/treatment	1	2	2	2	2
Pretreatment	2.87	3.36 (0.18)	2.44 (0.16)	3.18 (0.02)	3.28 (0.11)
Posttreatment	2.60	2.26 (0.09)	2.54 (0.36)	0.81 (0.81)	1.67 (0.27)
Mean change	-0.27	-1.10 (0.27)	0.10 (0.52)	-2.37 (0.79)	-1.61 (0.38)

[a]Virosan Bio-Ceutic, Boehringer Ingelheim Vetmedica., St Joseph, MO.
[b]LpH Ag, STERIS Corp., St. Louis, MO.
[c]Lysol, Reckitt Benckiser Inc., Wayne, NJ.

weigh the risk from food sources, and questions aimed at identifying this risk factor should be a routine part of salmonellosis case investigations. From households deemed to be at risk of environmental contamination, a vacuum cleaner bag should be collected and its contents assayed for *S. enterica*.

Carpet is a likely site of contamination in households and, once the carpet is contaminated, eliminating *S. enterica* by using conventional carpet cleaning methods is difficult, if not impossible. Use of a phenolic disinfectant resulted in the greatest reduction of *S. enterica* in carpet; however, this product may not be suitable for use in carpet due to the possibility of hazardous residues. Previous studies have reported the persistence of salmonellae when various disinfectants and cleaning strategies are used (4,13). The rapid buildup of bacteria in carpet under normal usage, the subsequent difficulty removing bacteria from carpet during cleaning, and the ability of bacteria to survive in carpet and other fabrics for many months has been documented (14,15).

The current study indicates that precautions are warranted for the home environments of personnel who regularly have contact with livestock or who have occupational exposure to *S. enterica.* Preventive measures such as having noncarpeted entry areas and removing footwear before entering living areas should be taken to minimize the chances of contaminating the home environment, especially when households have members who are predisposed to infection with enteropathogens by factors such as age or immunocompromised status.

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Persistence of W135 *Neisseria meningitidis* Carriage in Returning Hajj Pilgrims: Risk for Early and Late Transmission to Household Contacts

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After an outbreak of meningococcal disease caused by *Neisseria meningitidis* W135, associated with the Hajj pilgrimage in 2001, 15% of returning vaccinated pilgrims carried a single W135 clone, and 55% were still carriers 6 months later. Transmission to 8% of their unvaccinated household contacts occurred within the first few weeks, but no late transmission took place. Public health interventions are needed to protect household contacts.

The annual Islamic pilgrimage to Mecca and Medina in Saudi Arabia (Hajj pilgrimage) attracts approximately 2 million pilgrims from all over the world. Overcrowding during the 1-month-long religious rituals may facilitate rapid dissemination of meningococci (1–3). An international outbreak of meningococcal disease caused by *Neisseria meningitidis* W135 occurred in association with the Hajj pilgrimage in 2000 and 2001 (4,5), with a high attack rate not only among the pilgrims but also among household contacts of returning pilgrims (6–10).

Meningococcal carriers are the primary source of *N. meningitidis* transmission (11). Although vaccination can protect pilgrims against invasive meningococcal disease, it does not prevent acquisition of carriage (12). Pilgrims returning from the Hajj may have a high meningococcal carriage rate (13), and after the W135 outbreak during the Hajj pilgrimage 2001, we documented a high acquisition rate of a single clone of W135 in pilgrims (14). Returning pilgrims may therefore spread the organism to household contacts or even to the community at large.

Although researchers have shown that W135 can attain a high carriage rate (14,15), no data are available on the duration of carriage of W135. Persistence of carriage may represent a threat to the community and has important public health implications. We set out to determine the persistence of W135 meningococcal carriage in pilgrims and to quantify the ongoing risk of transmission to household contacts.

Methods

Vaccination records kept at a Moslem center in Singapore were reviewed to identify pilgrims on the Hajj pilgrimage in 2001. Returning pilgrims, all of whom had received pre-Hajj quadrivalent meningococcal polysaccharide vaccine, were contacted and invited to participate in the study.

Tonsillopharyngeal swabs specimens were taken from returning Hajj pilgrims 2 weeks after the Hajj pilgrimage 2001, and those found to be carriers of the W135 clone (henceforth referred to as "pilgrim carriers") had a repeat swab taken 5–6 months later. Antibiotics were not routinely administered to those who were identified as carriers, although some took antibiotics for other indications during the follow-up.

All household contacts of pilgrim carriers were asked to come for a throat swab within the first month and 5–6 months after the Hajj. In addition, all household contacts whose results of the initial throat swab were negative were asked to return for serial throat swabs taken 2 weeks, 1–2 months, and 2–3 months later.

Swab samples were transferred to a plate of selective culture medium (Oxoid GC, Basingstoke, U.K.). Culture plates were immediately put in candle jars and transferred to the laboratory within 2–4 hours of collection. Serogrouping by latex agglutination (Murex, Dartford, U.K.) and pulsed-field gel electrophoresis (PFGE) was performed on all meningococcal isolates. PFGE was performed by using previously described methods (16). The Tenover criteria were used to interpret the pattern of bands (17).

All subjects gave written informed consent. The study was approved by the Ethics Committee of Tan Tock Seng Hospital.

Results

Swab specimens were taken from 373 Malay pilgrims at a median time of 26 days (range 3–45) after return from the Hajj. The median age was 47 years (range 3–78 years), and 57% were female. Of the 373, a total of 61 (16%) were found to be meningococcal carriers (95% confidence interval [CI] 13% to 21%). Of these 61, 56 (92%) were of a single W135 clone by PFGE. Fifteen percent (95% CI: 12% to 19%) of the returning pilgrims were carriers of the W135 clone (Table). Forty pilgrim carriers returned for a repeat swab at 5–6 months (range 144–182 days) after the Hajj. Of those 40, 22 (55%) remained carriers of the W135 clone.

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[1]Originated the study idea; was responsible for study design, data collection, analysis, and interpretation; and wrote the final manuscript.
[2]Was responsible for the meningococcal cultures, serogrouping, and pulsed-field gel electrophoresis (PFGE), and contributed to the manuscript.
[3]Performed PFGE and made other contributions to the manuscript.
[4]Was responsible for data entry and statistical analysis.
[5]Contributed to the study design and data interpretation, and co-wrote the final manuscript.

Table. Meningococcal carriage rates in returning Hajj pilgrims and household contacts of pilgrim carriers

Meningococcal carriage rate	Returning Hajj pilgrims	Household contacts of pilgrim carriers[a]
	(n=373) (%)	(n=117) (%)
Overall	61 (16.4)	13 (11.1)
W135 clone	56 (15.0)	9 (7.7)

[a]A pilgrim carrier is a returning pilgrim with tonsillopharyngeal carriage of the W135 clone.

The household contacts of 42 of the pilgrim carriers (75%) agreed to provide throat swabs specimens. Swabs were taken from 117 (84%) of all existing 139 household contacts at a median time of 32 (range 5–45) days after the Hajj. The median age of household contacts was 14 years (range 1–52 years), and 65.3% were children <18 years of age. Of the 117 contacts, 13 (11%) were meningococcal carriers, and of those carriers 9 (69%) were found to carry the W135 clone (hereafter referred to as "contact carriers"). The 9 contact carriers belonged to eight households, and 11 (19%) of the pilgrim carriers transmitted the W135 clone to at least one household contact.

Of the 104 contacts with initially negative results, 26 had a repeat swab specimen taken at a median interval of 15 days (range 11–27 days) after the initial swab within the first month after the Hajj, and all results continued to be negative. Thirty-one of the 104 persons had repeat swabs taken at 1–2 months (median of 39 days, range 30–60), 32 at 2–3 months (median 65 days, range 62–72), and 79 at 5–6 months (median 160 days, range 144–182) after the Hajj, and none was found to have become a carrier (Figure).

Discussion

The high meningococcal carriage rate in returning pilgrims is consistent with the rate of serogroup A meningococcal infection found in pilgrims returning to the United States after the 1987 Hajj (13). This rate is also in keeping with studies in other situations in which large numbers of previously unrelated persons have come into close social contact (e.g., university freshmen), and dramatic increases in carriage rate have been shown to occur in the space of just a few days (18,19). However, the carriage rate of 16% in this study is markedly higher than the 2.6% found in pilgrims returning to the United States after the 2001 Hajj (20). This discrepancy is unlikely to be explained by differences in study methodology alone but probably results from differences in living conditions, degree of overcrowding, or social activities during the Hajj. Further detailed epidemiologic studies are warranted to determine exactly which aspect of the pilgrimage is responsible for the high transmission that we have documented. The proportion of overall carriage attributable to this single W135 clone (92% of all carriage) in our study is unusual. Even in the situation of a meningitis outbreak, the proportion of carriage of the hypervirulent strain responsible for invasive disease rarely exceeds the overall proportion of carriage in the population by a few percentage points (11,21,22).

In addition to our findings that W135 can attain a high carriage rate, we have shown that duration of carriage with this strain is lengthy (55% of carriers remain positive after 5–6 months). This long duration of carriage indicates that returning pilgrims may represent an ongoing threat to the community.

To our knowledge, this study is the first to investigate the extent of early transmission of *N. meningitidis* from pilgrim carriers to household contacts. We found that returning pilgrims carrying the W135 clone transmitted it to 7.7% of their household contacts, which is of the same order of magnitude as the transmission rate of meningococcal carriage from patients with invasive disease to household contacts (23,24). However, acquisition occurred only in the first month of contact with the returning pilgrim carriers, and none of the contacts with initially negative results acquired the strain after months of exposure. The absence of late transmission is an important new finding for which the explanation is currently unclear. Possibilities include the absence of particular epidemiologic risk factors for transmission in contacts with persistently negative results, host protective factors, or the attenuated virulence of the organism in the pilgrim carrier over time. Further studies are warranted to investigate our findings.

The absence of late transmission in our cohort is also consistent with our national epidemiologic data, which showed

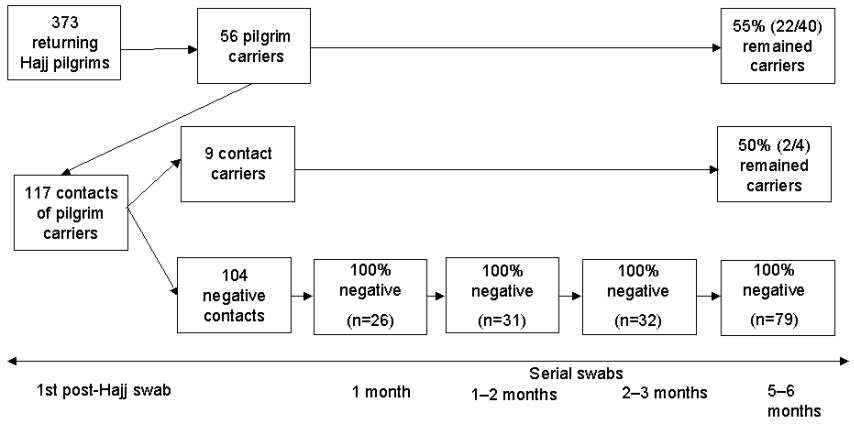

Figure. Results of serial tonsillopharyngeal swab specimens from returning Hajj pilgrims and their household contacts. A pilgrim carrier is a returning pilgrim with tonsillopharyngeal carriage of the W135 clone; a contact carrier is a household contact of a pilgrim carrier and carries the W135 clone.

that all the cases of invasive W135 meningococcal disease in contacts of returning Hajj pilgrims occurred within 2 months after the end of the Hajj pilgrimage 2001 and no further cases occurred later in the year (10,25). However, although persistence of carriage appears not to put the household contact at risk, this persistence may be an important threat to the community at large. Cases of W135 disease were identified in the United Kingdom several months after the end of the Hajj, but most of these case-patients had no identifiable direct contact with Hajj pilgrims (26).

Although vaccination may protect the pilgrims from invasive disease, our data show that returning pilgrims represent a sizeable reservoir of a highly transmissible and persistent W135 clone, which places their unvaccinated family contacts (and possibly the community at large) at risk of invasive disease. The appropriate public health response to this problem is unclear. One approach would be to eradicate carriage in pilgrims by administering antibiotics at the point of return to their home countries. However, more data on the impact of this intervention and on resistance and safety issues are needed before embarking on such a large-scale program. Vaccination of household contacts is a potential strategy, but it would be expensive and difficult to implement. Increasing the uptake of the quadrivalent meningococcal vaccine (now mandatory for all pilgrims) may have a beneficial effect in decreasing the carriage of W135 (6). Although the vaccine did not prevent acquisition of carriage in our cohort of Singaporean pilgrims, it may have a greater effect when the entire Hajj pilgrim population is vaccinated. Future studies are essential in order to determine the public health impact of such a vaccination program.

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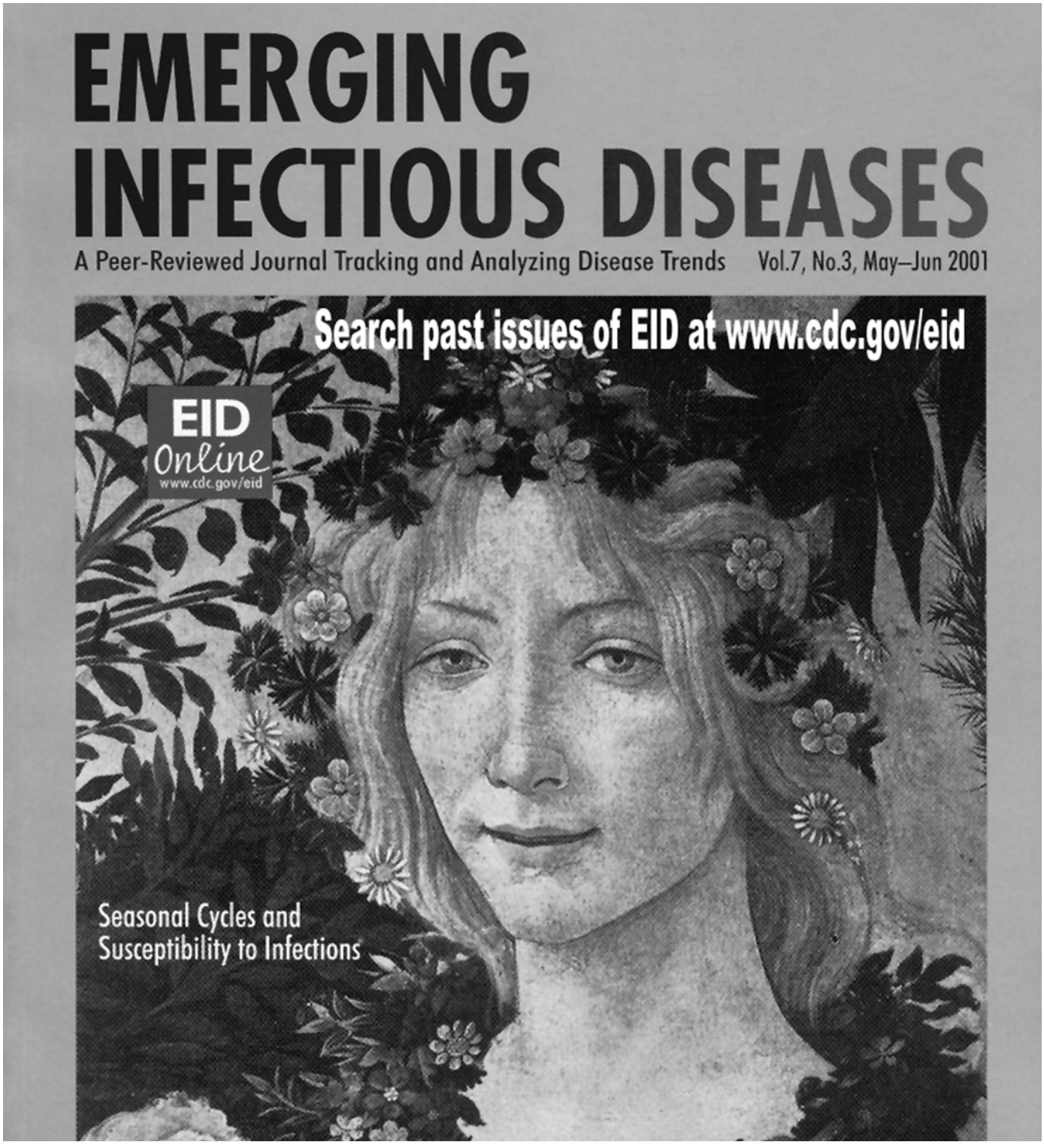

Single Multiplex Polymerase Chain Reaction To Detect Diverse Loci Associated with Diarrheagenic *Escherichia coli*

Catalina López-Saucedo,* Jorge F. Cerna,* Nicolas Villegas-Sepulveda,* Rocío Thompson,* F. Raul Velazquez,† Javier Torres,† Phillip I. Tarr,‡§ and Teresa Estrada-García*

We developed and tested a single multiplex polymerase chain reaction (PCR) that detects enterotoxigenic, enteropathogenic, enteroinvasive, and Shiga toxin–producing *Escherichia coli*. This PCR is specific, sensitive, and rapid in detecting target isolates in stool and food. Because of its simplicity, economy, and efficiency, this protocol warrants further evaluation in large, prospective studies of polymicrobial substances.

Escherichia coli causes disease in humans through diverse mechanisms (1). Classified on basis of their virulence traits, the most well-studied members of the diarrheagenic *E. coli* group include enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), and Shiga-toxin–producing *E. coli* (STEC), also called verocytotoxin-producing or enterohemorrhagic *E. coli*. ETEC produce secretory toxins (enterotoxins); EPEC adhere intimately to epithelial cells and induce host cell transmembrane signaling; EIEC invade eukaryotic cells; and STEC produce Shiga toxins.

Identifying diarrheagenic *E. coli* in the polymicrobial milieus of stool and food poses challenges. Occasionally, economically detectable phenotypes distinguish such organisms when they are abundant in human stools. For example, sorbitol- and lactose-nonfermenting colonies are typical of *E. coli* O157:H7 and EIEC (2,3), respectively. However, these phenotypes are nonspecific, and subsidiary testing is needed to confirm the isolate identity. In vitro assays that detect toxins, adherence, or invasion phenotypes can also identify candidate diarrheagenic *E. coli*. These determinations are often expensive, require special expertise, and employ various detection systems (e.g., cell culture, cytotoxicity assays). Applying such assays to enteric microbiologic diagnosis is cumbersome.

Nucleic acid hybridization techniques, exploited by colony hybridizations or polymerase chain reaction (PCR), apply a single detection method to a diversity of organisms. The application of nucleic acid amplifications requires selecting appropriate oligonucleotide primers and optimizing conditions to maximize sensitivity and specificity. The inclusion of reactions and conditions that apply to a variety of virulence loci so that multiple candidate pathogens can be sought in a single reaction makes this technology more efficient and economical. Such multiplex detection is an appropriate solution to the challenge of finding diarrheagenic *E. coli* in stools and in food. We describe the development of a multiplex PCR that detects four categories of diarrheagenic *E. coli* and the application of the assay to human diarrheal stools and food in Mexico City.

The Study

We developed a single multiplex PCR reaction to detect ETEC, EPEC, EIEC, and STEC, using specific previously described (4–6) or new primers (GIBCO-BRL, Gaithersburg, MD) for diverse virulence traits (Table 1). Because primers for loci that unambiguously distinguish pathogenic from nonpathogenic EAggEC have not yet been determined (1), we did not address this group in this study.

We prepared bacterial lysates by resuspending single colonies in 1 mL of deionized water (Milli-Q System, Millipore, Bedford, MA), boiling them 1 min, and then freezing them until needed. *E. coli* O86:H18 was the negative control in all assays. Each PCR tube contained 23 µL of reaction mix, comprised (in final concentrations) of Tris-HCl (10 mM, pH 8.3), KCl (50 mM), $MgCl_2$ (2 mM), gelatin (100 µg/mL), glycerol (5 % v/v), dATP, dCTP, dGTP, and dTTP (200 µM each), Ampli*Taq* polymerase (GIBCO-BRL) (0.5 U/23 µL), a mixture of the 14 primers (Table 1), and 2 µL of bacterial lysates. The final concentration of each primer in the reaction mix was determined by employing a DNA mix (Table 1) of the four prototype *E. coli* (7,10,11,13), until each of the seven PCR products exhibited a band of similar intensity after electrophoresis in a 2.5% agarose gel in Tris-borate-EDTA buffer and ethidium staining (Figure). The solutions were then subjected to the following cycling conditions: 50°C (2 min, 1 cycle); 95°C (5 min, 1 cycle); 95°C, 50°C, and 72°C (45 sec each temperature, 40 cycles); and a final extension step (10 min, 72°C) in a thermal cycler (iCycler System, Bio-Rad Laboratories, Inc., Hercules, CA). PCR products (4 µL) were visualized after electrophoresis and ethidium staining. The PCR sensitivity was determined by suspending one colony of each reference strain in individual 1-mL aliquots of sterile saline (0.85% w/v). Serial twofold dilutions in sterile saline were then made (to 1:256), and bacterial concentrations were determined by plating on MacConkey agar. Each dilution was also subjected to PCR analysis. *E. coli* 3030 (O86:H18) strain was used as a negative control during the characterization. In all further experiments, the DNA mix from the four prototype *E. coli*

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Table 1. Prototypes and reference strains of ETEC, EPEC, EIEC, and STEC tested in the multiplex PCR by using specific oligonucleotide primers for several loci[a]

E. coli category tested strains and serotypes	Locus	Primers	Amplicon size (bp)	Primer (pMol) in mix
ETEC H10407 O78:H11[b] (7) E9034A O8:H9 (8) B_2C O6:H16 (8) E8775A O25:H42[c] (9)	*lt*	F:5′GGC GAC AGA TTA TAC CGT GC3′(4) R:5′ CGG TCT CTA TAT TCC CTG TT3′(4)	450	5.0
ETEC H10407 O78:H11[b] (7) E9034A O8:H9 (8) B_2C O6:H16 (8) E8775A O25:H42[c] (9)	*st*	F:5′ATT TTT CTT TCT GTA TTG TCT T3′(4) R:5′CAC CCG GTA CAA GCA GGA TT3′(4)	190	6.47
EPEC E2348-69 O127:H6[b] (10) B171-8 O111:NM (10) 659-79 O119:H6 (10) E851/71 O142:H6 (10)	*bfp*A	F:5′AAT GGT GCT TGC GCT TGC TGC3′ (5) R:5′ GCC GCT TTA TCC AAC CTG GTA3′ (5)	324	2.5
EPEC E2348-69 O127:H6[b] (10) B171-8 O111:NM (10) 659-79 O119:H6 (10) E851/71 O142:H6 (10) STEC EDL933 O157:H7[b] (11) TB334C O85:NM (12) TB285A O126:H2 (12) TB226A O11:HN(12)	*eae*A	F:5′ GAC CCG GCA CAA GCA TAA GC3′ (6) R:5′CCA CCT GCA GCA ACA AGA GG3′ (6)	384	3.88
STEC EDL933 O157:H7[b] (11) TB334C O85:NM (12) TB285A O126:H2 (12) TB226A O11:HN (12)	*stx*1	F:5′CTG GAT TTA ATG TCG CAT AGT G3′[d] (GenBank accession no. M17358) R:5′AGA ACG CCC ACT GAG ATC ATC3′ (6)	150	3.88
STEC EDL933 O157:H7[b] (11) TB226A O11:HN (12)	*stx*2	F:5′GGC ACT GTC TGA AAC TGC TCC3′ (6) R:5′TCG CCA GTT ATC TGA CAT TCT G3′ (6)	255	2.5
EIEC E11 O124NM[b] (13) O124:H30 (14) O136:NM (14) O143:NM (14)	*ial*	F:5′GGT ATG ATG ATG ATG AGT CCA 3′[d] (GenBank accession no. D13663) R:5′ GGA GGC CAA CAA TTA TTT CC 3′[d]	650	10.25

[a]*E. coli*, *Escherichia coli*; ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; EIEC, enteroinvasive *E. coli*; STEC, Shiga-toxin–producing *E. coli*; PCR, polymerase chain reaction.
[b]*E. coli* prototype strains.
[c]Donated by the Public Health Laboratory Service, Central Health Laboratory, London, United Kingdom.
[d]These primers were designed by us from the gene bank sequences.

served as the positive control. The multiplex PCR was further characterized by using three additional reference strains for each category (Table 1).

Stools from 58 children <5 years of age hospitalized for diarrhea in July, August, and September, 1999, at the three main hospitals of the Instituto Mexicano del Seguro Social, Mexico City, were studied. The Institutional Review Board of the Institute approved this study, and parental informed consent was obtained for each patient. Standard diagnostic evaluations on these stools included culture for *Campylobacter, Salmonella, Shigella, Vibrio cholerae, Aeromonas,* and *Plesiomonas*; identification of *Rotavirus*, *Adenoviridae*, *Astrovirus*, and *Caliciviridae* by enzyme immunoassay; and microscopy for *Entamoeba histolytica*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isospora belli*, and *Giardia lamblia*. Five lactose-fermenting colonies and five sorbitol-nonfermenting colonies with morphology resembling that of *E. coli* (when present) were selected from standard and sorbitol MacConkey agar plates, respectively, speciated biochemically, and then subjected to multiplex PCR.

Because of our concern about food safety, we purchased 52 food items (hot chili sauces and taco dressings) from street vendors in Mexico City in July, August, and September, 1999, and analyzed them for the presence of *E. coli* (which indicate fecal contamination) and diarrheagenic *E. coli,* without enrichment. One gram of food was added to 1 mL of 0.85% sterile saline and vortexed, and serial 10-fold dilutions were prepared. To enumerate candidate *E. coli*, and identify diarrheagenic *E. coli*, 100 µL of each sample and dilutions were plated on MacConkey and sorbitol MacConkey agar plates. Five pink colonies from MacConkey and five colorless colonies from sorbitol MacConkey agar were tested for indole positivity and the lactose-fermenting phenotype (if selected from the sorbitol plate). Only indole-positive, lactose-fermenting

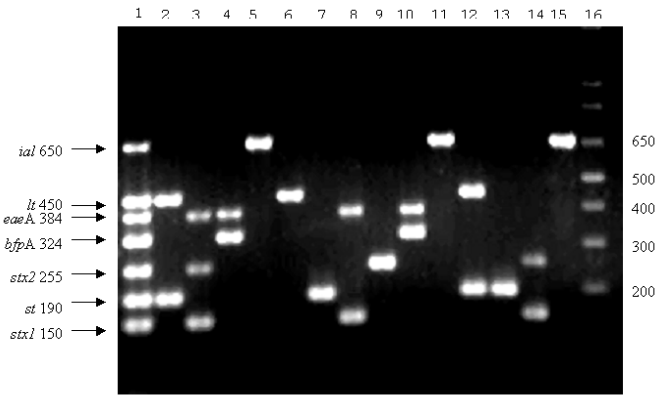

Figure. Polymerase chain reaction (PCR) products of each locus. Lane 1: sizes of the seven PCR products of each locus in base pairs, obtained when using a DNA mix of the four reference strains and the primers mix. PCR products obtained by using DNA of enterotoxigenic *Escherichia coli*, Shiga-toxin–producing *E. coli*, enteropathogenic *E. coli*, and enteroinvasive *E. coli* (lanes 2–5, respectively). Lane 6–11: PCR products obtained when using DNA of patients' isolates and the primers mix. Lanes 12–15: PCR products obtained when using DNA of food isolates and the primers mix. Lane 16: 1 kb molecular weight marker in base pairs.

colonies isolated from both media were then subjected to the multiplex PCR. STEC from patients and food were tested to determine if they expressed the O157 lipopolysaccharide antigen by using latex particle agglutination (Oxoid Limited, Basingstoke, UK).

Multiplex PCR detected the appropriate loci in each positive control strain; extraneous bands were not produced (Figure). When DNA from each of the four reference strains was mixed, the same bands appeared without nonspecific amplification (Figure). The minimum number of CFU detected were 320–1,526 for ETEC; 84–168 for EPEC; 120–1,556 for EIEC; and 20–194 for *E. coli* O157:H7.

Eleven (19%) of the 58 patients had candidate diarrheagenic *E. coli* in their stools (Table 2). In 6 (55%) of these 11 patients, no other enteric pathogens was identified, and in 3 patients target sequences were found in each of the selected *E. coli* colonies (Table 2). Thus, these candidate pathogens constituted the predominant aerobic coliform flora in some samples. None of the other 47 patients with diarrhea had *E. coli* containing the target loci in their stools. Twenty-two (42%) of the 52 food samples contained *E. coli*, and 7 (13%) contained candidate diarrheagenic *E. coli* (Table 2). No STEC isolated from patients or food expressed the O157 LPS antigen, and most were *eae* negative.

Conclusions

This multiplex PCR specifically and sensitively detected a diversity of loci in *E. coli* with ease, speed, and economy; its utility was demonstrated by using reference strains as well as clinical and food isolates. Conceivably, additional loci might

Table 2. Diarrheagenic *Escherichia coli* isolates in patient and food samples[a]

Samples	Diarrheagenic *E. coli* group	Identified genes	No. positive strains/ no. tested	Other pathogens isolated	CFU/gram food
Stool					
Patient 1	STEC	*stx1,eae* A	5/5	none	
Patient 2	STEC	*stx*2	5/5	none	
Patient 3	ETEC	lt	2/5	none	
Patient 4	STEC	*stx* 2	2/9	none	
Patient 5	STEC	*stx* 2	1/5	none	
Patient 6	EIEC	ial	1/5	none	
Patient 7	ETEC	lt	5/5	*Shigella flexneri*	
Patient 8	ETEC	st	2/5	*S. sonnei*	
Patient 9	EPEC	*bfp*A, *eae*A	1/5	*S. sonnei*	
Patient 10	ETEC	lt	1/5	*Rotavirus*, *S. sonnei*	
Patient 11	STEC	*stx*1, *eae* A	1/10	Rotavirus	
Food					
Green sauce	ETEC	lt, st	5/5		8.0×10^2
Green sauce	ETEC	lt, st	5/5		1.3×10^5
Raw cabbage	STEC	*stx*1, *stx*2	2/5		2.6×10^5
Green sauce	ETEC	st	1/5		2.6×10^4
Green sauce	EIEC	ial	1/5		6.0×10^2
Raw coriander	EIEC	ial	1/5		1.8×10^5
Raw lettuce	EIEC	ial	1/5		8.2×10^4

[a]STEC, Shiga-toxin–producing *Escherichia coli*; ETEC, enterotoxigenic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*.

be included because no signal attenuation occurred when a mixture of reference strains was assayed. The estimated cost per reaction for one strain is U.S. $2.00, compared to U.S. $15.00 for a colony blot analysis for one strain (data not shown). Furthermore, the signals from colony hybridizations are sometimes equivocal, in contrast to the unambiguous data obtained from our assay.

We believe that multiplex nucleic acid amplification to detect a panel of putatively pathogen traits should be considered as a replacement for tedious, less sensitive, and less specific detection technologies in clinical and food microbiologic analyses. This method should also be considered to be a more parsimonious use of PCR reagents than the individual locus PCR testing protocols described by others (15,16). Moreover, our approach does not rely on DNA extraction (16); boiling of cultures provides adequate nucleic acid to detect sequences of interest.

Comparing our protocol's sensitivity to that reported in other protocols is difficult because of differences in methods. Specifically, other techniques seek amplicons directly from stool cultures (17) or employ fecal DNA extraction (4), whereas we assessed isolated, randomly picked colonies. Nevertheless, our sensitivity ranges were within the range of previous reports (18,19), to the extent that we were able to compare them. Our approach also provides, simultaneously, an indication of the proportion of fecal gram-negative organisms that contain loci of interest.

Without a more extensive epidemiologic analysis, we cannot state with certainty that the positive *E. coli* isolated were the causes of the diarrhea in the children studied. However, in some samples, the PCR-positive organisms were well represented among the aerobic coliform flora selected for analysis. Such organisms were also well represented among the food isolates. Because these *E. coli* indicate fecal contamination, our findings present a disconcerting picture of the hygienic status of street-vended food in Mexico City. In fact, our colony selection protocol was biased towards high-frequency organisms because we sampled only five such strains. Surveys that examine several hundred colonies (20) or PCR amplification of supernatant of fecal or food outgrowths (17,21) or of extracted DNA (4) could detect target organisms at lower densities. Though the clinical and food safety implications of low levels of candidate diarrheagenic *E. coli* remain unclear, multiple studies have demonstrated that consumption of food sold by street vendors is a risk factor for acquiring diarrhea in Mexico (22–24) and elsewhere (25–27), and attempts to improve the safety of these ubiquitous vehicles would most likely improve public health.

We have demonstrated for the first time that multiplex PCR can detect a variety of diarrheagenic *E. coli* with relative ease. Such organisms are found in food vended in Mexico City and in local children with diarrhea. This feasible technology should be evaluated in larger, controlled, prospective studies of human diarrhea and in microbiologic studies of food to establish the current epidemiology of these pathogens, including the emerging strains of STEC.

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Ms. López-Saucedo is a candidate for a master of science degree in biology. Her research interests include clinical microbiology and epidemiology of diarrheal diseases.

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Risk for *Pneumocystis carinii* Transmission among Patients with Pneumonia: a Molecular Epidemiology Study

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We report a molecular typing and epidemiologic analysis of *Pneumocystis carinii* pneumonia (PCP) cases diagnosed in our geographic area from 1990 to 2000. Our analysis suggests that transmission from patients with active PCP to susceptible persons caused only a few, if any, PCP cases in our setting.

Pneumocystis carinii pneumonia (PCP) is an important cause of illness and death in immunocompromised patients, specifically HIV-infected patients, transplant recipients, and patients with oncohematologic diseases. In the absence of a reliable method of in vitro culture of the pathogen, many aspects of PCP epidemiology remain to be elucidated (1). Because of the evidence that contact with *P. carinii* occurs early in life, clinical infection in adults was thought to be mostly the result of reactivation of latent organisms. However, this concept has been challenged by evidence of the occurrence of de novo infection in HIV-infected persons (2,3). Possible sources include the environment, asymptomatic carriers, unrecognized infections, and patients with active PCP. Carriage by some persons with severe immunosuppression (4) or with chronic pulmonary disease (5) has been described recently. Direct transmission of *P. carinii* from patients with active PCP to susceptible persons is suggested by numerous reports of clusters of PCP cases, as well as by the demonstration of transmission of *P. carinii* in animal models (6). However, recent molecular typing failed to support this hypothesis in some studies (7–9). Indeed, different molecular *P. carinii* types were found in members of three different couples diagnosed with PCP (7), and the types were often different within clusters of PCP cases (8,9). Using molecular typing and epidemiologic analysis, we investigated the possibility of transmission of *P. carinii* between PCP patients who were seen in our region (about 500,000 inhabitants) during recent years. With few exceptions, all PCP patients are diagnosed at or referred to our hospital, Centre Hospitalier Universitaire Vaudois.

From October 1990 to August 2000, 1,299 bronchoalveolar lavage specimens were examined at our hospital by using the Gomori staining method; 230 (18%) were positive for *P. carinii* (including eight recurrent episodes), among which 131 (57%) were available for typing (127 patients, four recurrent episodes). The typing system consisted of amplification by polymerase chain reaction (PCR) of four variable regions of the *P. carinii* genome, followed by the detection of the polymorphisms using the single-strand conformation polymorphism technique (SSCP). The four genomic regions are: the internal transcribed spacer number 1 of the nuclear rRNA genes operon, the intron of the nuclear 26S rRNA gene, the variable region of the mitochondrial 26S rRNA gene, and the region surrounding the intron number 6 of the beta-tubulin gene. These four genomic regions were shown to be stable over prolonged periods of time by using SSCP (10). The interpretation of SSCP results for typing has been described (11,12). A *P. carinii* type is defined by a combination of four alleles corresponding to the four genomic regions. This system has been validated (10–12). The SSCP typing system is easier and faster than DNA sequencing of multiple loci and allows the analysis of large collections of specimens. Moreover, this system detects a higher proportion of coinfections than typing by DNA sequencing of several genetic loci, probably because it is more sensitive at detecting alleles present in low amounts (11,13). This higher rate of detection is important for epidemiologic studies. The disadvantage of SSCP is that specimens containing more than two *P. carinii* types (about 30% of the specimens) cannot be typed because of the complexity of the alleles' configuration (11).

The ages of the 127 patients ranged from 25 to 82 years (median 38), and 93 (73%) were men. Seventy-three percent (93/127) of the patients were HIV infected, and 27% (34) were immunosuppressed from other conditions. Twenty-three (18%) of the 131 PCP episodes corresponded to an infection with a single *P. carinii* type, 66 (50%) to a coinfection with two types, and 42 (32%) to a coinfection with more than two types. The four patients with two PCP episodes were infected with different types at each episode. Altogether, 35 different PCR-SSCP types were observed (numbered as in a previous publication [10]).

To evaluate the proportion of PCP cases that could have resulted from transmission of *P. carinii* from a patient with active PCP to a susceptible person, we analyzed the distribution over time of the *P. carinii* types observed in the patients carrying one or two types (89 episodes, 39% of the cases in the period). Described clusters of PCPs (14–16) suggest that the incubation period of *P. carinii* infection may range from 3 to 12 weeks, which is also in accordance with experiments in animals (17,18). Accordingly, we hypothesized that the incubation period of a newly acquired infection would range from 3 weeks to 3 months and that a patient with PCP might be infectious from 1 month before diagnosis until the end of treatment

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(usually 3 weeks after diagnosis). A patient can be both a receptor of *P. carinii* and a donor. The Figure represents the receptor-donor period for each *P. carinii* type identified in a patient (a patient with a coinfection has two types). The distribution of the types over time was relatively homogeneous, suggesting the absence of outbreaks due to a single type. In only 19 instances did the periods of two or more isolates of a given type overlap.

To investigate whether these overlapping periods could reflect transmission of *P. carinii* between the members of the cluster, we calculated, for each type, the multinomial probability that the observed clusters occurred by chance alone using Monte-Carlo simulation experiments (we assumed uniform distribution over time). For types 1, 10, and 11, the probability was small (4.6%, 5.6%, and 5.4%, respectively). This probability is even smaller when we consider only 1990–1995 (before antiretroviral tritherapy was introduced), a period during which more cases were observed (1.5%, 2.3%, and 1.5%, respectively). This probability suggests that some clusters may indeed be the result of interhuman transmission of *P. carinii* (or of infection from a common source). However, 16 of the 19 clusters involved patients infected with different coinfecting types, implying that interhuman transmission was less likely (either the donor would have not transmitted both types or the receptor would have acquired one type elsewhere, or both).

We further investigated the possibility of transmission between members of each cluster using available epidemiologic data limited to the location of patients' residence and documented time in the hospital. None of the patients involved in the 19 clusters lived in the same city sector or the same town. To investigate the possibility of encounters in the hospital, timing and location of consultations and hospitalizations were determined by review of the patients' medical charts and the outpatient clinic's schedule. An encounter was considered compatible with an interhuman transmission of *P. carinii* if an "infectious" and a "susceptible" patient (as defined above) were present in the same ward or facility of the hospital on the same day. Analyses of all identified clusters did not reveal any hospital encounters. Because the period at risk of acquisition and transmission chosen might have been too restrictive, we also analyzed four clusters of patients with identical typing results but with PCP episodes occurring up to 10 months apart. Again, no encounter in the hospital, as defined above, was found.

Because the analyzed specimens represent only 39% (89/230) of the PCP episodes of the period, the possibility of a selection bias can be raised. Such bias cannot be firmly excluded but statistical analysis argues against it, at least for the period before antiretroviral tritherapy was introduced (a period covering 51% [87/171] of the specimens available). Statistical analysis showed that the distribution over time of the available specimens was not significantly different from that of the cases that were unavailable (Wilcoxon rank sum test; p=0.55), suggesting that the results for this period are representative of the overall situation. On the other hand, the two

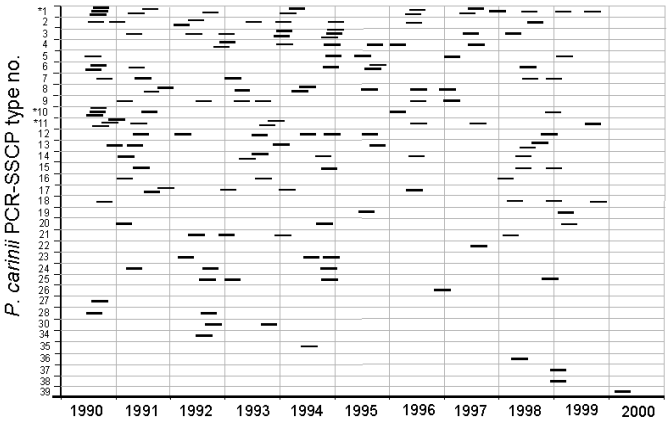

Figure. Distribution over time of *Pneumocystis carinii* types, as determined by polymerase chain reaction (PCR) – single-strand conformation polymorphism technique (SSCP), observed in Centre Hospitalier Universitaire Vaudois. Each occurrence is represented as a bar corresponding to the period of 15 weeks from 3 months before to 3 weeks after PCP diagnosis. Starred types may exhibit excessive clustering (see text).

distributions for the period from 1996 to 2000 were different because the proportion of specimens stored for typing increased regularly during the period (p=0.07; 75% [44/59] of the specimens available).

Our study reports on a large number of PCP episodes observed for >10 years. Although our retrospective analysis does not allow us to exclude encounters of the clustered cases outside the hospital, the results strongly suggest that transmission of *P. carinii* by a patient with active PCP to a susceptible person contributed to only a very small number, if any, of the PCP cases in our geographic area during this period. The broad diversity of observed types suggests that the patients acquired *P. carinii* from multiple unknown sources not addressed in the present study. Moreover, hospital epidemiologic data and molecular typing did not provide evidence of transmission of *P. carinii* inside the hospital between members of clusters infected with the same *P. carinii* type. Thus, the main source of *P. carinii* is unlikely to be represented by patients with active PCP.

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First Isolation of *Rickettsia slovaca* from a Patient, France

To the Editor: *Rickettsia slovaca* is a bacterium that infects *Dermacentor marginatus* ticks in central and western Europe. First detected in ticks, the bacterium was subsequently identified with genomic amplification by using polymerase chain reaction (PCR) followed by sequencing in a skin biopsy from a French patient (1). We describe the first isolation of the organism from a patient.

A 79-year-old woman from St. Etienne, France, found a tick on the parietal area of her scalp 6 days after she returned from a trip to rural southern Burgundy. The tick was removed and subsequently identified as an adult female *D. marginatus* tick. The patient saw a physician 1 day later for low-grade fever (38°C) and myalgia. She was given amoxicillin (3 g once a day), but the fever worsened. She was examined at University Hospital on September 24, 2001, 4 days later. At that time, the patient had a fever; the site of the tick bite showed a necrotic black lesion surrounded by an erythematous halo 4 cm in diameter. Right cervical lymphadenopathy and a papular rash consisting of 10 pink spots on the thorax and arms were observed. Routine blood tests were within normal ranges but asparate aminotransferase (53 IU; normal <45), creatine phosphokinases (140, normal <120), and lactate dehydrogenases (890, normal <620) were elevated. A skin biopsy from the patient's scalp, serum, and the tick were sent to Marseille to test for possible rickettsial infection. The patient was treated with doxycycline (200 mg once a day, 15 d), and her condition improved. At a check-up 1 month later, she complained of fatigue and insomnia; 2 months later, she had recovered completely, although alopecia still appeared at the site of the tick bite.

R. slovaca was demonstrated in the tick and the biopsy by using PCR with primers derived from the citrate synthase and the *rOmpA* genes as previously reported (2). *R. slovaca* was found in human embryonic lung cells (2), 3 days after the cells were injected with the skin-biopsied material. Seroconversion, determined by indirect immunofluorescence, occurred with titers to both *R. slovaca* and *R. conorii* of <1/8 and 1/128 in acute- and convalescent-phase sera (sampled 2 months later), respectively.

R. slovaca, first identified in dermacentor ticks from Slovakia, has subsequently been found in both *D. marginatus* and *D. reticulatus* in France, Switzerland, Portugal, Spain, Armenia, and Germany (3). Since the first human infections with *R. slovaca* were reported, patients with similar clinical signs have been observed in France and Hungary (4). Some of these cases have been confirmed by PCR and others by serology (3), although serologic titers are frequently low and show cross-reactions with other *Rickettsiae*. We have described the isolation of *R. slovaca* from a patient, which provides the first definitive evidence that *R. slovaca* is a human pathogen. Clinical signs of infection consist of a skin lesion at the site of a tick bite on the scalp (often a dermacentor tick) and regional lymphadenopathy that may be painful. Fever and rash develop subsequently, and the acute disease can be followed by fatigue and residual alopecia at the bite site. The disease may be prevalent within the distribution range of *D. marginatus* and *D. reticulatis* in southern, western, and central Europe. This new spotted rickettsiosis should be added to the list of recognized rickettsial diseases, mainly those caused by *R. africae* (5), *R. felis* (6) and *R. mongolotimonae* (7,8).

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Enteropathogenic *Klebsiella pneumoniae* HIV-Infected Adults, Africa

To the Editor: Although *Klebsiella pneumoniae* lives as a commensal in the intestine, this bacterium can occasionally cause diarrhea in HIV-negative persons (1–4). Some of these diarrheagenic strains encode thermostable or thermolabile toxins (2). One group of researchers showed that a

K. pneumoniae strain isolated from bloody diarrhea can bind to HeLa cells and cytoskeletal proteins, such as the actin that accumulates at the point of bacterium-host contact (3). However, this isolate did not contain any of the genes encoding virulence factors that have been ascribed to pathogenic *Escherichia coli* strains and are responsible for bloody diarrhea or dysenteric syndromes (3).

In Bangui, the rate of isolation of pure cultures of *K. pneumoniae* from the stools of immunocompromised HIV-infected adults with chronic diarrhea is increasing. This finding was observed during the routine biological analyses performed in the Pasteur Institute Medical Laboratory and is consistent of that made by Gassama et al. in Dakar (5). The role of *K. pneumoniae* in HIV-infected adults is not well documented. As no other known enteric pathogens were isolated from these samples, we conducted a case-control study in Bangui in 1999–2001 to determine the clinical significance of *K. pneumoniae*. The study population included 31 adults hospitalized with chronic diarrhea and 31 matched controls. (Because of civil unrest in Bangui due to military rebellions and the difficulties involved in recruiting controls, the study was performed on a small sample.) To be included in the study, the patients had to be HIV positive, be ≥18 years of age, have provided a stool sample containing *K. pneumoniae* on the day of recruitment, and have given informed consent. Inclusion criteria were the same for controls except that they did not have diarrhea on the day of recruitment or in the previous month. Controls were family members or neighbors of the patients, matched by age (within 5 years) and sex. Specimens from cases and controls were collected over the same 1-month period. Known enteric pathogens were identified by standard methods as described (6). Endoscopic examinations were used to diagnose pseudomembranous colitis in patients with bloody chronic diarrhea or watery chronic diarrhea. The median CD4+ cell count was 122 cells/mL in the patients and 436 cells/mL in the controls. AIDS-related symptoms were observed in all of the cases (Centers for Disease Control and Prevention [CDC] stage C2 or C3) and none of the controls (CDC stage A1). Of the 31 patients, 7 (22.6%) had bloody chronic diarrhea, 9 (29%) had watery chronic diarrhea, and 15 (48.4 %) had mild chronic diarrhea. Pseudomembranous colitis was diagnosed in nine patients (six with bloody diarrhea, three with watery chronic diarrhea) who had been taking several antibiotics, including ampicillin, for a long period (>1 month). Five *K. pneumoniae* colonies were randomly picked up from each case sample and examined. Control colonies were chosen if their appearance suggested *K. pneumoniae*. The mean number of strains tested was 4.99 for patients and 4.64 for controls (not significant, p=0.969). All of the enteric bacteria isolated from the patients and grown on nonselective bromocresol purple medium were *K. pneumoniae,* whereas an average of 10% to 20% of the enteric bacteria isolated from the controls were *K. pneumoniae*. We used assays typically used to identify the virulence factors of diarrheagenic *E. coli* (7) to characterize the virulence properties of the *K. pneumoniae* isolates, their genotypes, and their phenotypes (their ability to bind to cultured HEp-2 cells and to promote cytoskeleton modifications [fluorescent actin staining test], to invade epithelial cells, to produce various enterotoxins and cytotoxins, and to induce fluid accumulation in the intestines of newborn mice). The rabbit ligated ileal loops test was performed when the genetic or phenotypic (on Vero or Y1 cells) tests were positive for toxins. All isolates from 27 patients (7, 9, and 11 with bloody, watery, and mild chronic diarrhea, respectively) and two of the isolates from one control displayed an aggregative adherence phenotype on HEp-2 cells. This phenotype appeared to be significantly associated with chronic diarrhea (27/31 cases vs. 1/31 controls, χ=40.7, $p<10^{-6}$). All HEp-2–adherent *K. pneumoniae* isolated from six of the patients produced toxins. The culture supernatants of the HEp-2–adherent *K. pneumoniae* strains isolated from four of the patients with bloody chronic diarrhea and pseudomembranous colitis had cytotoxic effects on Vero and Y1 cells, as characterized by the rounding of cells after 24 h, followed by their detachment from the culture plate and death after 72 h. These effects were not neutralized by rabbit antisera raised against the Shiga toxin or the cholera toxin. The HEp-2–adherent *K. pneumoniae* strains isolated from two patients with watery chronic diarrhea were enterotoxigenic in ligated rabbit ileal loops. Only the HEp-2–adherent *K. pneumoniae* strains isolated from one patient with watery chronic diarrhea and pseudomembranous colitis (5 strains) and from four patients with mild chronic diarrhea (20 strains) carried sequences related to virulence genes from pathogenic enteroaggregative *E. coli*. These isolates were all positive for the *astA* gene, which encodes the EAST1 toxin, and for the genes that produce the AAF/I fimbriae.

K. pneumoniae is normally resistant to β-lactams. Multidrug-resistant *K. pneumoniae* have been reported (1). All *K. pneumoniae* isolates in this study were resistant to several antibiotics including cotrimoxazole and ampicillin, which are largely used in Bangui according to the recommendations of the World Health Organization (8). In addition to the nonspecific measures used to correct and prevent fluid, electrolyte, and nutritional imbalances, all persons with bloody and watery chronic diarrhea (including those with pseudomembranous colitis) and 5 of the 15 patients with mild chronic diarrhea (10 were lost to follow-up) were treated with ofloxacin (800 mg/day) or ceftriaxone (2 g/day), based on the results of antimicrobial susceptibility testing. The state of all patients with pseudomembranous colitis and mild chronic diarrhea, and of

five of the patients with watery chronic diarrhea (one patient died), improved within 10 days of treatment.

In Dakar, during the study describing ordinary and opportunistic enteropathogens associated with diarrhea in adults (5), stool samples were collected from five HIV-infected adults with watery chronic diarrhea. In all cases, heavy *K. pneumoniae* growth was observed on the primary culture media, and no other known pathogens were recovered. These *K. pneumoniae* strains were subjected to the same phenotypic and genotypic tests as the strains isolated in Bangui. HEp-2–adherent *K. pneumoniae* was identified in four of these five samples. The condition of all the patients rapidly improved after treatment with ofloxacin. In Bangui and Dakar, repeated stool cultures were negative for *K. pneumoniae* by the end of treatment, providing further evidence that these *K. pneumoniae* were of etiologic importance, especially the HEp-2–adherent *K. pneumoniae* strains.

Only seven patients (four with mild, two with watery, and one with bloody chronic diarrhea) had not taken antibiotics during the 2 weeks before stool collection. The stool specimens from these seven patients yielded pure primary cultures of HEp-2–adherent *K. pneumoniae* and no other bacterial enteric pathogens. None of these seven participants was diagnosed with pseudomembranous colitis. The HEp-2–adherent *K. pneumoniae* strains isolated from the two participants with watery chronic diarrhea induced the accumulation of fluid in ligated rabbit ileal loops, and the HEp-2–adherent strains isolated from three of the participants with mild chronic diarrhea carried the *astA* gene, which is associated with pathogenic EAEC. Among the five patients with pseudomembranous colitis, all of whom had received antibiotics before the onset of illness, we found that the four isolates from the patients with bloody chronic diarrhea were cytopathogenic; the one isolate from the patient with watery chronic diarrhea had the pathogenic marker for enteroaggregative *E. coli*. These findings suggest that not only is *K. pneumoniae* associated with chronic diarrhea in HIV-infected persons but also that infection with particular HEp-2-adherent *K. pneumoniae* subtypes may be associated with specific clinical illness.

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Granulomatous Lymphadenitis as a Manifestation of Q Fever

To the Editor: Q fever is a worldwide zoonosis caused by the obligate intracellular pathogen *Coxiella burnetii* (1). Human infection is usually the result of exposure to infected cattle, sheep or goats. Acute Q fever may be asymptomatic or manifest as a self-limiting febrile illness, pneumonia, hepatitis, or meningoencephalitis. Most cases of acute Q fever will resolve without sequelae, but endocarditis, granulomatous hepatitis, osteomyelitis, and endovascular infections are well-documented manifestations of chronic *C. burnetii* infection (1). Recently, various atypical manifestations of acute (2), and chronic (3) Q fever have been reported as well as changing clinical presentation of Q fever endocarditis (4) and changing epidemiology of Q fever (5). Researchers have suggested that heightened awareness of Q fever among doctors, coupled with improved diagnostic methods, could increase the medical knowledge about this difficult-to-diagnose and difficult-to-treat infection (4). We report two cases of granulomatous lymphadenitis associated with *C. burnetii* infection.

A 70-year-old man was admitted to the hospital because of weight loss, night sweats, and a continuous high-

grade fever of 2 months' duration. His past medical history was unremarkable, except for pulmonary tuberculosis treated 55 years earlier and chronic glaucoma. He lived in a rural area and had rare contact with cattle. On admission, his body temperature was 39.5°C; his right laterocervical lymph nodes were enlarged (3 cm x 4 cm) and inflamed. Blood values were unremarkable except for an elevated C-reactive protein level of 150 mg/L (normal<6). A computed tomography scan of the chest showed hilar calcifications and enlarged mediastinal lymph nodes. A biopsy of cervical lymph nodes indicated granulomatous lymphadenitis with foci of necrosis. *C. burnetii* DNA was detected on the lymph nodes with a *C. burnetii*–specific pair of primers that amplified an *htpAB*-associated repetitive element (6). Results of serologic testing by indirect immunofluorescence (IF) were positive for *C. burnetii* with immunoglobulin (Ig) G antibody titer to phase 1 and phase 2 antigen of 800 and 1,600, respectively, and IgM antibody titer to phase 2 antigen of 50.

A 44-year-old man was admitted to the hospital because of a continuous low-grade fever of 3 months' duration. He had worked as a farmer for 15 years and assisted in the birth of sheep and cattle. On admission, his body temperature was 38°C, and right inguinal lymph nodes were inflamed, measuring 4 x 4 cm. A lymph node biopsy showed granulomatous lymphadenitis with stellate abscesses surrounded by palisading epithelioid cells. Serologic testing by indirect IF was positive for *C. burnetii* with an IgG antibody titer to phase 1 antigen of 320.

For both patients, results of Ziehl staining and Lowenstein (Bio-Rad, Marne-La-Coquette, France) cultures of gastric aspirates (x 3) and lymph node specimens were negative for mycobaceria, as were the results of tuberculin skin tests. Other diseases were ruled out, including brucellosis, yersiniosis, bartonellosis, and chlamydial infections (by serologic testing) and fungal infections (parasitologic studies on lymph node tissue). Antinuclear antibodies were absent, and angiotensin-converting-enzyme values were normal. Both patients received doxycycline, 200 mg once a day, and rifampin, 600 mg twice a day, for 1 year, and the symptoms resolved (follow-up at 18 months for patient 1 and 9 months for patient 2, respectively). For patient 1, serologic testing after 1 year of treatment showed an IgG antibody titer to phase 1 antigen of 320.

Granulomatous lymphadenitis has been described during mycobacterial infections, tularemia, cat scratch disease, yersiniosis, lymphogranuloma venereum, histoplasmosis, coccidioidomycosis, and chronic granulomatous diseases (7). One well-documented case of acute Q fever with necrotic cervical lymphadenitis has been recently reported (8); to our knowledge, granulomatous lymphadenitis has never been reported during Q fever. In both cases reported here, *C. burnetii* was the likely etiologic agent, given the results of polymerase chain reaction and serologic studies (patient 1) or the patient's occupation and results of the serologic testing (patient 2). Moreover, for both, no other potential cause could be identified, and the response to doxycycline-rifampin regimen was favorable. We suggest that granulomatous lymphadenitis be added to the list of atypical presentations of Q fever.

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Has *Coxiella burnetii* (Q fever) Been Introduced into New Zealand?

To the Editor: New Zealand has been an exception to the panglobal distribution of *Coxiella burnetii* (1), the causative organism of Q fever, as shown in a 1990–1991 study (2) of 12,556 sheepdogs and 2,181 aborting cattle, all seronegative for *C. burnetii.* In 1997, the *Rabbit hemorrhagic disease virus* (RHDV) was illegally imported from Australia into Central Otago, New Zealand, for the purpose of rabbit control. The unknown source and purity of RHDV, and the potential use of infected rabbits or their organs to transport it, meant that *C. burnetii* could have been coincidentally introduced along with the RHDV-infected rabbit material. To establish whether this occurred, we examined serum

specimens from 97 participants enrolled in the RHDV human health study for antibodies to Q fever (3).

C. burnetii is a very infective organism; it can remain viable for long periods in harsh environmental conditions (1). The primary route of human exposure is aerosol dispersion (1), and airborne agricultural dusts containing the organism have been implicated in the infection of distant communities (4).

Wild rabbits are part of the extensive reservoir of *C. burnetii* in the animal kingdom (1) and have been linked with Q fever (5). In the United States, 53% of wild rabbits and 39% of wild jackrabbits were found to have antibodies to *C. burnetii,* and the organism has been isolated from both these species (6); in Nova Scotia, 49% of hares had antibodies to *C. burnetii* (7). In Australia, Q fever is estimated to result in at least 1,700 weeks of lost work time annually, primarily affecting people in the eastern states (8).

We could not find evidence of *C. burnetii* in Australian rabbits despite the frequency of Q fever in that country (8) and reports of extensive *C. burnetii* infection of rabbits in other countries (6). However, the presence of RHDV-infected rabbits in the part of Australia where Q fever is most often reported (8,9) suggested that the rabbit tissue imported to New Zealand in 1997 might have been infected with *C. burnetii.*

A local lawyer enrolled the study participants anonymously on the basis of their possible exposure to the illegally imported rabbit material. These participants provided serum samples and answered interviewer-administered questionnaires 15 weeks after the first confirmation of the virus (3). As a result of the controversy and potential legal proceedings surrounding the circumstances of the biosecurity breach, several questions were considered too sensitive and were not asked; these included the participants' travel history and exact details of their roles in processing the infected rabbit material. However, in many cases the participants volunteered additional information regarding their exposures.

Nearly all participants (86/97) had had contact with rabbits, and more than half (53/97) appeared to have had contact with RHDV-infected rabbit material. Anecdotal reports suggest that heavy exposures occurred during the harvesting and processing of infected rabbit body organs, spraying of infective organ mixtures onto bait, and distribution of these infective baits by air and ground over 136,000 hectares (340,000 acres).

Thirteen persons were considered to have had a variety of inhalation exposures. Four persons mentioned tasting, smelling, breathing, or having the spray blown in their face for 3–5 hours; another four participants referred to their involvement in spraying. Another four participants had concerns about their inhalation exposures, and one person was included on the basis of exposure while shoveling carrots mixed with infected rabbit blood and molasses. Many additional aerosol exposures likely occurred that the researchers could not specifically identify.

Of the 97 serum samples, 3 were classified as positive to *C. burnetii,* 1 as equivocal, and 93 as negative by using an enzyme-linked immunosorbent assay (PanBio IgG Cat No QFG 100, Brisbane, Australia). On the basis of a single test, determining how many of the results were false positive is difficult: an assay specificity estimate of 95.7% (10) suggests we might expect to correctly identify 93 negative serum samples in a population of 97 with no exposure to Q fever.

No evidence of an association between seropositivity and exposure to infective rabbit material was apparent. The most strongly positive result was found in a person who had described no major exposures. One positive result occurred among 39 persons with possible direct contact (i.e., open wound) exposure, and another among 17 persons who had eaten possibly infected rabbit material. None of the 13 persons with inhalation exposures, the 2 persons with needlestick injuries, or the 1 person with a definite bait consumption exposure had serum samples that were positive or equivocal. Likewise, none of the seven persons with reported health problems

had serum samples that were either positive or equivocal. The low prevalence of antibodies to *C. burnetii* in the participants in our study (3/97) indicates that most were very unlikely to have had contact with the organism. If the results are true positives, the source of the infection was quite likely outside of New Zealand. However, considering the heavy exposures associated with the cultivation and harvesting of RHDV in live rabbits and the known infectivity of Q fever, *C. burnetii* was not likely to have been introduced inadvertently to New Zealand at the same time as RHDV.

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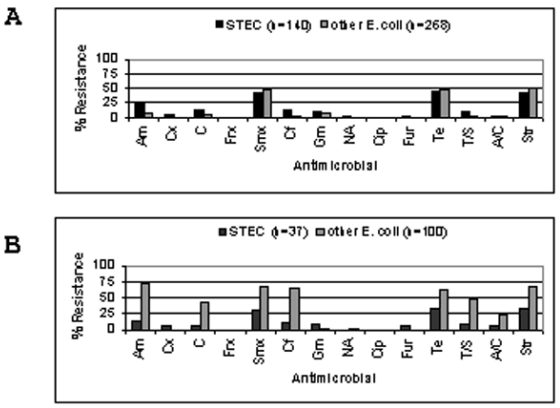
Correction, Vol.8, No.12

In the article, "Antimicrobial Resistance of *Escherichia coli* O26, O103, O111, O128, and O145 from Animals and Humans" by Carl M. Schroeder et al., errors occurred in the figure on page 1412. The corrected figure appears below and online at http://www.cdc.gov/ncidod/eid/vol8no12/02-0070.htm.

We regret any confusion these errors may have caused.

Figure 2. Comparison of antimicrobial resistance frequencies between Shiga toxin–producing *Escherichia coli* (STEC) and other *E. coli*. Of isolates from cattle, resistance frequencies were similar between STEC and other *E. coli* (A). In contrast, of isolates from humans, resistance frequencies were generally lower for STEC compared with other *E. coli* (B). Am, ampicillin; Cx, cefoxitin; C, chloramphenicol; Frx, ceftriaxone; Smx, sulfamethoxazole; Cf, cephalothin; Gm, gentamicin; NA, nalidixic acid; Cip, ciprofloxacin; Fur, ceftiofur; Te, tetracycline; T/S, trimethoprim-sulfamethoxazole; A/C, amoxicillin-clavulanic acid; Str, streptomycin.



Correction, Vol. 8, No. 10

In "Investigation of Bioterrorism-Related Anthrax, United States, 2001: Epidemiologic Findings" by Daniel B. Jernigan et al., errors occurred in the listing of the members of the Anthrax Epidemiologic Investigation Team on page 1019. Additional members of the National Anthrax Epidemiologic Investigation Team are:

Francisco Alvarado-Ramy, MacKenzie Andre, MaryKate Appicelli, Mick Ballesteros, Mark Beatty, Omotayo Bolu, Louise Causer, Soju Chang, Ilin Chuang, John Crump, Marvin DeBerry, Rachel Gorwitz, Michelle Goveia, Thomas Handzel, Josh Harney, Dan Hewett, Vincent Hsu, Young Hur, Marialena Jefferds, Joshua Jones, Kathleen Julian, Richard Kanwal, Jane Kelly, Dennis Kim, Judy Kruger, Richard Leman, Steve Lenhart, Jill Levine, Naile Malakmadze, Els Mathieu, Rob McCleery, Shawn McMahon, Manoj Menon, Kelly Moore, Jill Morris, James Andy Mullins, Melanie Myers, Timothy Naimi, Lori Newman, Chima John Ohuabunwao, Michael O'Reilly, Lisa Pealer, Chris Piacitelli, Joe Posid, John Redd, Mary Reynolds, Julia Rhodes, Louie Rosencrans, Lisa Roth, Denise Roth-Allen, Sharon Roy, Taraz Samandari, Dejana Selenic-Stanacev, Jina Shah, Tanya Sharp, Allison Stock, Lauralynn Taylor, Pauline Terebuh, Christopher Thomas, Beth Tohill, Barna Tugwell, Angela Weber, Dana White, Sara Whitehead, Wally Wilhoite, Leigh Winston, Brad Winterton, Katharine Witgert, William Wong, Susie Wootton, and Weigong Zhou

We regret any confusion these errors may have caused.

Polymicrobial Diseases

K.A. Brogden and J.M. Guthmiller, editors

ASM Press
Washington, D.C., 2002
ISBN: 1-55581-244-9
Pages: 446, Price: $115.95

Polymicrobial diseases involve multiple infectious agents and are referred to as complex, complicated, mixed, dual, secondary, synergistic, concurrent, polymicrobial, coinfections. This new book, a collection of 21 chapters written by a variety of authors, reviews mixed infections in animals and humans. The chapters are gathered into sections on polyviral diseases, polybacterial diseases, viral and bacterial infections, fungal infections, infections resulting from microbe-induced immunosuppression, and a concluding perspective. Polymicrobial diseases described include abscesses, AIDS-related opportunistic infections, conjunctivitis, gastroenteritis, hepatitis, multiple sclerosis, otitis media, periodontal diseases, respiratory diseases, and genital infections. Approximately two-thirds of the chapters deal with human diseases; the others discuss infections in cattle, goats, and pigs.

The chapters are generally well written with a focus on microbiology, pathogenesis, and to a lesser degree, treatment. The chapters on abscesses, multiple sclerosis, and mixed mycotic infections are especially informative. The chapter on abscesses provides a comprehensive review of the microbiology processes involved, the role of anaerobes in mixed infections, and animal models. The section on viruses and multiple sclerosis is provocative in its proposal that several viruses might coexist and interact to promote multiple sclerosis and other neurologic diseases. The list of candidate etiologic agents includes *Human herpesvirus*-6, human T-lymphotropic virus type 1, measles viruses, JC virus, Epstein-Barr virus, and herpes simplex virus-1. The chapter on mixed mycotic infections adequately discusses how fungi interact by mechanisms such as commensalism, opportunism, mixed colonization, coisolation, and dual and polymicrobial infection.

Growing two or more microbes in the laboratory in a clinical situation does not prove that a polymicrobial infection is the cause of the disease. The editors and authors do not provide a framework similar to that of Robert Koch or Bradford Hill, which one can use to decipher the role(s) of each candidate agent in a polymicrobial disease. A limited discussion is provided on the role of noninfectious factors, such as genetics of the host, retained "hardware," alcohol in hepatitis, or tobacco use in respiratory diseases. How each of the chapters was selected for inclusion and what other topics were considered is not clear.

The reference lists are one of the book's strengths but also a weakness. The lists are extensive, occupying about 30% of the book's pages. Prioritizing the outside readings on each topic would have been useful. Several of the chapters might have been combined, such as the two on periodontal diseases, those on retroviruses, and those on respiratory diseases in humans, cattle, and pigs. In the next edition, the authors might explore the polymicrobial etiology of Reye syndrome, autoimmune disorders, atherosclerosis, and cancers, such as Kaposi sarcoma, hepatocellular sarcoma, and cervical cancer. I recommend the book to those who think beyond the "single agent, single disease" framework and imagine multifactorial causes for those diseases currently listed as "etiology unknown."

Harry W. Haverkos

Food And Drug Administration, Rockville, Maryland

Manual of Commercial Methods in Clinical Microbiology

Allan L. Truant

American Society for Microbiology
Washington, D.C., 2002
ISBN: 1-55581-189-2
Pages: 502, Price: $115.95

During the past 25–30 years, tremendous strides have been made in the development of various commercial methods designed to simplify the isolation (in some cases) and the detection or identification (in most cases) of many different microbes in the laboratory. During these years, the time-honored conventional test methods have served the overall science of

microbiology well. However, in a clinical microbiology laboratory, speed and accuracy are essential because the specimen must be processed and the results returned to the requesting physician by yesterday, if possible. Thus, many of the commercial methods reviewed in this book were developed mainly for use in the clinical microbiology laboratory, providing both rapid and accurate results with a minimum of hands-on use.

To my knowledge, this reference manual is the first resource that covers all subdisciplines of clinical microbiology. The book contains 18 chapters, including separate chapters on molecular microbiology, emerging infectious diseases, information management, and veterinary clinical microbiology, as well as chapters on bacterial identification and antimicrobial susceptibility testing, blood cultures, mycology and mycobacteriology, virology, and parasitology. A chapter on licensure and regulation of commercial products is also included, which I found helpful. In addition, the book provides an appendix that lists the manufacturers and distributors for many of the systems described in the book. The authors include a description of the sensitivities, specificities, and predictive values of the tests from peer-reviewed sources. Another chapter of interest focuses on future technology for the clinical microbiology laboratory. My only suggestion is that future editions of this text include a chapter on the history of how all these commercial tests came into being, instead of various authors alluding to this point in their respective chapters.

Each chapter is well referenced, and many chapters contain tabular material that is, for the most part, easy to read and understand. The photography is adequate, although several photographs are blurred and lack clear definition. Although the intended audience for this book is primarily clinical microbiologists and other professionals who work in these environments, I suspect that many physicians, including infectious disease specialists, will find this book especially valuable when deciding what tests to order for their patients, especially in light of the high costs of health care.

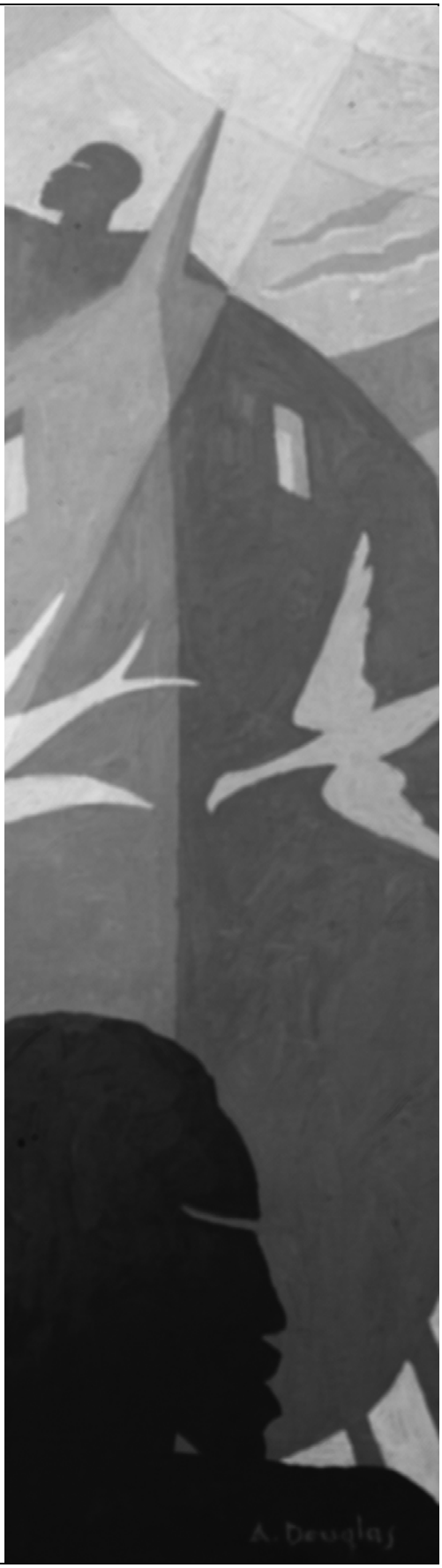
As with any multi-authored text, some unevenness in the writing is expected. However, I believe that the overall scope and format of this book are quite useful, and that readers will find this manual a valuable, comprehensive source of information. The authors are to be commended for tackling such an enormous project and successfully presenting it in such a readable format.

William J. Martin

Tucson, Arizona

About the Cover

Aaron Douglas (1899–1979)
Noah's Ark, 1927
Oil on masonite, 4' X 3'
Fisk University Galleries, Nashville, Tennessee



Aaron Douglas, a native of Kansas, studied art in Nebraska before going to Paris and finally settling in New York City, where he became part of the flourishing art scene of the 1920s and 1930s known as the Harlem Renaissance. Douglas drew inspiration from this powerful cultural movement that encompassed all fields of art and advocated celebration of African cultural identity and heritage (1).

Douglas, whose diverse artistic inclinations included cubism (a stylistic invention of Georges Braque and Pablo Picasso) and African sculpture, was greatly influenced by German-born American artist Winold Reiss (1886–1953). Reiss, son of celebrated architectural designer Fritz Reiss, was a master painter of folk themes. His work captured ethnic and local characteristics in portraits of powerful universal appeal and showcased Pueblo and African cultures in the same way Paul Gauguin's work showcased Far Eastern culture (2). Reiss encouraged Aaron Douglas to bring cultural identity to the forefront of his art.

In a series of seven paintings based on a book of poems by James Weldon Johnson, God's Trombones: Seven Negro Sermons in Verse, Douglas successfully breathed cultural life into work inspired by cubism and African sculpture motifs. Primitive and mystic elements added intensity and complexity to these modern compositions and enhanced their multidimensional scope.

Noah's Ark, the painting featured on this month's cover of Emerging Infectious Diseases, is characterized by the formal, analytical innovations of cubism. A narrow range of sober hues (greens, beiges, blues, whites) allows uninterrupted concentration on the strict geometric definition of space. The transparent, overlapping geometric forms define the desired perspective. The ark is thrust to the foreground, preceded only by the prominent African mask, which firmly anchors the viewer into a geographic and chronological milieu. Sharp lightening strikes, terse animals heading for cover, and a focused crew advancing the vessel's final course bespeak highest emergency.

Cataclysmic disasters fill the pages of human history, from Moses to "the little Dutch boy." The stuff of nightmares, these disasters touch a chord because they reach beyond individual tragedy to massive plight of global proportions. Like other universal themes, the ark derives its appeal from broad applicability: haven of last resort protects those inside from impending disaster for the greater good.

Our times, plagued by (among other pestilences) the relentless emergence of global communicable disease, have constructed their own version of the ark, quarantine. But in this modern version, those sealed inside the vessel are not saved; they perish for the common good. Modern quarantine (as practiced in the prevention of mad cow disease, foot and mouth disease, and other epizootics) protects the whole from the damaged parts. Still, the infected are sealed in the ark to ensure long-term survival of the herd.

Marcia Smalls and Polyxeni Potter

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Upcoming Infectious Disease Conferences

February 10-14, 2003

10th Conference on Retroviruses and Opportunistic Infections
Boston, MA
Website: www.retroconference.org

March 17-20, 2003

27th National Immunization Conference
Chicago, IL
Contact: Suzanne Johson DeLeon
(404) 639-8225
E-mail: msj1@cdc.gov
Website: http://www.cdc.gov/nip/nic

April 6-8, 2003

Society for Healthcare Epidemiology of America (SHEA) Annual Meeting
Arlington, VA
Contact: SHEA Meetings Dept.
(856) 423-7222 ext. 350
Website: www.shea-online.org

May 7, 2003

Certificate of Knowledge in Travel Medicine Examination
International Society of
Travel Medicine Conference
New York, New York
Contact: Brenda Bagwell
E-mail: exam@istm.org
Phone: 770-736-7060
Fax: 770-736-6732
Website: http://www.istm.org

May 7-11, 2003

8th Conference of the International Society of Travel Medicine
New York City
Contact: Lisa Astorga,
Conference Manager
E-mail: lastorga@talley.com
Website: www.istm.org

May 10-13, 2003

13th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)
Glasgow UK
Contact: Administrative Secretariat +41 61 686 77 11
E-mail: info@akm.ch
Website: www.escmid.org/eccmid2003

May 27-30, 2003

Global Health Council's 30th Annual Conference
Our Future on Common Ground: Health and the Environment
Washington, DC
Website: http://www.globalhealth.org/

EMERGING INFECTIOUS DISEASES
A Peer-Reviewed Journal Tracking and Analyzing Disease Trends Vol.9, No.2, February 2003

Upcoming Issue

For a complete list of articles included in the February issue, and for articles published online ahead of print publication, see http://www.cdc.gov/ncidod/eid/upcoming.htm

Look in the February issue for the following topics:

Preparing for a Bioterrorist Attack: Legal and Administrative Strategies

B Virus Infection in Humans and Macaques:
The Potential for Zoonotic Human Disease

Emerging Pattern of Rabies Deaths and Increased Viral Infectivity

Elimination of Epidemic Methicillin-Resistant *Staphylococcus aureus* from a University Hospital and District Institutions, Finland

Endemic Babesiosis in Another Eastern State: New Jersey

Health and Economic Impact of Surgical Site Infections Diagnosed after Hospital Discharge

Equine Amplification and Virulence of Subtype IE Venezuelan Equine Encephalitis Viruses Isolated during the 1993 and 1996 Mexican Epizootics

Influence of Role Models and Hospital Design on Hand-Hygiene of Healthcare Workers

Viral Encephalitis in England, 1989–1998: What Did We Miss?

Photorhabdus: An Emerging Bacterial Pathogen?

Emerging Infectious Diseases thanks the following reviewers for their support through thoughtful, thorough, and timely reviews from the journal's inception to the present. We apologize for any names we may have omitted inadvertently.

F.M. Aarestrup
P. Aavitsland
K.D. Abbott
S. Abbott
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D. Acheson
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J. Ackelsberg
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M. Bennett
J.E. Bennett
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J.V. Bennett
D. Bennett
M. Bennish
G. Berbers
E.A. Berger
O. Bergh
C. Bern
K. Bernard
J. Bernardo
J.M. Bernhardt
D. Bernstein
R. Berry
G. Bertoni
D. Bessen
J. Besser
R. Besser
L.R. Beuchat
L. Beutin
W. Beyer
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B. Biggerstaff
J. Bille
S. Binder
G.S. Birkhead
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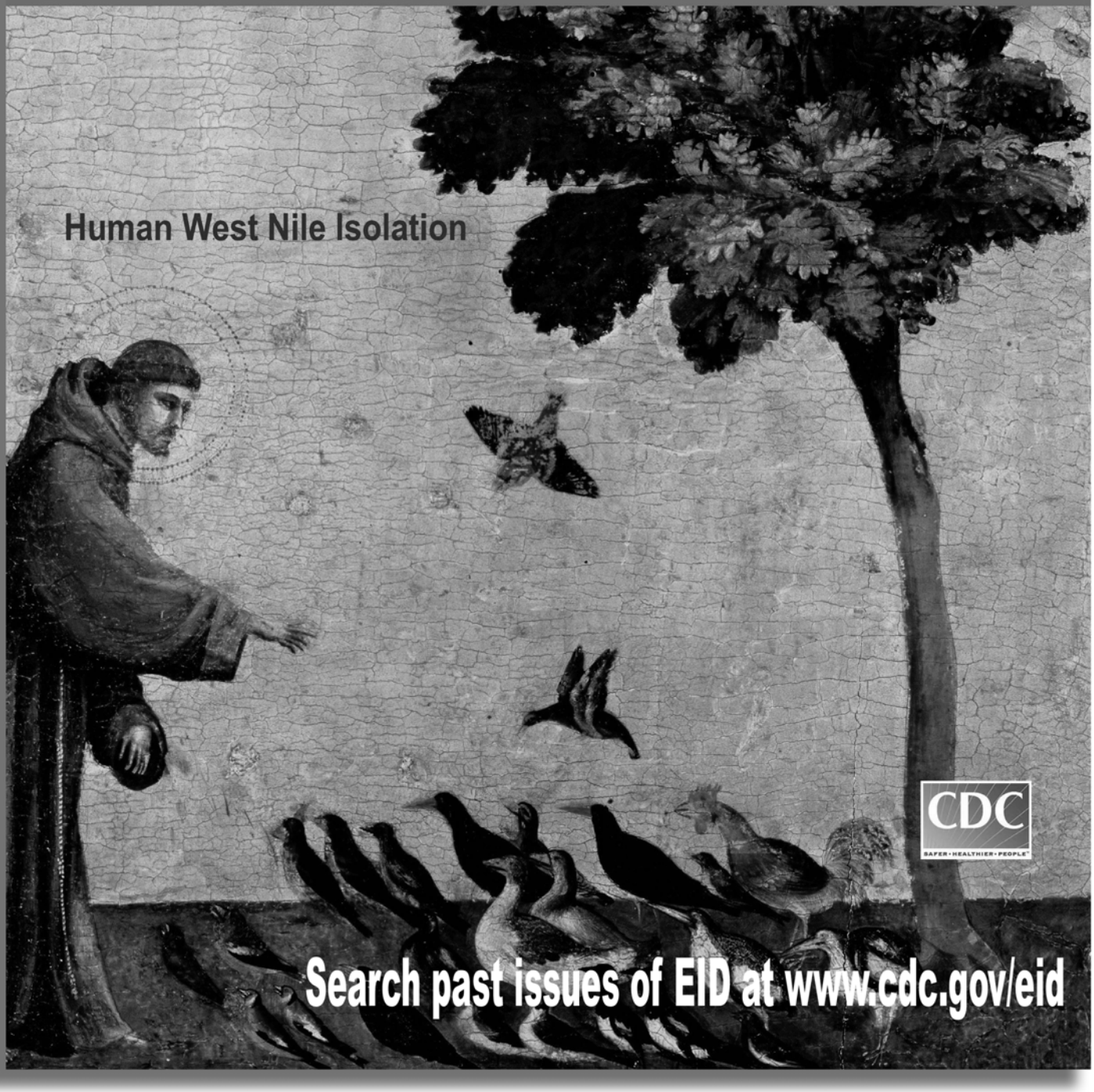

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JOURNAL BACKGROUND AND GOALS

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.

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Emerging Infectious Diseases launches Web-based manuscript submission and peer review

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Emerging Infectious Diseases, now in its 8th year of publication, welcomes submissions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, demography, sociology, and other disciplines. For information on the types of articles published in Emerging Infectious Diseases, see http://www.cdc.gov/ncidod/eid/instruct.htm.

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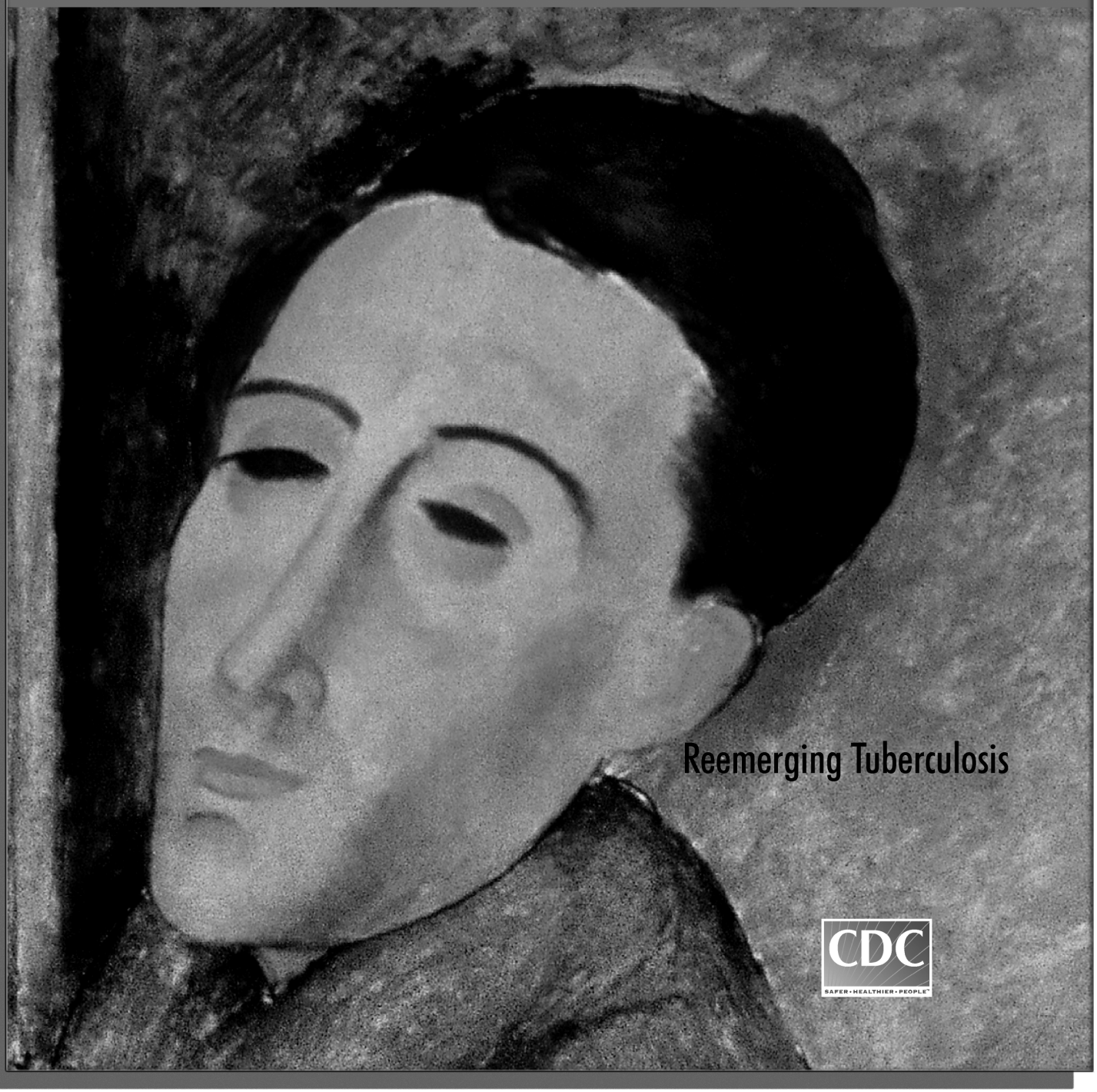

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