Amphibians   Orphan Vaccines   Antimicrobial Resistance

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Emerging Infectious Diseases and Amphibian Population Declines

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We review recent research on the pathology, ecology, and biogeography of two emerging infectious wildlife diseases, chytridiomycosis and ranaviral disease, in the context of host-parasite population biology. We examine the role of these diseases in the global decline of amphibian populations and propose hypotheses for the origins and impact of these panzootics. Finally, we discuss emerging infectious diseases as a global threat to wildlife populations.

Emerging infectious diseases have been reported increasingly as causes of death in free-living wild animals (1). These diseases are a particular threat to wildlife species whose population, habitat, or range has been diminished or artificially manipulated to promote species survival (e.g., captive breeding, translocation, and release programs) (2-4). An early example of an emerging disease panzootic was the introduction of rinderpest in African domestic cattle in 1889 (5). More recently, epizootics and panzootics of wildlife have been increasingly reported in terrestrial (1) and marine (6) habitats and are probably underreported (1,4,7-9). Recent advances in theoretical and experimental host-parasite ecology have demonstrated a major role for infectious agents in the population biology of wild animals (10,11). We discuss recent data on two newly emerging infectious diseases of amphibians and, by reference to host-parasite ecology, propose hypotheses to explain their origin and impact.

Amphibian Population Declines

Global declines in amphibian population are perhaps one of the most pressing and enigmatic environmental problems of the late 20th century

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(12-19). While some declines are clearly due to habitat destruction, others are not associated with obvious environmental factors. Causal hypotheses include the introduction of predators or competitors, increased ultraviolet (UV-B) irradiation, acid precipitation, adverse weather patterns, environmental pollution, infectious disease, or a combination of these. Transdermal water uptake and gaseous exchange and a biphasic life cycle are important aspects of amphibian biology. These factors led to the hypothesis that amphibians act as sentinels for global environmental degradation (12,18). However, this role has yet to be demonstrated, and many causal factors may be present (12,19,20).

Of particular concern are population declines in ecologically pristine areas, such as the montane tropical rain forests of Australia and Central America, where human impact from agriculture, deforestation, or pollution is thought to be negligible. Here, long-term data demonstrate recent and catastrophic amphibian population declines, often resulting in the complete loss of amphibian species (local extinction of multiple species) from large swaths of habitat (20-25). These declines include the disappearance and presumed extinction of the recently discovered golden toad (*Bufo periglenes*) of Costa Rica (23) and as many as seven Australian amphibian species, including two species of gastric-brooding

frog (*Rheobatrachus* spp.) (20). These data, along with recent findings of amphibian mass deaths in these areas, suggest that such local extinctions are not normal population fluctuations or metapopulation dynamics.

Investigations in Australia, the United Kingdom, and North and Central America (26-31) have repeatedly found two diseases as the causes of amphibian mass deaths globally (Table 1): chytridiomycosis in the rain forests of Australia and Central and South America and some parts of North America (28-32) and iridoviral infections in the United Kingdom, the United States, and Canada (26,27,33-36). Both

Table 1. Mass deaths caused by chytridiomycosis and ranaviral disease in wild populations of amphibians

	Locality and date of mass deaths	Species affected and impact[a]	References
Chytridiomycosis	E. & S. Australia (1993-1999)[b]	Multiple montane rain forest and temperate species. Mass deaths, local extinctions, population declines. Near-extinction of *Taudactylus acutirostris*. Hypothesized link with global extinction of two species of gastric brooding frog (*Rheobatrachus* spp.).	28-30
	W. Australia (1998-1999)[c]	Multiple species, predominantly the western green (or motorbike) frog (*Litoria moorei*). Mass deaths, population declines.	29,31
	Costa Rica and Panama (1994-99)	Multiple montane rain forest species. Mass deaths, local extinctions, population declines. Hypothesized link with global extinction of golden toad, *Bufo periglenes*.	20,23, 28,29
	Ecuador (1999)	Montane rain forest *Atelopus* species, *Telmatobius niger*, and *Gastrothecus pseustes*. Unknown impact.	29
	Arizona (1996-1997)	Leopard frog (*Rana yavapiensis* & *R. chiricahuensis*). Mass deaths.	29
	S. Arizona (1999)	Leopard frog (*Rana* sp.). Mass deaths.	31,32
	Colorado (1999)	Boreal toad (*Bufo boreas*). Mass deaths.	[d]
	Colorado (1970s)	Leopard frog (*Rana pipiens*). Mass deaths.	32[d]
	Sierra Nevada, California (1970s)	Yosemite toad (*Bufo canorus*). Mass deaths.	32[e]
Ranaviral disease	United Kingdom (1992-1999[f])	Common frog (*Rana temporaria*). Mass deaths, possibly population declines.	5,16,26, 33,34
	Arizona (1995)	Sonoran tiger salamander (*Ambystoma tigrinum stebbinsi*). Mass deaths in this endangered species.	27
	N. Dakota (1998)	Tiger salamander (*A. tigrinum*). Mass deaths.	35
	Maine (1998)	Tiger salamander (*A. maculatum*). Mass deaths.	35
	Utah (1998)	Tiger salamander (*A. tigrinum*). Mass deaths.	35
	Saskatchewan, Canada (1997)	Tiger salamander (*A. tigrinum diaboli*). Mass deaths.	36

[a]Mass deaths did not occur in all cases of wild amphibians infected by chytridiomycosis. *Bufo americanus* from Maryland and *Acris crepitans* from Illinois have been found infected with chytridiomycosis without observed deaths (37,38). In Australia, chytridiomycosis has been reported from small numbers of amphibians without evidence of clinical signs or deaths in both upland and lowland species (R. Speare, L. Berger, unpubl. obs.).
[b]Retrospective studies have identified chytridiomycosis as the cause of death in wild frogs in five Australian states from as early as 1989 (29).
[c]This recent outbreak was more than 2,000 km from the closest recorded chytridiomycosis-linked amphibian die-offs (31). It is thought that chytridiomycosis may now be enzootic in many areas of Australia, but still in the process of spreading to naïve populations. A role for chytridiomycosis in other recent W. Australian declines is suspected due to similarities in the pattern of declines and presence of the *Batrachochytrium* carcasses from W. Australia since 1992.
[d]D.E. Green, unpubl. obs.
[e]D.E. Green, unpubl. obs. Historically collected specimens recently examined histologically revealed chytridiomycosis as a contributing factor to the cause of death in 2 of 12 animals.
[f]A.A. Cunningham, unpubl. obs.

diseases have been classified as emerging (1). The parasitic infection recently implicated as the cause of amphibian deformities in North America has not been associated with mass deaths or population declines (31).

Chytridiomycosis—an Emerging Panzootic Fungal Disease of Amphibians

Chytridiomycosis is a fungal disease first described in 1998 from moribund and dead adult amphibians collected at sites of mass deaths in Australia and Panama from 1993 to 1998 (28). Here, long-term ecologic study sites reported catastrophic amphibian population declines in Big Tableland, Queensland (39,40), and Fortuna and Cerro Pando, Panama (24,25,28). No significant pathogens were found on routine parasitologic, bacteriologic, mycologic, or virologic examinations of tissue samples (28). Fresh skin smears and histologic sections of the epidermis, however, consistently contained large numbers of developing and mature sporangia of a new genus of chytrid fungus (phylum Chytridiomycota) (Figures 1, 2). Sporangia were also present within the keratinized mouthparts, but not the epidermis, of sympatric tadpoles (tadpoles lack epidermal keratin) (28,29). No significant morphologic differences between chytrids infecting Australian and Central American amphibians were found by transmission electron microscopy, and the pathogen was identified as a member of the order Chytridiales by analysis of zoospore ultrastructure and 18s rDNA sequence data (28). Chytrids are ubiquitous fungi that develop without hyphae and are found in aquatic habitats and moist soil, where they degrade cellulose, chitin, and keratin (41). Parasitic chytrids mainly infect plants, algae, protists, and invertebrates (41); the amphibian pathogen is the only example of a chytrid parasitizing vertebrates (28).

Clinical signs of amphibian chytridiomycosis include abnormal posture, lethargy, and loss of righting reflex. Gross lesions, which are usually not apparent, consist of abnormal epidermal sloughing and (more rarely) epidermal ulceration; hemorrhages in the skin, muscle, or eye; hyperemia of digital and ventrum skin, and congestion of viscera (29). Diagnosis is by identification of characteristic intracellular flask-shaped sporangia and septate thalli within the epidermis (Figures 1, 2).
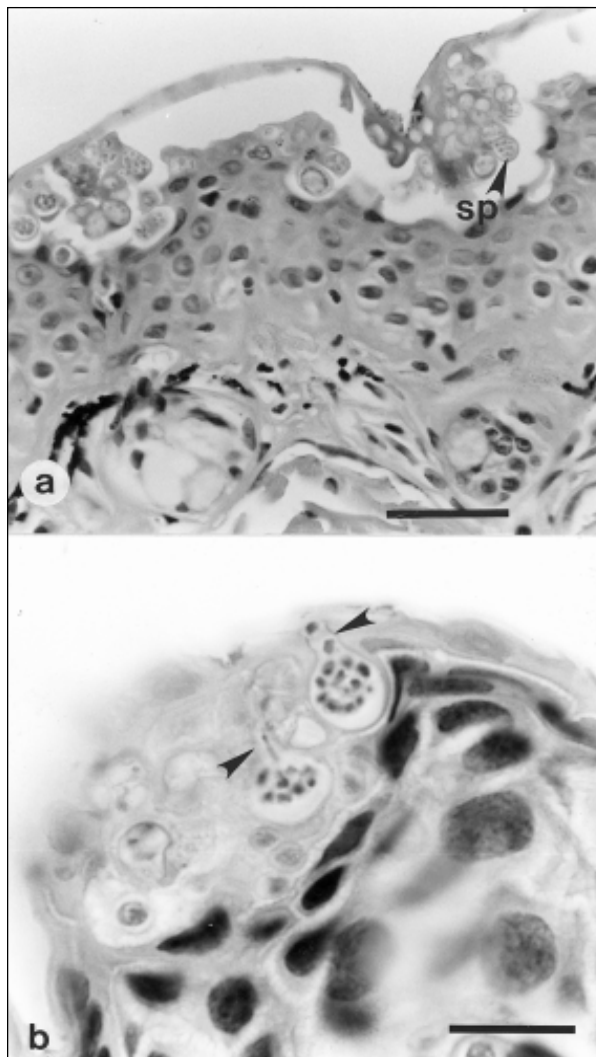
Figure 1a: Ventral abdominal skin of *Bufo haematiticus* from western Panama. The superficial keratinized layer of epidermis (*stratum corneum*) contains numerous intracellular spherical-to-ovoid sporangia (spore-containing bodies) of *Batrachochytrium* sp. The mature sporangia (sp, arrows) are 12-20 µm (n = 25) in diameter and have refractile walls 0.5-2.0 µm thick. Most sporangia are empty, having discharged all zoospores, but a few sporangia contain two to nine zoospores. This stratum corneum is markedly thickened adjacent to groups of parasitized cells and in some cases, the superficial layer has become detached. No chytrids are present in the *stratum spinosum*, *stratum basale*, dermis, dermal glands, and blood vessels. Note the absence of hyphae and lack of an inflammatory cell response in the deeper layers of epidermis and the dermis. Hematoxylin and eosin stain. Bar = 35 µm. 1b. Ventral skin of upper hind limb of *Atelopus varius* from western Panama. Two sporangia containing numerous zoospores are visible within cells of the *stratum corneum*. Each flask-shaped sporangium has a single characteristic discharge tube (arrow) at the skin surface. Exiting zoospores are visible in the discharge tubes of both sporangia. Hyperkeratosis is minimal in this acute infection. Tissues were fixed in neutral-buffered 10% formalin, paraffin-embedded, sectioned at 6 µm thick and stained with hematoxylin and eosin. Bar = 35 µm.
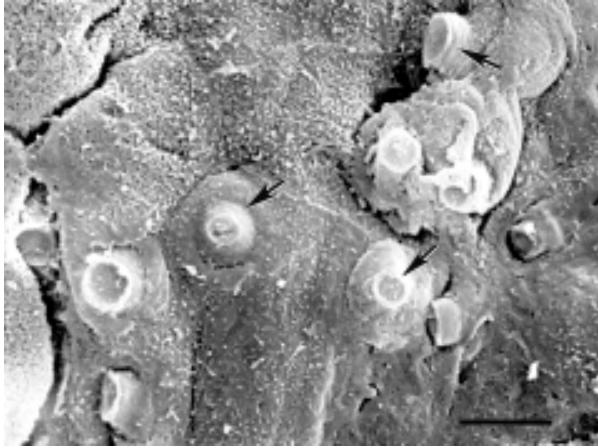
Figure 2. Scanning electron micrograph of digital skin of a wild frog (*Litoria lesueuri*, from Queensland, Australia) that died of cutaneous chytridiomycosis. Many cells within this area of the superficial layer of the epidermis contain mature sporangia, and unopened discharge tubes are visible protruding through infected cells. The skin was fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated, critical-point-dried, sputter coated with gold, and examined with a JEOL JSM 840 scanning electron microscope at 5 kV. All specimens are from animals that were naturally infected and died due to chytridiomycosis in montane rain forest regions of Panama and Australia. Bar = 5 µm.
Photo courtesy of L. Berger, reprinted with permission from A. Campbell (29).

Its occurrence solely in keratinized tissue suggests that the chytrid uses amphibian keratin as a nutrient. A hyperkeratotic and hyperplastic response of the epidermis to infection (restricted to the *stratum corneum* and *stratum granulosum*) usually coincides with the immediate location of chytrid developmental stages. Inflammatory cell response is negligible. An isolate cultured from captive dendrobatid frogs has recently been used to fulfil Koch's postulates as a fatal pathogen of frogs and has been described as a new genus and species, *Batrachochytrium dendrobatidis* (42). Three mechanisms by which chytridiomycosis causes death have been proposed (28): 1) epidermal hyperplasia impairs essential cutaneous respiration or osmoregulation; 2) a fungal toxin is absorbed systemically (although a lack of clinical signs in infected larvae suggests otherwise); and 3) these factors are combined (28,37).

Comparison of histologic, ultrastructural, and 18s rDNA sequence data indicates that the chytrids found in wild Australian (28) and captive Central American (T.Y. James, D. Porter, J.E. Longcore, pers. comm.) amphibians belong to the genus *Batrachochytrium*, are probably conspecific, and form a distinct monophyletic clade within the Chytridiales.

Emergence of Chytridiomycosis

Retrospective histologic surveys of museum specimens of montane, riparian anurans from protected sites in Central America and Australia, conducted 1 to 10 years before the population declines, showed no evidence of chytrid infection, which suggests that chytridiomycosis has recently emerged on two continents (28). The relatively synchronous discovery of chytridiomycosis in Australia and Central America in association with amphibian population declines is striking. The data suggest that *Batrachochytrium* 1) may be endemic to these regions and the amphibian deaths and declines attributed to it have only recently been discovered; 2) may be endemic and has recently become pathogenic (e.g., through an increase in the organism's prevalence or virulence, or a decrease in the host's defenses), or 3) may have been introduced recently into these geographic regions and is now parasitizing novel host species.

The pattern of amphibian deaths and population declines associated with chytridiomycosis is characteristic of an introduced virulent pathogen dispersing through a naïve population (7,10,39). In Australia, a distinct geographic and temporal progression in population declines has occurred (20), moving northward at a mean rate of 100 km per year (39). In Central America, a progression from northern Costa Rica to western Panama occurred from 1996 to 1998 (24,25). The uneven progression of declines in Australia (40) may reflect gaps in surveillance. Small-scale irregularities, however, characterize the epidemiology of many pandemics (43,44), within which individual epidemics progress at different rates in different areas. In amphibian populations, unevenness may be due to differences in ecologic factors (e.g., population density, habitat, age structure); differences among pathogen strains; stochastic factors, such as the time of introduction; or a combination of these factors. In Australia and Central America, population declines have been catastrophic, occurring over a few months, with dramatic population loss and high rates of adult deaths (20,24,25,28,39,40,45).

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Such high depopulation rates are characteristic of introduced virulent pathogens (10). Conversely, in coevolved host-pathogen relationships, a degree of herd immunity to the pathogen and lower virulence (infectivity and death rates) are normally observed. The low host specificity of amphibian chytridiomycosis (more than 30 species of wild amphibians from seven families in Central America and Australia [28,29]) also suggests that the disease was not enzootic in those montane rain-forest populations. Ability to infect a range of host species is a characteristic of many invading pathogens (10) and is less common in endemic microparasites that have coevolved with their hosts.

The most parsimonious hypothesis for the origin of chytridiomycosis panzootics in Australia and Central America is the introduction of disease into populations of previously unexposed amphibians. Introduction of pathogens, termed pathogen pollution (1), is increasingly recognized as a significant threat to global biodiversity (1,6,46) and forms an integral part of human history (10,47). There are precedents for the introduction of fungal pathogens (including chytrid parasites) that cause high death rates (41,48). Mechanisms by which wildlife pathogens can be introduced are common; for example, a consequence of the increasing mobility of humans is the global translocation of wildlife, plants, soil, and ballast water (1,4,49,50). Freshwater fish and amphibians are also transported globally. In Australia, chytridiomycosis-infected cane toads (*Bufo marinus*), a recently introduced species, have been found (29), and in North America, bullfrogs (*Rana catesbeiana*) and other species of amphibians have been translocated or introduced widely. Some authors have suggested that tourists or fieldworkers surveying amphibian populations may have facilitated the dissemination of *Batrachochytrium* (19), although this has not been demonstrated. *Batrachochytrium* may have coevolved with some amphibians (e.g., lowland) species, populations of which remain unaffected. Recent disturbances of rain forest habitats may have introduced this parasite into naïve populations in Central America and Australia, leading to mass deaths. This range of disease outcomes parallels many diseases of humans, e.g., measles and smallpox, which produce a range of effects on persons in disease-endemic regions and cause massive deaths when introduced into naïve populations (48).

The occurrence of chytridiomycosis in free-living North American amphibians (Table 1) suggests a less obvious pattern of dissemination than in Central America and Australia. This irregularity may be due to a paucity of data, the pathogen's being enzootic to the United States, or the pathogen's introduction a number of years before. Historical reports of declines in the United States include postmetamorphic death syndrome, which progressed in waves through populations of amphibians, causing 90% to 100% death rates in recently metamorphosed animals and low death rates in larvae (50). Recent reports of chytridiomycosis-linked die-offs in *Bufo boreas* markedly resemble these previous die-offs. This observation and the finding that chytridiomycosis caused similar die-offs (of *B. canorus* and *R. pipiens*) in the 1970s (Table 1) (32), support the last of the above hypotheses. In the United States an amphibian pathogen (histologically very similar to *Batrachochytrium* but identified as *Basidiobolus ranarum*) has been described in wild Wyoming toads (*Bufo hemiophrys baxteri*) (51) and captive dwarf African clawed frogs (*Hymenochirus curtipes*) (52). As the latter species was widely introduced in ornamental garden ponds throughout the United States in the late 1980s, it may be involved in the dissemination of *Batrachochytrium*.

Chytridiomycosis as the Cause of Population Declines

The ability of a pathogen to cause local population declines resulting in local host extinction requires a mechanism of persistence at low host densities. In epidemiologic models, highly virulent parasites rapidly suppress the host population density below a threshold value required to maintain transmission, resulting in the pathogen's extinction and recovery of the host population (7,10). Microparasites such as *Batrachochytrium*, with their relatively short duration of infection and high death rates, have an increased threshold population density and are usually less able to persist. Many parasites have evolved life history strategies for persistence (10) and the presence of reservoir hosts may augment the impact of other introduced wildlife diseases on host populations (46). The aclinical presence of *Batrachochytrium* in the keratinized mouthparts of amphibian larvae implicates this life-cycle stage as a reservoir host for the pathogen. This form of infection may

enable *Batrachochytrium* to persist in reduced amphibian populations (Figure 3). In both Australia and Central America, chytrid infection was observed in larval mouthparts months after initial adult deaths: the larvae of many tropical amphibian species survive 12 to 18 months—and some temperate species as long as 3 years—before metamorphosing. Examples of larval infection enhancing pathogen-mediated population declines and leading to host population extinctions have been reported for invertebrates (53).

Persistence may be further enhanced by saprophytic development (Figure 3). *Batrachochytrium* can be cultured in vitro on tryptone agar without the addition of keratin or its derivatives (37,42), and it will grow for at least one generation on cleaned epidermal keratin or on amphibians that have died of the infection (42). *Batrachochytrium* may survive and reproduce as a saprophytic organism in the environment, at least for short periods. Keratin (from decaying carcasses, shed skin, and other sources) is widely distributed in the environment, and chytrids that use this substrate are

well known (42). Furthermore, the ability to develop and reproduce saprophytically is common to many other fungal (54) (including chytrid [41]) and bacterial (55) pathogens. An epidemiologic model of a host-parasite system for pathogens that can reproduce saprophytically clearly shows a lowering of the host threshold population, allowing the pathogen to drive the host to extinction (55). Development of *Batrachochytrium* for even short periods outside its amphibian host may greatly increase its impact and accelerate population declines. Long-term presence as a saprophyte may explain the lack of recolonization of streams from which amphibians have been extirpated in both Australia (29) and Central America (24,25).

The impact of chytridiomycosis may be enhanced by the ecologic characteristics of certain host species. In Australia, chytridiomycosis-linked deaths have occurred in both declining and nondeclining species (28,29). Species with declining populations belong to a similar ecologic guild: regionally endemic rain forest specialists with low fecundity that
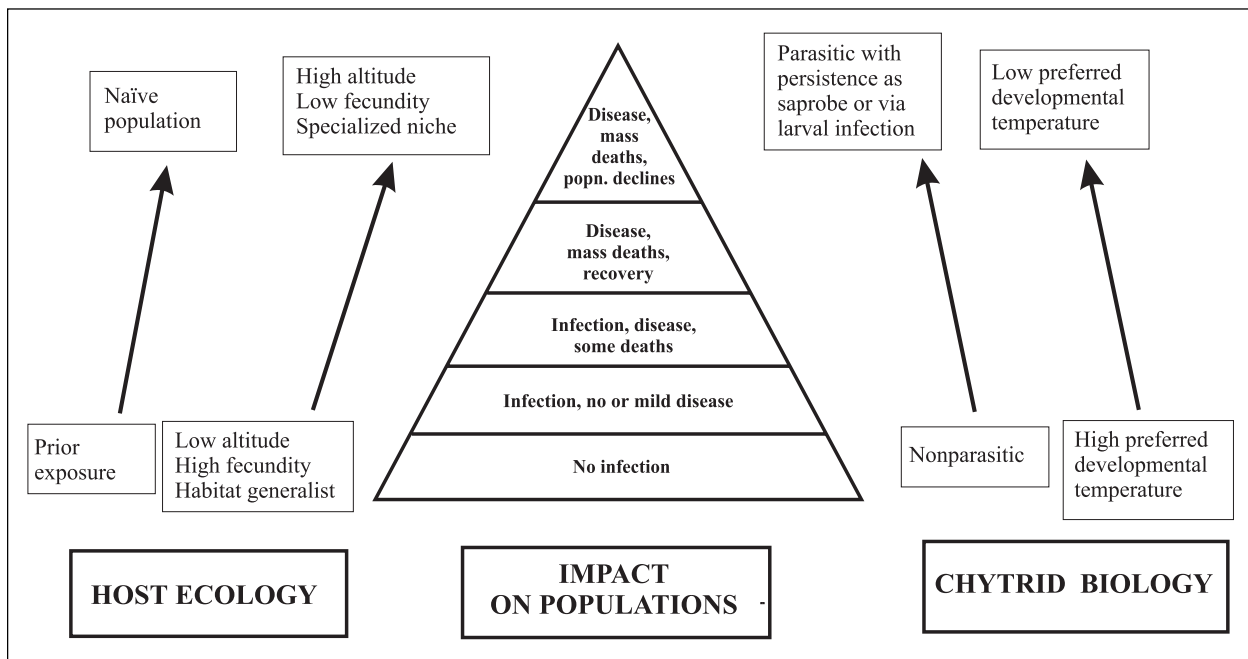
Figure 3. Diagrammatic representation of the range of disease outcomes in populations of amphibians affected by a *Batrachochytrium*-like pathogen. Factors that hypothetically predispose some amphibian populations to declines are illustrated. In this model, host ecologic traits (left side of pyramid) and parasite biologic traits (right side of pyramid) combine to produce declines in a specific group of amphibian species that have low fecundity, are stream-breeding habitat specialists, and occur in montane regions. These characteristics predispose them to population declines after introduction of a waterborne pathogen with a low preferred developmental temperature and ability to persist at low host population densities.[1]

[1]Note that the relative number of mass deaths decreases with increasing impact on population.

reproduce in streams and live at high altitudes (22). These characteristics, which are largely shared by declining Central American amphibians (24,25), are predictors of increased impact from chytridiomycosis. Species that reproduce in streams are probably more susceptible to a waterborne pathogen than terrestrial breeders. Low fecundity (56) and habitat specialization indicate a reduced ability to recover from population declines caused by stochastic events, including disease introduction. Laurance, McDonald, and Speare (39) suggested that the relation between high-altitude populations and declines may be due to a pathogen with a lower preferred developmental temperature. Preliminary data on cultured *Batrachochytrium* are consistent with this hypothesis: it develops most rapidly at 23ºC in culture, with slower growth at 28ºC and reversible cessation of growth at 29ºC (42). The growth rate of *Batrachochytrium* in the skin (and therefore virulence) and the survival of zoospores outside the host (and therefore transmission rate) are likely to be lower in amphibians from the warmer lowland regions. The ability of the pathogen to survive saprophytically in the environment and for the disease to persist may also be enhanced in the cooler montane regions. These laboratory data may explain why chytridiomycosis has been associated with population declines in North American amphibians in montane localities (31,32) and after periods of cool weather at many U.S. and Australian sites (29,31).

Potential Environmental Cofactors in the Emergence of Chytridiomycosis

Multiple factors (host, pathogen, environmental) may be involved in chytridiomycosis emergence. Some authors have hypothesized that infectious disease is only the proximate cause of declines and that environmental factors such as increased UV-B, chemical pollution, climate change, or stress may have predisposed amphibian populations to opportunistic pathogens (13,32,57). Recent work at Monteverde, Costa Rica, suggests that atmospheric warming, with a resultant elevation of the average altitude of the base of the orographic cloud bank and an increase in dry periods, is causally linked to amphibian declines at this site (57). Although no pathologic studies of amphibians were undertaken, overcrowding during periods of drought

may have allowed chytridiomycosis to cause substantial deaths (57). Further work is required to test this hypothesis, since a drier climate would also predict a lower overall impact from chytridiomycosis—a disease transmitted by flagellated, waterborne zoospores. The evidence suggests that cofactors are not required for chytridiomycosis to cause amphibian mass deaths. Chytridiomycosis is highly pathogenic to captive-bred amphibians exposed in captivity where control animals remained healthy (28,42). Further experimental infections using extremely small inocula (100 zoospores) also proved fatal (29).

Some deaths among wild amphibians have been attributed to immunosuppression, predisposing them to infectious disease (13,32). In the chytridiomycosis-related deaths, chytridiomycosis was consistently found as the cause of death, and the range of opportunistic infections expected to occur in immunocompromised animals was not found (28,29). An increase in UV-B irradiation may influence amphibian declines (58), but in the subtropical regions of Australia and Central America, data demonstrate no significant increase in UV irradiation (29,59). Even so, the potential effect of increased irradiation on montane riparian rain forest amphibians is uncertain, since these animals lay eggs under rocks or in sand banks and adults are rarely exposed to direct sunlight (24,29). Furthermore, in these regions, the species most likely to be affected by UV increases (arboreal amphibians, which bask or lay exposed eggs) are not in decline (20). Despite extensive research, chemical pollution (20,25), habitat destruction (22), or climate change (57) have not, so far, been causally linked either to the Australian declines or to those at Central American sites other than Monteverde. No other possible cofactors, such as sympatric pathogens, have been found.

Emerging Viral Diseases of Amphibians

Iridoviruses have been implicated as the cause of amphibian mass deaths worldwide, with novel iridoviruses of amphibians recently identified from a number of regions (Tables 1, 2). The Iridoviridae encompass five recognized genera: *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Lymphocystivirus,* and goldfish virus 1-like viruses (71). Of these, the genus *Ranavirus* contains pathogens of fish, amphibians, and reptiles (Table 2; Figure 4).

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Characteristics of Ranaviral Disease

Ranaviruses are often highly virulent and cause systemic infections in amphibians. Experiments with Bohle iridovirus and Gutapo virus suggest that tadpoles are the most susceptible developmental stage for ranavirus infection, and death rates of 100% occur (72). Infected metamorphs die without overt signs of infection, and infected adults show either no overt signs or, occasionally, a general weakness. Histologically, acute necrosis of hematopoietic and lymphoid tissues and of leukocytes occurs in most organs of infected animals (72). Epizootiologic data on tadpole edema virus infections of North American amphibians are scanty, although the virus was isolated mostly from diseased animals

Table 2. Iridoviruses[a,b] of herpetofauna (34)

Host	Virus[c]	Country or region where isolated	Ref.
Amphibian iridoviruses			
Leopard frog (*Rana pipiens*)	Frog virus 3 considered type for sympatric isolates frog virus 1, 2, 9-23	North America, United States	60
Red-spotted new eft *Notophthalamus viridescens*)	T6-20	North America, United States	61
Bullfrog (*Rana catesbeiana*)	Tadpole edema virus	North America, United States	62
Edible frog (*Rana esculenta*)	Rana esculenta iridovirus	Europe (Croatia)	63
Ornate burrowing frog (*Limnodynastes ornatus*)	Bohle iridovirus	Australia	64
Cane toad (*Bufo marinus*)	Gutapo iridovirus	South America (Venezuela)	65
Common frog (*Rana temporaria*)	Rana UK virus	Europe, United Kingdom	33
Common toad (*Bufo bufo*)	Bufo UK virus	Europe, United Kingdom	34
Red-legged frog larvae (*Rana aurora*)	Redwood Creek virus	California, United States	66,67
Tiger salamander (*Ambystoma tigrinum stebbinsi*)	*A. tigrinum* virus	Arizona, United States	27
Tiger salamander (*A. t. mavortium*)	Regina ranavirus	Saskatchewan, Canada	36
Ranid frog (*Rana grylio*)	Rana grylio virus	China	68
Tiger salamander (*A. tigrinum*)	Not yet named	N. Dakota, United States	35
Spotted salamander (*A. maculatum*)	Not yet named	Maine, United States	35
Tiger salamander (*A. tigrinum*)	Not yet named	Utah, United States	35
Reptile iridoviruses			
Box turtle (*Terrapene c. carolina*)	Turtle virus 3	Maryland, United States	69
Central Asian tortoise (*Testudo horsfieldi*)	Tortoise virus 5	North America, United States	69
Soft-shelled turtle (*Trionyx sinensis*)		China	70
Green tree python (*Chondropython viridis*)	Wamena virus	Australia	A.D. Hyatt (unpubl. obs.)
Gopher tortoise (*Gopherus polyphemus*)		North America, United States	69

[a]Erythrocytic viruses, which are antigenically unrelated to ranaviruses and are not associated with amphibian mass deaths or declines, are not included. Further work is required to evaluate their significance.
[b]There is little variation in the major capsid protein (a major antigen of this group of viruses) within the genus *Ranavirus* (<4% difference at the nucleotide and amino acid level; Hyatt, unpubl. obs). This high degree of homology is interesting, as some of these viruses do not appear to be species specific. No discriminating neutralizing antibodies exist, and ranaviruses are identified and characterized by a range of techniques, including antigen capture enzyme-linked immunosorbent assay, polyacrylamide gel electrophoresis, restriction endonuclease digestion, polymerase chain reaction, and sequencing and in situ hybridization (67,71).
[c]Where no name has been given, the virus has not yet been named.
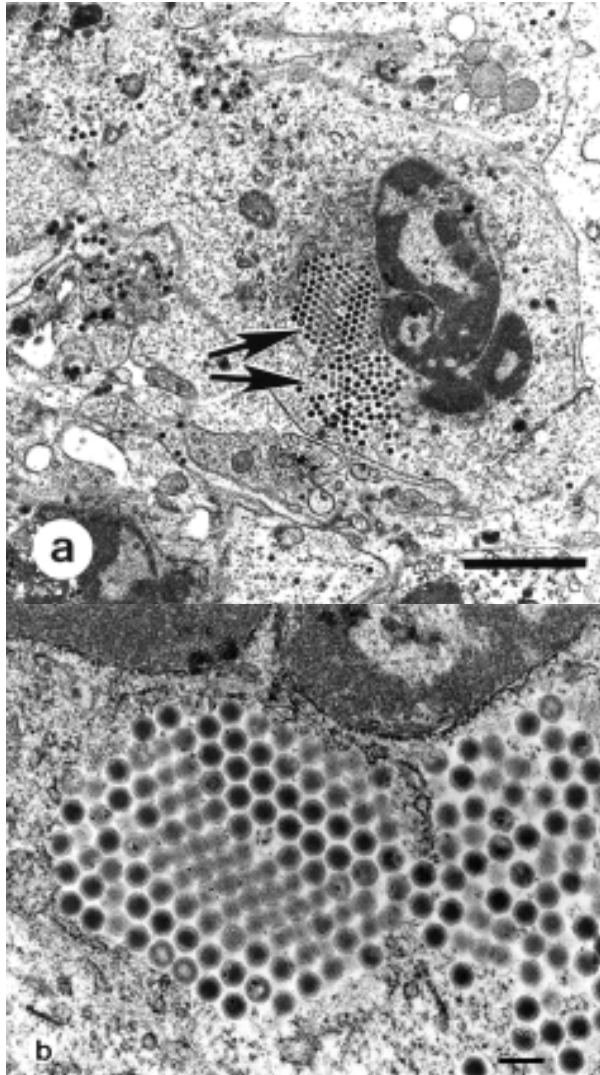
Figure 4. Transmission electron micrographs of iridovirus cultured from the liver of a naturally diseased common frog (*Rana temporaria*) by using a fathead minnow epithelial cell line. 4a. Virus-infected cell. Large isocahedral viruses are conspicuous within the cytoplasm (arrows). Bar = 2 µm. 4b. Paracrystalline array of iridovirus. Bar = 200 µm.

(60-62). Experimental infections show that bullfrog (*R. catesbeiana*) tadpoles infected with tadpole edema virus develop an acute lethal disease after a short incubation. The virus invades the liver, kidneys, and digestive tract and causes hemorrhage in skeletal tissue, pathologic findings similar to those described in *B. marinus* infected with the ranaviruses frog virus 3, Bohle iridovirus, or Gutapo virus (72). Tadpole edema virus was uniformly lethal to three species of experimentally infected North American toad (61,62). Experimental inoculation with tadpole edema virus caused high rates of deaths in toads (100%, 4 to 17 days postinfection in young and adult *Bufo* sp. and newly metamorphosed *Scaphiopus* sp.), 40% death rates in metamorphosing bullfrogs (*R. catesbeiana*), and no deaths in newly hatched salamanders (*Ambystoma* sp.). For the last two species, the low virulence suggests either some prior exposure and acquired immunity or a degree of innate resistance.

Pathologic data from ranaviral infections in wild amphibians may be complicated by secondary bacterial infection. Cunningham et al. (26) described two syndromes in *Rana temporaria* collected at sites of mass deaths in the United Kingdom: a hemorrhagic syndrome affecting the skeletal musculature and the alimentary and reproductive tracts and an ulcerative skin syndrome with dermal ulceration and often necrosis of the distal limbs, but without hemorrhage in the muscles or viscera. In some areas, frogs were found with lesions common to both syndromes (26). These lesions are characteristic of red-leg, a syndrome thought to be caused by bacterial infection; however, Cunningham et al. (26) concluded that the lesions they described were caused by primary iridoviral infection, with or without secondary bacterial infection. Secondary bacterial infection was also reported in wild-collected, diseased *A. t. stebbinsi* infected with *Ambystoma tigrinum* virus (27). This infection resulted in rapid onset (5 to 7 days) of apparent epidermal hyperplasia, then dermal hemorrhage, followed by visceral hemorrhage and death rates of up to 45% (27).

Factors Associated with the Emergence of Ranaviruses

The epizootiology of ranaviral disease in amphibians is poorly understood. Data from closely related ranaviral infections of fish, however, suggest a number of factors which may explain their recent emergence. Epizootic hematopoietic necrosis virus is a ranaviral pathogen of fish and the causative agent of epizootic hematopoietic necrosis, a notifiable disease under the Office Internationale des Epizooties (73,74). Sequencing data suggest that this disease is unique among the ranaviruses, is probably indigenous to Australia, and has a wide geographic range. Dissemination may be partly due to the virus's ability to remain infectious

under adverse conditions and for prolonged periods (Table 3).

The resistant nature of epizootic hematopoietic necrosis virus suggests that amphibian iridoviruses may survive long periods at the bottom of ponds, particularly during winter. Jancovich et al. (27) demonstrated that water containing *Ambystoma tigrinum* virus-infected *Ambystoma* became uninfective after 2 weeks at 25°C, and and epizootics of *Ambystoma tigrinum* virus and regina ranavirus often followed periods of cold weather (27,36). Epizootic hematopoietic necrosis virus may be spread by fomites such as fishing nets, boats, and fishing rods and through artificial stocking of ponds for recreational fishing (76). Birds have the potential to mechanically transfer virus on their feathers, feet, or bills, or by regurgitation of ingested infected material (76). Similar modes of spread are likely to occur with the amphibian ranaviruses. The occurrence of epizootic hematopoietic necrosis may be correlated with poor husbandry, including overcrowding, inadequate water flow, and fouling of local environments with feed from fish farms (77).

Recent movements of amphibians may have disseminated ranaviral diseases. The North American bullfrog (*Rana catesbeiana*), the host of tadpole edema virus, has been widely introduced in the western United States and South America. In the United Kingdom, ranaviruses may be disseminated by the common practice of translocation of amphibian egg masses and larvae by humans. Zupanovic et al. (65,78) reported the existence of ranaviruses and antibodies to ranaviruses in the cane toad (*Bufo marinus*) in Venezuela. In Australia, where *B. marinus* was introduced to Queensland in 1935, ranaviral antibodies can be identified in this species throughout its range (78). The range of *B. marinus* has expanded rapidly in Australia, and the toad threatens environmentally sensitive wetlands in the Kakadu National Park. The effects of toxicity, predation, and competition of this relentlessly expanding species are well documented, and its potential to disseminate novel viral pathogens to native amphibians should also be considered in evaluating its ecologic impact. So far, a ranavirus (Bohle iridovirus) has been isolated from only one native Australian amphibian, the ornate burrowing frog (*Limnodynastes ornatus*) (79).

Experimental infection with Bohle iridovirus causes illness and death in a range of frogs and toads, fish, and reptiles (L. Owens, pers. comm.) (80,81). Gutapo virus, isolated from Venezuelan *B. marinus* (65), is also able to infect other amphibian species (72). Despite Bohle iridovirus' low host specificity, current levels of surveillance have not implicated it in diseases of free-ranging Australian animals other than those from which it was originally isolated. The ability of Bohle iridovirus to infect fish implicates the widespread introduction of fish for recreational purposes as a potentially significant factor in the dissemination of amphibian ranaviruses. Similarly, Redwood Creek virus, a ranavirus found to cause death of the endangered red-legged frog (67), can infect both amphibians and fish.

Impact of Ranaviral Disease on Amphibian Populations

The link between amphibian population declines and ranaviral disease is less clear than that with chytridiomycosis. However, these highly virulent ranaviruses are a potential threat to amphibian populations, especially populations isolated from previous disease outbreaks (and thus lacking specific immunity) and species with low fecundity. In particular, the high death rates of tadpoles infected by ranaviruses predict a negative impact on populations. Using the model of Lampo and De Leo (82), a tadpole death rate of 80% to 90% would remove 80% to 90% of the animals that would otherwise survive metamorphosis, resulting in an approximately 80% reduction in adult populations (Lampo, pers. comm.). Although such an epizootic might not endanger the long-

Table 3. Longevity of infectious epizootic hematopoietic necrosis virus (72,74,75)

Treatment	Survival time (days)	Ref.
Animal tissues		
-20°C	>730	75
-70°C	>730	75
In solution		
Distilled water (temperature not specified)	97	75
4°C, cell maintenance media	>1124	74
-20°C, cell maintenance media	>1124	74
-70°C, cell maintenance media	>1124	74
On dry surfaces	>113, <200	75

term survival of a highly adaptable, highly fecund species such as *B. marinus*, species that inhabit specialized ecologic niches or have low fecundity might be adversely affected.

Recent outbreaks of iridoviral disease have been particularly notable in *Rana temporaria* (United Kingdom) and *Ambystoma tigrinum* (Arizona, Saskatchewan). These outbreaks involved of extensive deaths and the annual recurrence of the diseases. Common ecologic themes occur for these amphibian populations. The natural habitat of *A. t. stebbinsi* has been entirely destroyed by drainage after human colonization of the southwestern United States, and relict populations are now confined to manmade watering holes for cattle. Other outbreaks of ranaviral disease in *A. tigrinum* have been associated with altered habitats and artificial ponds. Similar habitat destruction has occurred in the United Kingdom, where most outbreaks of iridovirus infection are reported from artificial ponds (16). High population densities of frogs (16) and salamanders (27) occur in these bodies of water, facilitating the transmission of viruses and predisposing the amphibian populations to mass deaths.

Implications of Emerging Infectious Diseases for Amphibian Population Declines and Wildlife Conservation

The geographic spread of chytridiomycosis threatens populations of endemic and endangered amphibians in rain forests of Australia and Central and South America. Chytridiomycosis has now been reported from 38 amphibian species in 12 families, including ranid and hylid frogs, bufonid toads, and plethodontid salamanders (28,32,37,38,42,83). Although many of these records are from captive animals, the data demonstrate that chytridiomycosis is fatal to species originating from Europe, Africa, Madagascar, the Americas, and Oceania and attest to its potential impact should the disease be introduced into these areas. The emergence of amphibian ranaviruses raises similar concerns. Their ability to infect a wide range of amphibian and fish hosts (different vertebrate classes), global distribution, and high virulence clearly establish them as a global threat to amphibian populations. Despite the recent geographic spread and impact of chytrids and ranaviruses, these diseases probably do not account for all the reports of declining amphibian populations, and

evidence exists for other causal factors, such as habitat loss (12). Although chytridiomycosis causes high death rates in a range of species and habitats, it has caused population declines of amphibians only in certain species confined to montane rain forests, while the emergence of ranaviral disease seems to be associated with disturbed or degraded habitats.

Hypotheses regarding the origins and impact of emerging infectious diseases on amphibians are being tested. A number of *Batrachochytrium* and iridovirus isolates have been cultured, and analysis of their phylogenetic and pathologic features in natural and experimental infections, as well as their biologic characteristics (e.g., ability to survive in the environment, evolution of virulent strains) has already begun. Basic host and parasite ecologic data, such as prevalence and duration of infection and presence of reservoir hosts, are being collected. Data from these studies may enable the formulation of management plans to limit the diseases' impact. Development of chemotherapeutic regimens and diagnostic tests (enzyme-linked immunosorbent assay, in situ hybridization) may enable rapid progress toward these goals (29). National and international structures for the rapid dissemination of information between scientists, politicians, and the public may be crucial in combating the threat of these globally emergent pathogens. However, large geographic areas (e.g., Africa and much of Asia) have not yet been surveyed for declining amphibian populations or for the occurrence of these pathogens. Raising awareness of this threat should be one of the highest priorities for the immediate future.

Many introduced pathogens have affected human populations (10,47). However, far fewer studies of introduced diseases among wildlife have been published, and usually only those producing obvious population losses are studied vigorously (1-6).

The loss of amphibian populations to the point of local extinction is a striking effect of chytridiomycosis in Australia and Central America. However, the effects of introduced wildlife diseases may be more far-reaching and subtle, with knock-on (ripple) effects permeating throughout the ecosystem (1,2,7,82,84). In many tropical and some temperate areas, amphibians make up a significant proportion—sometimes most—of these vertebrate biomass (85), and their loss is likely to have unpredictable effects on

populations of other species. For example, loss of herbivorous amphibian larvae may cause an overgrowth of algae in montane tropical streams, with further knock-on effects. In Australia and Central America, certain species of snakes prey exclusively on amphibians and are likely to suffer significant population declines, in the same way that the red fox population was drastically reduced in the United Kingdom after the introduction of myxomatosis (10). Such insidious effects following disease introduction and an underestimation of their historical incidence suggest that this pathogen pollution may be as serious a conservation threat as habitat destruction and chemical pollution (1).

Acknowledgments

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References

1. Daszak P, Cunningham AA, Hyatt AD. Emerging infectious diseases of wildlife—threats to human health and biodiversity. Science 1999. (In press).
2. Lyles AM, Dobson AP. Infectious disease and intensive management: population dynamics, threatened hosts, and their parasites. J Zoo Wildl Med 1993;24:315-26.
3. Viggers KL, Lindenmayer DB, Spratt DM. The importance of disease in reintroduction programmes. Wildlife Research 1993;20:687-98.
4. Cunningham AA. Disease risks of wildlife translocations. Conservation Biology 1996;10:349-53.
5. Plowright W. The effects of rinderpest and rinderpest control on wildlife in Africa. Symposia of the Zoological Society of London 1982;50:1-28.
6. Harvell CD, Kim K, Bukholder JM, Colwell RR, Epstein PR, Grimes DJ, et al. Emerging marine diseases—climate links and anthropogenic factors. Science 1999;285:1505-10.
7. Dobson AP, May RM. Disease and conservation. In: Soulé M, editor. Conservation biology: The science of scarcity and diversity. Sunderland (MA): Sinauer Assoc Inc.; 1986. p. 345-65.
8. Scott ME. The impact of infection and disease on animal populations: Implications for conservation biology. Consservation Biology 1988;2:40-56.
9. Daszak P, Cunningham AA. Wildlife disease biology comes of age. Oryx 1998;32:238-9.
10. Anderson RM, May RM. The invasion, persistence and spread of infectious diseases within animal and plant communities. Philos Trans R Soc Lond B Biol Sci 1986;314:533-70.
11. Tompkins DM, Begon M. Parasites can regulate wildlife populations. Parasitology Today 1999;15:311-3.
12. Blaustein AR, Wake DB. Declining amphibian populations: A global phenomenon. Trends in Ecology and Evolution 1990;5:203-4.
13. Carey C. Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. Conservation Biology 1993;7:355-62.
14. Drost CA, Fellers GM. Collapse of a regional frog fauna in the Yosemite area of the California Sierra Nevada, USA. Conservation Biology 1996;10:414-25.
15. Cunningham AA, Langton TES, Bennett PM, Drury SEN, Gough RE, Kirkwood JK. Unusual mortality associated with poxvirus-like particles in frogs (*Rana temporaria*). Vet Rec 1993;133:141-2.
16. Cunningham AA, Langton TES, Bennett PM, Lewin JF, Drury SEN, Gough RE, et al. Investigations into unusual mortalities of the common frog (*Rana temporaria*) in Britain. In: Proceedings of the 5th International Colloquium on the Pathology of Reptiles and Amphibians; 1995 March 3; The Netherlands. p. 19-27.
17. Gupta BK. Declining amphibians. Current Science 1998;75:81-4.
18. Weygoldt P. Changes in the composition of mountain stream frog communities in the Atlantic Mountains of Brazil: Frogs as indicators of environmental deteriorations? Studies of Neotropical Fauna and Environments 1989;24:249-55.
19. Halliday TR. A declining amphibian conundrum. Nature 1998;394:418-9.
20. Mahony M. The decline of the green and golden bell frog *Litoria aurea* viewed in the context of declines and disappearances of other Australian frogs. Australian Zoologist 1996;30:237-47.
21. Richards SJ, McDonald KR, Alford RA. Declines in populations of Australia's endemic tropical forest frogs. Pacific Conservation Biology 1993;1:66-77.
22. Williams SE, Hero J-M. Rainforest frogs of the Australian Wet Tropics: Guild classification and the ecological similarity of declining species. Proc R Soc Lond B Biol Sci 1998;265:597-602.
23. Pounds JA, Fogden MPL, Savage JM, Gorman GC. Tests of null models for amphibian declines on a tropical mountain. Conservation Biology 1997;11:1307-22.
24. Lips KR. Decline of a tropical montane fauna. Conservation Biology 1998;12:106-17.
25. Lips KR. Mass mortality and population declines of anurans at an upland site in Western Panama. Conservation Biology 1999;13:117-25.
26. Cunningham AA, Langton TES, Bennett PM, Lewin JF, Drury SEN, Gough RE, et al. Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). Philos Trans R Soc Lond B Biol Sci 1996;351:1529-57.

27. Jancovich JK, Davidson EW, Morado JF, Jacobs BL, Collins JP. Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. Diseases of Aquatic Organisms 1997;31:161-7.

28. Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, et al. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proc Natl Acad Sci U S A 1998;95:9031-6.

29. Berger L, Speare R, Hyatt AD. Chytrid fungi and amphibian declines: Overview, implications and future directions. In: Campbell A, editor. Declines and disapparances of Australian frogs. Environmental Australia, Canberra, Australia: Environmental Australia; 2000. p.21-31.

30. Daszak P, Cunningham AA. Extinction by infection. Trends in Ecology and Evolution 1999;14:279.

31. Morell V. Are pathogens felling frogs? Science 1999;284:728-31.

32. Carey C, Cohen N, Rollins-Smith L. Amphibian declines: An immunological perspective. Dev Comp Immunol 1999;23:459-72.

33. Drury SEN, Gough RE, Cunningham AA. Isolation of an iridovirus-like agent from common frogs (*Rana temporaria*). Vet Rec 1995;137:72-3.

34. Hyatt AD, Gould AR, Zupanovic Z, Cunningham AA, Hengstberger S, Whittington RJ, et al. Characterisation of piscine and amphibian iridoviruses. Arch Virol 2000. In press.

35. Docherty D, Chinchar VG, Meteyer C, Brannian R, Hansen W, Wang J, Mao J. A diagnostic evaluation of three salamander mortality events associated with an iridovirus and subsequent genomic comparison of the virus isolates obtained. In: Proceedings of the 48th Annual Wildlife Disease Association Conference; 1999 Aug 8-12; Athens, Georgia.

36. Bollinger TK, Mao J, Schock D, Brigham RM, Chinchar VG. Pathology, isolation and preliminary molecular characterization of a novel iridovirus from tiger salamanders in Saskatchewan. Journal of Wildlife Diseases 1999;35:413-29.

37. Pessier AP, Nichols DK, Longcore JE, Fuller MS. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). J Vet Diagn Invest 1999;11:194-9.

38. Milius S. Fatal skin fungus found in U.S. frogs. Science News 1998;July 4th:7.

39. Laurance WF, McDonald KR, Speare R. Epidemic disease and the catastrophic decline of Australian rain forest frogs. Conservation Biology 1996;10:406-13.

40. Alford RA, Richards SJ. Lack of evidence for epidemic disease as an agent in the catastrophic decline of Australian rain forest frogs. Conservation Biology 1997;11:1026-9.

41. Powell MJ. Looking at mycology with a Janus face: A glimpse at chytridiomycetes active in the environment. Mycologia 1993;85:1-20.

42. Longcore JE, Pessier AP, Nichols DK. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 1999;91:219-27.

43. Garnett GP, Homes EC. The ecology of emergent infectious disease. Bioscience 1996;46:127-35.

44. Quinn TC. Global burden of the HIV pandemic. Lancet 1996;348:99-106.

45. Laurance WF, McDonald KR, Speare R. In defense of the epidemic disease hypothesis. Conservation Biology 1997;11:1030-4.

46. McCallum H, Dobson A. Detecting disease and parasite threats to endangered species and ecosystems. Trends in Ecology and Evolution 1995;10:190-4.

47. Dobson AP, Carper ER. Infectious diseases and human population history. Bioscience 1996;46:115-26.

48. Burdon JJ. Fungal pathogens as selective forces in plant populations and communities. Australian Journal of Ecology 1991;16:423-32.

49. Vitousek PM, D'Antonio CM, Loope LL, Westbrooks R. Biological invasions as global environmental change. American Scientist 1996;84:468-78.

50. Scott NJ. Postmetamorphic death syndrome. Froglog 1993;7:1-2.

51. Taylor SK, Williams ES, Thorne ET, Mills KW, Withers DI, Pier AC. Causes of mortality of the Wyoming toad. Journal of Wildlife Diseases 1999;35:49-57.

52. Groff JM, Mughannam A, McDowell TS, Wong A, Dykstra MJ, Frye FL, et al. An epizootic of cutaneous zygomycosis in cultured dwarf African clawed frogs (*Hymenochirus curtipes*) due to *Basidiobolus ranarum*. J Med Vet Mycol 1991;29:215-23.

53. Richard A, Cory J, Speight M, Williams T. Foraging in a pathogen reservoir can lead to local host population extinction: A case study of lepidoptera-virus interaction. Oecologia 1999;118:29-38.

54. Cox RA. Coccidioidomycosis. In: Fungal infections and immune responses. Murphy JW, Friedman H, Bendinelli M, editors. New York: Plenum Press; 1993. p. 173-211.

55. Godfray HCJ, Briggs CJ, Barlow ND, O'Callaghan M, Glare TR, Jackson TA. A model of insect-pathogen dynamics in which a pathogenic bacterium can also reproduce saprophytically. Proc R Soc Lond B Biol Sci 1999;266:233-40.

56. Bennett PM, Owens IPF. Variation in extinction risk among birds: Chance or evolutionary predisposition? Proc R Soc Lond B Biol Sci 1997;264:401-8.

57. Pounds JA, Fogden MPL, Campbell JH. Biological response to climate change on a tropical mountain. Nature 1999;398:611-5.

58. Kiesecker JM, Blaustein AR. Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. Proc Natl Acad Sci U S A 1995;92:11049-52.

59. Madronich S, de Gruijl FR. Skin cancer and UV radiation. Nature 1993;366:23.

60. Granoff A, Came PE, Rafferty KA. The isolation and properties of viruses from *Rana pipiens*: Their possible relationship to the renal adenocarcinoma of the leopard frog. Ann N Y Acad Sci 1965;126:237-55.

61. Clark HF, Brennan JC, Zeigel RF, Karzon. Isolation and characterization of viruses from the kidneys of *Rana pipiens* with renal adenocarcinoma before and after passage in red eft (*Triturus viridescens*). J Virol 1968;2:629-40.

62. Wolf K, Bullock GL, Dunbar CE, Quimby MC. Tadpole edema virus: A viscerotrophic pathogen for anuran amphibians. J Infect Dis 1968;118:253-62.

63. Fijan N, Matasin Z, Petrinec Z, Valpotic I, Zwillenberg LO. Isolation of an iridovirus-like agent from the green frog *Rana esculenta* L. Veterinarski Archiv Zagreb 1991;61:151-8.

64. Hengstberger SG, Hyatt AD, Speare R, Coupar BEH. Comparison of epizootic haematopoietic necrosis and Bohle iridoviruses, recently isolated Australian iridoviruses. Diseases of Aquatic Organisms 1993;15:93-107.

65. Zupanovic Z, Musso C, Lopez G, Louriero C-L, Hyatt AD, Hengstberger S, et al. Isolation and characterization of iridoviruses from the giant toad *Bufo marinus* in Venezuela. Diseases of Aquatic Organisms 1998;33:1-9.

66. Mao J, Green DE, Fellers G, Chinchar VG. Molecular characterization of iridoviruses isolated from sympatric amphibians and fish. Virus Res 1999;63:45-52.

67. Mao J, Hedrick RP, Chinchar VG. Molecular characterization, sequence analysis and taxonomic position of newly isolated fish iridoviruses. Virology 1997;229:212-20.

68. Zhang QY, Li ZQ, Gui JF. Studies on morphogenesis and cellular interactions of *Rana grylio* virus in an infected fish cell line. Aquaculture 1999;175:185-97.

69. Westhouse RA, Jacobsen ER, Harris RK, Winter KR, Homer BL. Respiratory and pharyngo-esophageal iridovirus infection in a gopher tortoise (*Gopherus polyphemus*). J Wildl Dis 1996;32:682-6.

70. Chen Z-X, Zheng J-C, Jiang Y-I. A new iridovirus from soft-shelled turtle. Virus Res 1999;63:147-51.

71. Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, Martelli P, et al. Iridoviridae. In: Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, Martelli P, et al., editors. Virus taxonomy: The classification and nomenclature of viruses. The sixth report of the international committee on the taxonomy of viruses. New York, Wien: Springer-Verlag; 1995. p. 95-9.

72. Hyatt AD, Parkes H, Zupanovic Z. Identification, characterisation and assessment of Venezuelan viruses for potential use as biological control agents against the cane toad (*Bufo marinus*) in Australia. Report to the Australian Federal Government and Environment Australia. 1998.

73. Ahne W, Bremont M, Hedrick RP, Hyatt AD, Whittington RJ. Iridoviruses associated with epizootic haematopoietic necrosis (EHN) in aquaculture. World Journal of Microbiology and Biotechnology 1997;13:367-73.

74. Hyatt AD, Whittington RJ. National diagnostic tests for the detection of epizootic haematopoietic necrosis virus (EHNV) and certification of EHNV-free fish. Final report (project 92/66). Fish Research Development Council of Australia; 1997.

75. Langdon JS. Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in redfin perch, *Perca fluviatilis* L., and 11 other teleosts. Journal of Fish Diseases 1989;12:295-310.

76. Whittington RJ, Kearns C, Hyatt AD, Hengstberger S, Rutzou T. Spread of epizootic haematopoietic necrosis virus (EHNV) in redfin perch (*Perca fluviatilis*) in Southern Australia. Aust Vet J 1996;73:112-4.

77. Whittington RJ, Hyatt AD. Diagnosis and prevention of epizootic haematopoietic necrosis virus infection. Proceedings of the National Research Institute of Aquaculture International Workshop: New approaches to viral diseases of aquatic animals; 1997 Jan 21-24; Kyoto, Japan. National Research Institute of Aquaculture; 1997.

78. Zupanovic Z, Lopez B, Hyatt AD, Green B, Bartran G, Parkes H, et al. Giant toads *Bufo marinus* in Australia and Venezuela have antibodies against "ranaviruses". Dieases of Aquatic Organisms 1998;32:1-8.

79. Speare R, Smith JR. An iridovirus-like agent isolated from the ornate burrowing frog *Limnodynastes ornatus* in northern Australia. Diseases of Aquatic Organisms 1992;14:51-7.

80. Cullen BR, Owens L, Whittington RJ. Experimental infection of Australian anurans (*Limnodynastes terraereginae* and *Litoria latopalmata*) with Bohle iridovirus. Diseases of Aquatic Organisms 1995;23:83-92.

81. Moody NJC, Owens L. Experimental demonstration of the pathogenicity of a frog virus, Bohle iridovirus, for a fish species, barramundi *Lates calcarifer*. Diseases of Aquatic Organisms 1994;18:95-102.

82. Lampo M, De Leo GA. The invasion ecology of the toad *Bufo marinus*: From South America to Australia. Ecological Applications 1998;8:388-96.

83. Nichols DK, Pessier AP, Longcore JE. Cutaneous chytridiomycosis: an emerging disease? Proceedings American Association of Zoo Veterinarians 1998:269-71.

84. Lessios HA. Mass mortality of *Diadema antillarum* in the Caribbean: What have we learned? Annual Reviews in Ecological Systematics 1988;19:371-93.

85. Burton, TM, Likens GE. Salamander populations and biomass in the Hubbard Brook Experimental Forest, New Hampshire. Copeia 1975;3:541-6.

Development of Orphan Vaccines:
An Industry Perspective

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The development of vaccines against rare emerging infectious diseases is hampered by many disincentives. In the face of growing in-house expenditures associated with research and development projects in a complex legal and regulatory environment, most pharmaceutical companies prioritize their projects and streamline their product portfolio. Nevertheless, for humanitarian reasons, there is a need to develop niche vaccines for rare diseases not preventable or curable by other means. The U.S. Orphan Drug Act of 1983 and a similar proposal from the European Commission (currently under legislative approval) provide financial and practical incentives for the research and development of drugs to treat rare diseases. In addition, updated epidemiologic information from experts in the field of emerging diseases; increased disease awareness among health professionals, patients, and the general public; a list of priority vaccines; emergence of a dedicated organization with strong leadership; and the long-term pharmacoeconomic viability of orphan products will be key factors in overcoming the complexity of orphan status and the limited need for vaccine.

The Problem

In considering the development of a new vaccine, preventive immunization, generally considered the most cost-effective health intervention, should be ranked against other strategies for disease control, such as case management (treatment of disease) or control of environmental factors linked to vector prevalence and dynamics (e.g., overpopulation, rural-to-urban migration, economic status, vector control, inadequate domestic water supply or sewage disposal) (1). Evaluating vaccination options and economic impact is particularly important for vaccines against low-prevalence or geographically contained emerging infectious diseases with limited demand, for which development costs may not be recovered. Thus, consensus should be reached on the mid- to long-term public health significance (e.g., vector dynamics and potential control, age prevalence and targets, risk categories, case-fatality rates, and possible future epidemiologic scenarios) of any vaccine-preventable disease. Without clear premises and long-term commitment, the

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development of vaccines for rare infectious diseases or those of narrow scope (e.g., geographically limited but regionally important diseases such as arboviral or diarrheal diseases) or for which development costs offset the market potential, called here orphan vaccines, may be considered a precarious venture that most organizations would hesitate to pursue.

Disincentives for Orphan Vaccine Development

Competing Costs

Vaccine development involves a substantial investment in time, effort, and resources. Any private- or public-sector vaccine research and development process involves choices concerning the allocation of resources at all levels, including personnel and management. The costs from research to licensure, the risks inherent in vaccine development (e.g., technological constraints, regulatory approval) and the short- and long-term market financial evaluations (e.g., net present value, return on investment [2]) are key factors in the decision to develop a vaccine against a rare disease. In addition, long-term market evaluation and return on investment are often difficult to estimate because of the

unpredictable nature of disease outbreaks and vector dynamics. Growing in-house expenditures associated with research and development projects in a complex legal and regulatory environment prompt most pharmaceutical companies to prioritize their projects and streamline their product portfolio (3). The same is true in the public health sector where the appearance of an orphan vaccine would increase the already tough competition for resources, as evidenced by the present shortcomings in developing countries' use of current and candidate Expanded Program of Immunization (EPI) vaccines (hepatitis B, measles, yellow fever, *Haemophilus influenzae* type b).

Vaccine Pricing

It has been repeatedly shown that one of the most accurate predictors of the successful use of an EPI vaccine, such as hepatitis B, is not necessarily the endemicity of the disease but instead the vaccine cost per dose (4,5). Thus, the research, development, production, marketing, and distribution of a safe and effective vaccine should be assessed to determine if its potential cost per dose would be acceptable in an already difficult marketplace (4). The limited economic prospects and size of the market, with probably no prospect for economies of scale in production, are particularly relevant in the vaccine industry. Economic models of vaccine production have shown an inverse relationship between the number of doses produced and the cost per dose (6). As a consequence, a tiered pricing strategy has been endorsed by the World Health Organization (WHO), in which high-cost but low-volume vaccine sales in industrialized countries could subsidize the low cost and larger volume of sales in developing countries, although this may not be feasible if the quantity of vaccines needed in developing countries is low (6,7).

Patent Protection and Product Liability

Introduction of new vaccines relies heavily on the strategic use of intellectual property rights to reassure investors that a candidate vaccine will provide a fair return on invested funds. The lack of patent protection or legal framework for intellectual property rights in some developing countries interferes with the long-term viability of a vaccine. In Western countries, liability issues associated with a

candidate vaccine and its intended population (8) also affect development costs.

Orphan Drugs and Vaccines Situation in the United States

The United States was the first nation to propose a legal framework to overcome the disincentives to developing orphan drugs and encourage their development and availability (9,10). The Preamble on Orphan Drugs to the legislation passed by the U.S. Congress contained the following points: 1) Many diseases and conditions (so-called orphan diseases) exist that affect very small numbers of persons; however, the overall group of patients affected by such diseases totals 20 million or more in the United States; 2) adequate drugs for orphan diseases have not been developed; 3) pharmaceutical companies may reasonably expect to generate relatively small sales in comparison to the cost of developing an orphan product; and 4) costs of developing such drugs should be reduced, and financial incentives should be provided.

The legislation defines two classes of orphan diseases. The first class comprises diseases that affect fewer than 200,000 Americans. In this case, sales of a drug, vaccine, diagnostic test, or blood product intended for use in such a disorder would be insufficient to offset the costs incurred during development and marketing of the product. This program is directed at public health needs beyond the U.S. borders, providing a stimulating factor for the U.S. pharmaceutical community to develop products to meet the needs of populations elsewhere. The second class of orphan diseases affects more than 200,000 Americans but has no potential recovery costs from U.S. sales. Thus, the program may also apply to specific subpopulations of patients with a more common disease for which the sponsor does not expect to offset development and marketing costs in the first 7 years of sales.

Concerning vaccines, the U.S. Food and Drug Administration (FDA) stipulated that, when establishing the claim for orphan status, the intended population should reflect the number of persons who would receive the vaccine annually as of the date of designation.

Orphan Drug Incentives

To further encourage orphan drug availability, accompanying market-oriented incentives

for orphan drug development were issued by the Office of Orphan Products Development, under the auspices of FDA (11). The sponsor makes the request for orphan drug status (before filing a New Drug Application or a Product License Application) on the basis of information and circumstances at the time the request is submitted.

Funds for research through Orphan Products Grants Programs benefit from a 50% deduction tax credit for clinical trial expenses (9) and a market exclusivity of 7 years. Protocol assistance in the form of written recommendations from the secretary of the Department of Health and Human Services for the nonclinical and clinical investigations needed for marketing approval are provided to accelerate the approval process. In this respect, a flexible approach has been adopted for the development of orphan drugs. For example, the preclinical dossier (i.e., the pharmaceutical and pharmacotoxologic data included in the registration file) may not have to include data on animal toxicity, and teratogenicity or carcinogenicity results may be waived in some cases (12). This flexibility in the registration requirements can be applied in certain cases to expedite the approval process but cannot be used in instances where it could compromise the safety of the consumer.

The legislation states that the clinical dossier of an orphan drug or vaccine should be built on a realistic assessment of the qualitative and quantitative nature of the studies that can be performed. This measure is relevant because the orphan nature of the disease and its prevalence in regions with limited medical facilities and services may make it difficult to recruit a large enough number of qualified participants for a clinical trial. On the other hand, the drawback of basing a clinical dossier on a limited amount of data is the obvious difficulty in evaluating the safety profile of an orphan product with sufficient statistical confidence. On average, orphan drugs may be associated with greater hazard than other products. For example, during clinical testing, 31% of orphan drugs on the market had more pronounced adverse effects than nonorphan medicinal products (13). Likewise, after FDA approval, 13% of orphan products provoked more side effects than anticipated.

To encourage development of novel orphan compounds, FDA stipulated that two products would be considered the same (and thus the latter one would not qualify for the incentives in the Orphan Drug Act), unless the second product was shown to be clinically superior to the first. This stipulation provides a clear incentive for the original manufacturer of a product likely to be reproduced, who funds the full costs of research and development. For example, in the case of two live, attenuated viral vaccines, only the first would be granted orphan status for a given preventive indication, unless the second vaccine proved clinically superior.

Liability Coverage

Although not definitely clarified, proposals have been made to solve some specific liability issues, including design defects, duty to warn, negligence in testing or manufacturing, and defining responsibility for no-fault injury (13). The National Vaccine Compensation Program (issued in 1986), which provides no-fault compensation for vaccine-related injuries, is financed by a trust fund created by an excise tax on every dose of vaccine sold (14).

Orphan Drugs and Vaccines in Europe

In 1994, the European Commission (the legislative body of the European Union [EU]) stated its interest in orphan diseases. In 1998, with close collaboration of the French Ministry of Health (15) and the European Medicine Evaluation Agency, a text was approved recommending the creation of a European Office for Orphan Drugs along the same lines as the U.S. Office of Orphan Products Development.

The proposed European criteria for classification of a drug as an orphan drug (including vaccines) are almost identical to the U.S. criteria, except that they are based on a disease prevalence of 5 per 10,000 Europeans (falling between the United States [7 per 10,000] and Japan [2.5 per 10,000]), when no current methods of diagnosis, prevention or treatment, or major contribution to current patient care exist.

The legislation will provide incentives to the European pharmaceutical industry in terms of research and development assistance (protocol assistance, normal evaluation, possible form of centralized but fast-track approval procedures), fee waiver, tax credits, funds from the European Orphan Product Grant Program, and market exclusivity for 10 years (interim period 6 years) and will encourage national policies (subsidiary

principle, e.g., the French compassionate use authorization [Autorisation Temporaire Utilisation]) (16).

The role of patient groups in increasing awareness of orphan drug development has been widely recognized for pharmaceutical orphan drugs in the United States and has been emphasized in the European project. The potential end-users of an orphan product may not be aware of therapeutic or preventive options. The European Office of Orphan Products Development will therefore support the establishment of groups of persons with the same rare conditions to play a role in increasing awareness of the disease within the population and will coordinate their activities at national and community levels. It remains to be seen how this initiative will apply to vaccine-preventable infectious diseases in communities where individuals or groups may not be aware of the risk for infection and thus the value of the vaccine.

To clarify the extent of patent protection and the right to benefit from the orphan incentive package, the European Commission (DG24 committee) recently defined "similarity" between orphan products as the same substance, or a substance that differs from the original substance in molecular structure, source material, or manufacturing process, or an organism (living or nonliving) that is comparable with the original substance or organism in terms of biologic action and properties (including efficacy and safety) and ability to act through the same mechanism. In the same way as the U.S. legislation, this would favor the development of novel orphan products by the innovative company.

In June 1999, the European Parliament's committee on the environment, public health, and consumer protection adopted a report by one of its senior members in favor of the Policy on Orphan Drugs and proposed some amendments to widen the scope of the legislation. Among other changes, the committee requested more flexibility in the proposed provisions for clinical trials, allowing (under specific conditions) availability of the product before final authorization is granted. The committee proposed extending the definition of orphan drug status to cover products intended for serious and chronic diseases. It also recommended, as in the United States, additional incentives for developing medicinal or biologic products for diseases that occur mainly in tropical regions but rarely within EU territory. Finally, the committee called for an Orphan Medicinal Product Innovation Promotion Fund to be financed from the sales of orphan drugs after the proposed 10-year period of market exclusivity. The European Orphan Drug Policy could be enacted early in the year 2000.

Orphan Drugs in Other Industrialized Countries

After the U.S. Orphan Drug Act, similar legislation was enacted in Japan in 1993. An Australian orphan drugs program based on the U.S. program began in 1998 (17). Since then, the Therapeutic Goods Administration has designated two biological drugs as orphans—rabies immunoglobulin and recombinant enzyme imiglucerase for replacement therapy in patients with Gaucher disease. A cross-national comparison of orphan drug and vaccine policies has been made for different countries, including Japan, Canada, France, Sweden, and the United Kingdom (12).

Orphan Vaccines in Developing Countries

The availability and use of orphan vaccines in developing countries are complex since these countries have yet to ensure optimum use of existing priority vaccines (17). The limitations and obstacles involved in expanding the use of these priority vaccines are further multiplied for orphan vaccines of limited need. Within the framework of WHO, the Children's Vaccine Initiative set the development of vaccines with commercial prospects as a priority measure (7). This cost-oriented definition reflects mainly the difficulty of developing vaccines and drugs for tropical diseases, even those as prevalent as malaria (19,20). The Children's Vaccine Initiative's role has been problematic for various reasons (21), and this structure has faced increasing difficulties in maintaining its visibility.

Nevertheless, other noneconomic factors (3) could justify an industry's decision to develop and market an orphan vaccine: desire to enhance the company's ethical profile by fulfilling a medical or social need; capacity to develop, produce, and market the drug; a larger company strategy (e.g., part of a product range); and possible additional uses that would increase the drug's future economic viability. The latter point may be less relevant for vaccines, which are usually tailor-made to their infectious agents.

In the development of any vaccine against an emerging infectious disease, certain general

rules apply (4,6,18,22), for example, developing strong research and development capacity, obtaining reliable scientific results and training in industrialized and developing countries; bulk filling arrangements; licensing technology; negotiating partnerships for specific products; joint venture agreements with western research and development manufacturers (economic value of the alliance); identifying the neediest countries on the basis of a banding strategy that classes countries according to their gross national product per capita, thereby allowing tiered pricing among them (6,23); and creation of funding mechanisms. Some could argue that from an industry perspective, if all of these criteria cannot be met, the vaccine should not be developed.

No trade-off on the quality of an orphan vaccine is ethically justified or accepted. For the pharmaceutical industry, therefore, the costs incurred in development, ensuring tight quality controls, and establishing industrial good manufacturing procedures for an orphan vaccine are similar to those incurred with a traditional vaccine. For this reason, the development of any orphan vaccine should be broadly supported by measures to increase the awareness of immunization benefits at three levels—the decision-makers, the caregivers, and the patients.

Increasing Orphan Vaccine Availability

Development of orphan vaccines is guided by the limited need for or market potential of the product, with the accompanying regulations, as well as the specific characteristics of the vaccine and those who need it (24). Because of the pitfalls related to these limitations, few orphan vaccines have reached the neediest populations. For example, in the United States, by the end of 1997, 837 medicinal products had been designated orphan drugs; 152 of these obtained authorization. This number was a clear improvement over that of the previous 14 years, during which 34 medicinal orphan products obtained authorization. However, our website review found only eight vaccines registered with orphan status (seven for therapeutic indications [e.g., cancer and sickle cell anemia], one to prevent an Asiatic infectious disease—Japanese encephalitis virus) and, to our knowledge, none has yet obtained final authorization. In addition, "It is not vaccines that save lives but vaccination." Even when orphan vaccines are available, we have to examine the feasibility of getting them to the intended population.

Various strategies, proposals, and recommendations for overcoming limitations inherent in orphan vaccine development and availability are listed in the Table.

Providing Information, Prioritizing, and Securing Demand

Although funding is a major obstacle to orphan vaccine development, it may not be the only impediment to the introduction of new vaccines (25). Reliable information on the epidemiology, disease severity, and effect on public health is essential to substantiating the need for a vaccine and may not be available to support the development decision. Market forces may not always be good cultivators of vaccines, which, unlike some chemical drugs, are not big money-making products. For this reason, the public and decision makers should know about the benefits of immunization, to increase disease awareness, and support an orphan vaccine initiative.

Facilitating Vaccine Research and Development and National and Regional Approvals

An accelerated procedure for final authorization and exemptions from all or part of the registration fee can reduce development costs, staffing requirements, and time to market and render the development of an orphan vaccine more attractive for the sponsor. Local initiatives may also speed the authorization process.

Ensuring Market and Funding Visibility, Production, and Distribution

Finally, increasing patent protection and the defined period of market exclusivity reduces investment risks for manufacturers. Furthermore, funding for orphan projects may be advanced from private bodies looking to capitalize on an ethical business image. Indeed, the private sector looks to take on an increasingly important role in international health development, especially in poorer countries (26). Increased world travel and the risk for transport of pathogens across borders (27) support tiered pricing between the western traveler and the disease-endemic country. In addition, an orphan infectious agent in a remote developing country requiring an orphan vaccine

with limited need could, over time, become an emerging disease worldwide. HIV is a case in point: a disease originating in Africa that has successfully spread to the industrialized world.

In Argentina, strong political and governmental support, aided by the U.S. Army Medical Research Institute of Infectious Diseases collaboration, ultimately culminated in a successful Candid 1 vaccination campaign against Argentinean hemorrhagic fever in agricultural workers (28). Close collaboration between the pharmaceutical sector, WHO, and the Chinese government resulted in the development of the antimalarial drug artemisin (29).

Table. Solutions and proposals for accelerating orphan vaccine availability

1. Provide information, prioritize, and secure demand
   Increase awareness of disease: set-up of special interest groups (patients, parents, professionals), expert groups, and national forums.
   Acquire epidemiologic data on selected infectious diseases to guide decision-making: obtain access to data registries with comparable case-definitions across countries, and obtain information from specialized units and experts, scientific literature, patient organizations,and pharmaceutical manufacturers associations.
   Establish the suitability of vaccine prevention vs. other options: realistic comparisons of vaccination with patterns and costs of other alternatives, such as treatment or vector control.
   Ensure political support for orphan vaccine initiatives and organize tripartite partnerships between public, private, and nongovernmental sectors.

2. Facilitate vaccine research and development and national/regional approvals
   Promote innovative research and development technologies that could be applied to blockbuster vaccines or, alternatively, promote low-cost traditional vaccine technologies.
   Encourage public/private sector links: academic/industrial research groups.
   Set international standards of quality, safety, and efficacy and define minimum amount of data required for licensure.
   Make recommendations on appropriate schedules, target ages.
   Promote national and regional ex-U.S. and European Community incentives on Orphan Drug Policies (Latin America, Asia).
   Expand and harmonize orphan drug policies as part of the ICH process (decrease time to regulatory approval).

3. Ensure market/funding visibility, production and distribution
   Reduce investment risks for manufacturers by providing realistic demand estimates.
   Fund development of orphan vaccines for developing countries through various institutional bodies, such as CVI, WHO, UNICEF, PAHO, WB, USAID, NIH, CDC, PATH, other donor bodies, and nongovernmental organizations and foundations (e.g., Gates Foundation) on the basis of target assistance for the neediest countries based on total gross national product.
   Strengthen political and public health collaboration between orphan programs (European Community, United States) and other countries to create a supranational office dedicated to orphan vaccines (World Office of Orphan Vaccine Development or CVI) that could harmonize and coordinate funding (from research to manufacturing) from various sources.
   Identify and expand the pool of the committed purchasers based on expected coverage criteria.
   Promote and support protection of intellectual property.
   Clarify compensation programs that may assume responsibility for liability.
   Evaluate tiered pricing (high/low) feasibility at two levels:
   Multinational: traveler or military vaccines in industrialized countries, endemic community vaccines in developing countries.
   National: a private market for the high GNP per capita subgroup, a public market for the low GNP per capita subgroup.
   Establish manufacturing strategies, such as campaigning to subsidize orphan vaccine cost investments by large volume sales of EPI vaccines.
   Strengthen the vaccine distribution network for the targeted population.

ICH, International Conference on Harmonization; CVI, Children's Vaccine Initiative; WHO, World Health Organization; UNICEF, United Nation's Children's Fund; PAHO, PanAmerican Health Organization; WB, World Bank; USAID, U.S. Agency for International Development; NIH, National Institutes of Health; CDC, Centers for Disease Control and Prevention; PATH, Program for Appropriate Technology in Health; EPI, Expanded Program of Immunization.

Conclusions

The dilemmas intrinsic to the development and distribution of orphan vaccines against emerging infectious diseases reflect many of the issues faced by policy makers worldwide with regards to cost, quality of care, access to care, and the role of government intervention in regulating the health-care market (30). In view of the current globalization of trades and markets, worldwide orphan vaccine policies and a specialized organization with a strong leadership and commitment similar to the Children's Vaccine Initiative project for a National Vaccine Authority may be needed (6,18). This kind of organization could be responsible for establishing a list of priority orphan vaccines and indicating reasons for not including other vaccines. The organization could also oversee all stages of vaccine development and have access to funds that could rapidly be mobilized. Such a global structure could serve as a forum for discussing the current limitations on orphan vaccine development and availability. Nevertheless, the problems recently faced by the Children's Vaccine Initiative indicate the difficulties in mounting and maintaining such a worldwide initiative.

Acknowledgments

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References

1. Dodet B, Saluzzo J. Factor in the emergence of arbovirus diseases. (Les Pensières): Veyrier du Lac Elsevier Science 1997; 8-10 Decembre 1996.
2. Brealey RA, Myers SC. In: Principles of corporate finance. 3rd ed. Singapore: McGraw-Hill; 1988.
3. Stucki J. The development of orphan drugs—a pharmaceutical company perspective. Cooperative approaches to research and development of orphan drugs 1985;95-104.
4. Aylward B, Kane M, Batson A, Scott R. A framework for the evaluation of vaccines for use in expanded programme on immunization. Vaccine 1994;12:1155-9.
5. Shepard DS, Walsh JA, Kleinau E, Stansfield S, Bhalotra S. Setting priorities for the Children's Vaccine Initiative: a cost-effectiveness approach. Vaccine 1995;13:707-14.
6. Milstien J, Batson A. Accelerating availability of new vaccines: the role of the international community. Drug Inf J 1998;32:175-82.
7. CVI (Children's vaccine initiative). The CVI strategic plan—managing opportunity and change: a vision of vaccination for the 21st century. OMS 1997;CVI/GEN(97-04):1-92.
8. Trannoy E. Will ethical and liability issues and public acceptance allow maternal immunization? Vaccine 1998;16:1482-5.
9. Haffner M. Orphan products: origins, progress, and prospects. Annu Rev Pharmacol Toxicol 1991;31:603-20.
10. Haffner M, Kelsey J. Evaluation of orphan products by the U.S. Food and Drug Administration. Int J Tech Assess Health Care 1992;8:647-57.
11. Cramer R. Orphan drugs improved medical treatment of rare diseases. Alabama J Med Sci 1998;25:257-67.
12. Thamer M, Brennan N, Semansky R. A cross-national comparison of orphan drug policies: implications for the U.S. Orphan Drug Act. J Health Pol Policy Law 1998;23:265-90.
13. Scharf S. Orphan drugs: the question of product liability. Am J Law Med 1985;10:491-513.
14. Bloom B. The United States needs a national vaccine authority. Science 1994;265:1378-80.
15. Brandissou S, Yagoubi N, Hasselot N. Les médicaments orphelins: problème de santé publique et enjeux économiques. Thérapie 1996;51:647-53.
16. Benzi G, Ceci A. Drugs trying to get the patents: there will be incentives for the European scientific community to develop research in the field of the orphan drugs. Pharmacol Res 1997;35:89-93.
17. Hillcoat BL. Rare diseases and "orphan" drugs [editorial]. Med J Aust 1998;169:69-70.
18. Mahoney RT, Maynard JE. The introduction of new vaccines into developing countries. Vaccine 1999;17:646-52.
19. Local initiative aids in fight against malaria [editorial]. Lancet 1998;352:212.
20. Ollario P. Will the fight against tropical diseases benefit from orphan drug status? Trop Med Int Health 1997;2:113-5.
21. Muraskin W. The politics of international health: the children's vaccine initiative and the struggle to develop vaccines for the third world. Albany (NY): State University of New York Press; 1998.
22. Milstien J, Batson A, Meaney W. A systematic method for evaluation of the potential viability of local vaccine producers. Vaccine 1997;15:1358-63.
23. Batson A. Sustainable introduction of affordable new vaccines: the targeting strategy. Vaccine 1998;16:S93-8.
24. Bloom B. Bumps on the vaccine road. Science 1994;265:1371-3.
25. Hausdorff WP. Prospects for the use of new vaccines in developing countries: cost is not the only impediment. Vaccine 1996;14:1179-86.

26. Public-private partnerships—business as usual [editorial]. Lancet 1998;352:212.

27. Spier R. Report on a meeting of a working party sponsored by the European Commission to discuss the ethical, legal and social aspects of research on vaccines and vaccination. Vaccine 1999;17:400-2.

28. Maiztegui J, MacKee K, Barrera Oro J, Harrison L, Gibbs P, Feuillade M, et al. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. J Infect Dis 1998;177:277-83.

29. Helenport J, Roche G. What have been the strategies for the registration, positioning and control of medical information for im artemether? The rational use of Qinghaosu and its derivatives. 1998; Les Pensières (19-22 April):141-7, Fondation Merieux Eds.

30. Beutels P. Economic evaluations applied to HB vaccination: general observations. Vaccine 1998;16:S84-92.

Antimicrobial Resistance with *Streptococcus pneumoniae* in the United States, 1997–98

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From November 1997 to April 1998, 1,601 clinical isolates of *Streptococcus pneumoniae* were obtained from 34 U.S. medical centers. The overall rate of strains showing resistance to penicillin was 29.5%, with 17.4% having intermediate resistance. Multidrug resistance, defined as lack of susceptibility to penicillin and at least two other non-ß-lactam classes of antimicrobial drugs, was observed in 16.0% of isolates. Resistance to all 10 ß-lactam drugs examined in this study was directly related to the level of penicillin resistance. Penicillin resistance rates were highest in isolates from middle ear fluid and sinus aspirates of children <5 years of age and from patients in ambulatory-care settings. Twenty-four of the 34 medical centers in this study had participated in a similar study 3 years before. In 19 of these 24 centers, penicillin resistance rates increased 2.9% to 39.2%. Similar increases were observed with rates of resistance to other antimicrobial drugs.

Before 1990, most clinical isolates of *Streptococcus pneumoniae* in the United States were susceptible to a variety of antimicrobial drugs, including penicillin (1,2). In the early 1990s, however, antimicrobial resistance began to emerge (3-7), and ß-lactam resistance as a result of altered penicillin binding proteins was recognized (8-11). Resistance to other non-ß-lactam drugs, such as the macrolides, clindamycin, tetracycline, chloramphenicol, and trimethoprim/sulfamethoxazole (TMP/SMX), began to increase (4-6). Therapeutic failures in patients with pneumococcal infections treated with previously effective drugs were reported (12).

During November 1994 through April 1995, we assessed the prevalence of antimicrobial resistance with *S. pneumoniae* at 30 U.S. medical centers (4). Among 1,527 isolates of *S. pneumoniae*, 14.1% had intermediate resistance, and 9.5% were fully resistant to penicillin. Aggregate rates of intermediate and high penicillin resistance were 2.1% to 52.9%. In addition, high rates of resistance were noted with other antimicrobial drugs.

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From November 1997 to April 1998, we surveyed 34 U.S. medical centers to assess changes in antimicrobial resistance rates with *S. pneumoniae* during the 3 years since the 1994-95 study. Twenty-four of these centers had also participated in the earlier investigation. Similar patient populations were sampled, and identical test methods were used. In addition, we assessed the relationship between various demographic factors and resistance and undertook a systematic analysis of multidrug resistance. Finally, macrolide and fluoroquinolone resistance was characterized at a molecular level.

The Study

From November 1, 1997, to April 30, 1998, 1,601 isolates of *S. pneumoniae* were recovered in 34 U.S. medical centers. All isolates included in this study were from consecutive patients. With the exception of specimens from the lower respiratory tract, all isolates were from normally sterile body sites (i.e., blood, cerebrospinal fluid, middle ear fluid, sinus aspirates, pericardial fluid, and pleural fluid). Isolates from lower respiratory tract specimens were included only if they were of clinical significance.

In the study centers, isolates were subcultured onto 5% sheep blood agar plates and

incubated overnight at 35°C to 37°C in 5% to 7% CO_2. Colony growth was collected on a rayon swab and immediately immersed in a transport tube containing 12 ml of semisolid Ames transport medium with charcoal (Difco Laboratories, Detroit, MI). Transport tubes were then shipped overnight to the University of Iowa College of Medicine for additional analysis (Appendix). The recovery rate from this transport system was 100%. Twelve concentrations each of 23 antimicrobial drugs were tested against 1,601 isolates of *S. pneumoniae*.

Overall, 17.4% of isolates had intermediate and 12.1% had full resistance to penicillin (Table 1). Overall nonsusceptible rates with ceftriaxone and cefuroxime (intermediate plus fully resistant)

Table 1. In vitro activity of 23 antimicrobial agents for 1,601 isolates of *Streptococcus pneumoniae*

Antimicrobial agent	Penicillin-susceptible strains (n = 1,127)					Penicillin-intermediate strains (n = 278)				
	MIC_{50}	MIC_{90}	MIC range	% I	% R	MIC_{50}	MIC_{90}	MIC range	% I	% R
Penicillin	0.015	0.03	≤0.004 - 0.06	--	--	0.5	1	0.12 - 1	--	--
Amoxicillin	0.015	0.03	≤0.004 - 0.12	0.0	0.0	0.5	2	0.015 - 4	34.5	14
Amox/clav	0.015	0.03	≤0.004 - 0.12	0.0	0.0	0.5	2	0.015 - 4	31.7	16.9
Ceftriaxone	0.015	0.03	≤0.008 - 0.5	0.0	0.0	0.5	1	0.015 - 4	20.1	0.7
Cefuroxime	0.03	0.12	≤0.015 - 2	0.2	0.1	2	4	0.12 - 8	6.8	55.8
Cefpodoxime	0.03	0.06	≤0.015 - 4	–	–	1	4	0.03 - 8	--	--
Cefprozil	0.06	0.12	≤0.03 - 1	–	–	2	8	0.06 - 16	–	–
Cefixime	0.25	0.5	≤0.06 - 16	–	–	8	16	0.25 - 32	–	–
Loracarbef	0.5	1	≤0.06 - 4	–	–	16	128	0.25 - >128	–	–
Cefaclor	0.5	2	≤0.06 - 2	–	–	8	64	0.12 - >128	–	–
Ceftibuten	4	8	≤0.25 - >64	–	–	64	>64	4 - >64	–	–
Clarithromycin	≤0.03	≤0.03	≤0.03 - >64	0.5	5.2	≤0.03	>64	≤0.03 - >64	2.2	35.3
Erythromycin	0.06	0.06	≤0.03 - >64	0.3	5.7	0.06	>64	≤0.03 - >64	0.7	37.4
Azithromycin	0.06	0.12	≤0.03 - >64	0.2	5.6	0.12	>64	≤0.03 - >64	0.7	37.8
Clindamycin	0.06	0.06	≤0.008 - >8	0.1	1.1	0.06	>8	≤0.008 - 8	0.0	12.9
Trovafloxacin	0.06	0.12	0.015 - 8	0.0	0.2	0.06	0.12	0.015 - 0.25	0.0	0.0
Tetracycline	0.12	0.25	≤0.03 - 64	0.2	2.5	0.25	32	≤0.03 - >64	0.7	27.7
TMP/SMX	0.12	1	0.06 - 32	6.5	5.9	2	8	≤0.03 - >32	19.8	42.4
Chloramphenicol	2	4	≤0.5 - 16	–	0.8	4	16	≤0.05 - 16	–	11.9
Rifampin	≤0.12	≤0.12	≤0.12 - 0.5	0.0	0.0	≤0.12	≤0.12	≤0.12 - 2	0.7	0.0
Linezolid[a]	1	2	0.12 - 2	–	–	1	2	0.25 - 2	–	–
Quin/dalfo	0.25	0.5	0.06 - 8	0.0	0.2	0.25	0.5	0.06 - 1	0.0	0.0
Vancomycin	0.25	0.5	0.03 - 0.5	0.0	0.0	0.25	0.5	0.06 - 0.5	0.0	0.0

Antimicrobial agent	Penicillin-resistant strains (n = 196)					All strains (n = 1,601)				
	MIC_{50}	MIC_{90}	MIC range	% I	% R	MIC_{50}	MIC_{90}	MIC range	% I	% R
Penicillin	2	4	2 - 8	--	--	0.015	2	≤0.004 - 8	17.4	12.1
Amoxicillin	2	8	1 - 8	9.7	90.3	0.03	2	≤0.004 - 8	7.2	13.5
Amox/clav	2	8	1 - 8	6.6	93.4	0.03	2	≤0.004 - 8	6.3	14.4
Ceftriaxone	1	2	0.5 - 8	68.4	31.6	0.03	1	≤0.008 - 8	10.9	4
Cefuroxime	4	8	2 - 32	0.0	100	0.03	4	≤0.015 - 32	1.3	22
Cefpodoxime	4	8	1 - >32	--	--	0.06	4	≤0.05 - >32	–	--
Cefprozil	8	16	2 - 64	–	–	0.12	8	≤0.03 - 64	–	--
Cefixime	32	64	2 - 128	–	–	0.25	16	≤0.06 - 128	–	--
Loracarbef	128	>128	32 - >128	–	–	1	128	≤0.06 - >128	–	--
Cefaclor	128	>128	16 - >128	–	–	0.5	64	≤0.06 - >128	–	--
Ceftibuten	>64	>64	≤16 - >64	–	–	44	>64	≤0.25 - >64	–	–
Clarithromycin	2	>64	≤0.03 - >64	3.6	64.8	≤0.03	4	≤0.03 - >64	1.2	17.7
Erythromycin	4	>64	≤0.03 - >64	0.5	68.4	0.06	8	≤0.03 - >64	0.4	18.9
Azithromycin	8	>64	≤0.03 - >64	1.5	67.3	0.12	16	≤0.03 - >64	0.4	18.7
Clindamycin	0.06	>8	≤0.008 - >8	0.5	21.4	0.06	0.06	≤0.008 - >8	0.1	5.6
Trovafloxacin	0.06	0.12	0.03 - 4	0.5	0.5	0.06	0.12	0.015 - 8	0.1	0.2
Tetracycline	16	32	0.06 - 64	0.5	51.5	0.12	16	≤0.03 - >64	0.3	12.9
TMP/SMX	4	16	0.06 - 32	21.9	71.9	0.25	4	≤0.03 - 32	10.7	20.4
Chloramphenicol	4	16	≤0.5 - >16	–	37.2	2	4	≤0.5 - >16	–	7.2
Rifampin	≤0.12	≤0.12	≤0.12 - >4	0.0	0.5	≤0.12	≤0.12	≤0.12 - >4	0.1	0.1
Linezolid	1	2	0.5 - 2	–	–	1	2	0.12 - 2	–	--
Quin/dalfo	0.25	0.5	0.12 1	0.0	0.0	0.25	0.5	0.06 - 8	0.0	0.1
Vancomycin	0.25	0.5	0.06 - 0.5	0.0	0.0	0.25	0.5	0.03 - 0.5	0.0	0.0

[a]Because of the lack of NCCLS breakpoints for linezolid, resistance rates were not determined.
Amox/clav, amoxicillin/clavulanate; TMP/SMX, trimethoprim/sulfamethoxazole; Quin/dalfo, quinupristin/dalfopristin.
MIC, minimum inhibitory concentration; I, intermediate resistance; R, resistant.

were 14.9% and 23.3%, respectively. Because National Committee for Clinical Laboratory Standards (NCCLS)-approved breakpoints are lacking for the six other cephalosporins examined in this study (cefpodoxime, cefixime, ceftibuten, cefprozil, cefaclor, and loracarbef), rates of resistance were not determined for these drugs. However, when MIC values were compared, cefpodoxime was the most active.

Comparison of MIC values of the three macrolides we examined showed that clarithromycin was consistently twice as active as erythromycin, which in turn was consistently twice as active as azithromycin (Table 1). Overall rates of resistance, however, based on NCCLS breakpoints, which differ for these agents, were similar (18%-19%).

Compared with erythromycin as an indicator of macrolide activity, 302 (18.9%) isolates had MICs $\geq$1 µg/ml and thus were classified as resistant (Table 1). Among these, 217 (71.9%) had erythromycin MICs $\leq$32 µg/ml; the remaining 85 strains (28.1%) had erythromycin MICs $\geq$64 µg/ml. Of the 217 strains with erythromycin MICs $\leq$32, 214 had clindamycin MICs $\leq$0.25 and thus were categorized as clindamycin susceptible. Thirty-five of these strains, randomly selected, were examined by polymerase chain reaction (PCR) for the presence of *ermAM* and *mefE* genes. Of the 85 strains with erythromycin MICs $\geq$64 µg/ml, 83 had clindamycin MICs $\geq$8. Thirty-eight of these isolates, chosen randomly, were *ermAM* positive; 12 were also positive for *mefE* (Table 2). Five resistant strains were also characterized for the presence of macrolide resistance determinants (Table 2). Finally, the three isolates (Table 2) negative for both the *ermAM* and *mefE* genes were also negative by PCR for other known determinants of macrolide/lincosamide resistance in gram-positive bacteria (*ermA, ermC, ereA, ereB, msrA,* and *linA* genes).

Of the 1,601 isolates examined, one had intermediate resistance to trovafloxacin with an MIC 2 µg/ml; three strains (0.2%) were resistant, two with trovafloxacin MICs 4 µg/ml and one with a trovafloxacin MIC 8 µg/ml. The single strain with intermediate resistance had a ciprofloxacin MIC of 16 and an asp83→tyr substitution in the C subunit of topoisomerase IV, as well as a ser84→tyr substitution in the A subunit of DNA gyrase. The three trovafloxacin-resistant strains had ciprofloxacin

Table 2. Characterization of 302 *Streptococcus pneumoniae* isolates that were erythromycin resistant (MICs 1$\geq$ µg/ml)

Erythromycin MIC	No. of strains	No. with clindamycin MICs of				
		0.25	0.5	1	2	8
1	15	15[a]	0	0	0	0
2	52	51[b]	0	0	0	1[c]
4	53	53[d]	0	0	0	0
8	68	68[e]	0	0	0	0
16	23	22[f]	1[g]	0	0	0
32	6	5[h]	0	0	0	1[i]
64	85	0	0	1[j]	1[k]	83[l]

[a]Four isolates characterized for *ermAM* and *mefE*; all four *ermAM*-/*mefE*+.
[b]Seven isolates characterized; all seven *ermAM*-/*mefE*+.
[c]This isolate was *ermAM*+/*mefE*-.
[d]Seven isolates characterized; one *ermAM*-/*mefE*-; six *ermAM*-/*mefE*+.
[e]Seven isolates characterized; all seven *ermAM*-/*mefE*+.
[f]Four isolates characterized; all four *ermAM*-/*mefE*+.
[g]This isolate was *ermAM*-/*mefE*-.
[h]Three isolates characterized; all *ermAM*-/*mefE*+.
[i]This isolate was *ermAM*+/*mefE*-.
[j]This isolate was *ermAM*-/*mefE*+.
[k]This isolate was *ermAM*-/*mefE*-.
[l]Thirty-eight of these isolates were characterized; 26 were *ermAM*+/*mefE*-; 12 were *ermAM*+/*mefE*+.

MICs $\geq$32 µg/ml and a ser79→phe substitution in the C subunit of topoisomerase, as well as a ser84→phe substitution in the A subunit of DNA gyrase. None of these four strains had detectable mutations in the QRDR of *par E*.

Overall rates of resistance, expressed as the percentage of isolates intermediate or resistant, for selected other agents are described in Table 1: tetracycline, 13.2%, TMP/SMX, 31.1%, chloramphenicol, 7.2%, and rifampin, 0.2%. Linezolid was uniformly active over a narrow range of MICs (i.e., 0.12 to 2 µg/ml). Two strains among the 1,601 examined in this study were resistant to quinupristin/dalfopristin; one had an MIC 4 µg/ml and the other 8 µg/ml. Vancomycin was uniformly active against the 1,601 isolates of *S. pneumoniae* in this survey, with MICs $\leq$0.5 µg/ml.

The in vitro activity of all ß-lactams (penicillins, ß-lactamase inhibitor combinations and cephalosporins), macrolides, clindamycin, tetracycline, TMP/SMX, and chloramphenicol was lowest with high-level penicillin-resistant strains of *S. pneumoniae* and greatest with penicillin-susceptible isolates. This trend was not apparent with linezolid, trovafloxacin, rifampin, quinupristin/dalfopristin, or vancomycin.

The prevalence of resistance to selected agents was assessed according to the specimen

source of isolates, the patient's age, and the health-care setting (Table 3). In general, the highest resistance rates for all antimicrobial drugs were observed in middle ear fluid and sinus aspirate isolates and in isolates from patients ≤5 years old and from patients seen in ambulatory-care settings.

When patterns of multidrug resistance were analyzed, coresistance to penicillin, the macrolides, tetracycline, TMP/SMX, and chloramphenicol was observed in 5.4% of isolates (Table 4). Resistance to the first four of these drugs but not to chloramphenicol was seen with 3.7% of strains. The most common pattern of multidrug resistance (intermediate level or resistant to penicillin and resistant to TMP/SMX, but susceptible to the macrolides, tetracyclines, and chloramphenicol) was observed in 6.9% of isolates.

In the 34 participating medical centers, the lowest and highest overall rates of penicillin resistance (intermediate plus resistant) were 12.8% and 64.6% (Table 5). Eight medical centers had 10% to 18% penicillin resistance; 11 centers had 19% to 26%; 5 had 27% to 36%; 7 had 37% to

45%; 3 had >46%. Although no distinct geographic clustering was identified, the highest overall rates of penicillin resistance were in the

Table 4. Frequency of isolation of strains of *Streptococcus pneumoniae* with various patterns of antimicrobial resistance[a]

Pattern of resistance[b]					
Peni-cillin	Erythro-mycin	Tetra-cycline	TMP/SMX	Chloram-phenicol	No.
R	S	S	S	S	94
R	S	S	R	S	111
R	S	S	R	R	1
R	S	R	S	S	4
R	S	R	R	S	8
R	S	R	R	R	15
R	R	S	S	S	9
R	R	S	R	S	75
R	R	S	R	R	3
R	R	R	S	S	10
R	R	R	R	S	57
R	R	R	R	R	87

[a]Total number of isolates tested = 1,601.
[b]Resistance includes both intermediate and resistant strains. R, resistant; S, susceptible; TMP/SMX, trimethoprim/ sulfamethoxazole.

Table 3. Recovery of *Streptococcus pneumoniae* strains with intermediate and high levels of resistance, by specimen source and patient characteristics

Characteristic	Total no. of isolates	No. (%) of resistant isolates for the following antimicrobial drugs											
		Penicillin		Ceftriaxone		Erythromycin		Tetracycline		TMP/SMX		Chloramphenicol	
		I	R	I	R	I	R	I	R	I	R	I	R
Specimen source[a]													
LRT	773	144 (18.6)	103 (13.3)	91 (11.8)	36 (4.7)	5 (0.6)	152 (20.9)	1 (0.1)	116 (15.0)	89 (11.5)	149 (19.3)	--	67 (8.7)
Blood	509	68 (13.4)	41 (8.1)	35 (6.9)	16 (3.1)	1 (0.2)	59 (11.6)	2 (0.4)	33 (6.5)	40 (7.9)	87 (17.1)	--	17 (3.3)
URT	238	50 (21.0)	48 (20.2)	44 (18.5)	11 (4.6)	0 (0.0)	77 (32.4)	2 (0.8)	53 (22.3)	28 (11.8)	74 (31.1)	--	28 (11.8)
BF/CSF	60	12 (20.0)	3 (5.0)	4 (6.7)	1 (1.7)	0 (0.0)	9 (15.0)	0 (0.0)	3 (5.0)	1 (1.7)	13 (21.7)	--	2 (3.3)
Other	21	4 (19.0)	1 (4.8)	1 (4.8)	0 (0.0)	0 (0.0)	5 (23.8)	0 (0.0)	1 (4.8)	2 (9.5)	3) (14.3)	--	1 (4.8)
Age group (years)													
≤5	432	88 (20.4)	79 (18.3)	72 (16.7)	23 (5.3)	1 (0.2)	115 (26.6)	3 (0.7)	71 (16.4)	62 (14.4)	128 (29.6)	--	42 (9.7)
6-20	97	14 (14.4)	6 (6.2)	6 (6.2)	1 (1.0)	0 (0.0)	12 (12.4)	0 (0.0)	11 (11.3)	11 (11.3)	14 (14.4)	--	4 (4.1)
21-50	407	76 (18.7)	42 (10.6)	39 (9.6)	18 (4.4)	2 (0.5)	71 (17.4)	0 (0.0)	56 (13.8)	32 (7.9)	80 (19.7)	--	22 (5.4)
>50	652	96 (14.7)	68 (10.4)	57 (8.7)	22 (3.4)	3 (0.5)	102 (15.6)	2 (0.3)	66 (10.1)	65 (10.0)	102 (15.6)	--	46 (7.1)
Service													
Inpatient	969	172 (17.8)	111 (11.5)	104 (10.7)	37 (3.8)	3 (0.3)	171 (17.6)	3 (0.3)	107 (11.0)	85 (8.8)	192 (19.8)	--	61 (6.3)
Outpatient	628	106 (16.9)	85 (13.5)	71 (11.3)	27 (4.3)	3 (0.3)	131 (20.9)	2 (0.3)	99 (15.8)	86 (13.7)	134 (21.3)	--	54 (8.6)

[a]BF/CSF, body fluids/cerebrospinal fluid; LRT, lower respiratory tract; URT = upper respiratory tract.
TMP/SMX, trimethoprim/sulfamethoxazole.

Table 5. Resistance rates of selected antimicrobial drugs, by study center

Medical center and location	No. of isolates	Peni-cillin		Ceftria-xone		Erythro-mycin		Tetra-cycline		Chloram-phenicol		TMP/SMX	
		% I	% R	% I	% R	% I	% R	% I	% R	% I	% R	% I	% R
Children's Hospital & Medical Center, Seattle, WA	50	26.0	12.0	10.0	8.0	0.0	30.0	0.0	24.0	--	10.0	8.0	34.0
Veteran's Affairs Medical Center, Portland, OR	24	12.5	4.2	8.3	0.0	0.0	12.5	0.0	12.5	--	0.0	4.2	25.0
UCSF Medical Center, San Francisco, CA	47	4.3	8.5	6.4	4.3	0.0	12.8	2.1	14.9	--	6.4	6.4	19.1
UCLA Medical Center, Los Angeles, CA	55	27.3	14.5	20.0	1.8	1.8	18.2	0.0	20.0	--	10.9	25.5	21.8
Pathology Medical Laboratories, San Diego, CA	51	15.7	2.0	3.9	0.0	0.0	13.7	0.0	11.8	--	2.0	9.8	9.8
University of Utah Medical Center, Salt Lake City, UT	53	13.2	9.4	9.4	1.9	0.0	17.0	0.0	7.5	–	5.7	13.2	20.8
Denver Health, Denver, CO	26	7.7	7.7	11.5	0.0	0.0	7.7	0.0	11.5	--	11.5	3.8	15.4
Good Samaritan Medical Center, Phoenix, AZ	64	11.1	29.6	24.1	11.1	0.0	35.2	0.0	20.4	--	5.6	11.1	38.9
University Hospital, Albuquerque, NM	51	11.8	5.9	5.9	0.0	0.0	13.7	0.0	7.8	--	3.9	9.8	11.8
Texas Children's Hospital, Houston, TX	48	39.6	25.0	26.0	10.4	0.0	43.8	2.1	22.9	--	14.6	20.8	41.7
Parkland Health & Hospital System, Dallas, TX	36	19.4	11.1	11.1	2.8	0.0	27.8	0.0	22.2	--	11.1	16.7	22.2
Mayo Clinic, Rochester, MN	48	16.7	6.3	6.3	4.2	0.0	20.8	2.1	10.4	--	6.3	10.4	16.7
University of Iowa Hospitals and Clinics, Iowa City, IA	49	16.3	22.4	16.3	4.1	0.0	22.4	0.0	20.4	--	12.2	14.3	22.4
Children's Hospital, Milwaukee, WI	55	12.7	7.3	7.3	3.6	0.0	10.9	1.8	5.5	--	3.6	12.7	14.5
Evanston Hospital, Evanston, IL	35	11.4	2.9	2.9	0.0	0.0	14.3	0.0	5.7	--	0.0	5.7	8.6
Rush-Presbyterian St. Luke's Medical Center, Chicago, IL	41	14.6	4.9	4.9	2.4	0.0	19.5	0.0	17.1	--	7.3	2.4	19.5
Clarian Health Methodist Hospital, Indianapolis, IN	55	18.2	7.3	3.6	0.0	1.8	16.4	0.0	7.3	--	3.6	10.9	12.7
Washington University, St. Louis, MO	55	12.7	16.4	14.5	3.6	0.0	12.7	0.0	9.1	--	5.5	18.2	12.7
Henry Ford Health System, Detroit, MI	60	21.7	8.3	10.0	3.3	0.0	10.0	0.0	8.3	--	6.7	15.0	10.0
The Cleveland Clinic Foundation, Cleveland, OH	60	10.0	13.3	11.7	3.3	0.0	20.0	1.7	10.0	--	6.7	11.7	15.0
Temple University Hospital, Philadelphia, PA	42	7.1	14.3	4.8	9.5	2.4	9.5	0.0	7.1	--	4.8	2.4	9.5
Geisinger Medical Center, Danville, PA	49	30.6	8.2	12.2	0.0	0.0	20.4	0.0	20.4	--	12.2	10.2	14.3
SUNY Health Science Center, Syracuse, NY	50	12.0	8.0	8.0	2.0	0.0	8.0	0.0	8.0	--	6.0	12.0	8.0
University of Rochester Medical Center, Rochester, NY	50	10.0	10.0	10.0	2.0	2.0	10.0	0.0	8.0	--	6.0	8.0	14.0
Columbia Presbyterian Medical Center, New York, NY	53	18.9	1.9	3.8	0.0	0.0	3.8	0.0	7.5	--	5.7	5.7	7.5
Dartmouth Hitchcock Medical Center, Lebanon, NH	50	8.0	8.0	4.0	4.0	0.0	10.0	0.0	6.0	--	6.0	6.0	22.0
Hartford Hospital, Hartford, CT	51	17.6	9.8	9.8	0.0	2.0	5.9	0.0	7.8	--	2.0	11.8	17.6
Beth Israel Deaconess Medical Center, Boston, MA	41	9.8	4.9	2.4	2.4	0.0	14.6	0.0	9.8	--	2.4	7.3	9.8
Children's Hospital, Washington, DC	28	17.9	17.9	21.4	0.0	0.0	28.6	0.0	17.9	--	3.6	7.1	28.6
University of North Carolina Hospital, Chapel Hill, NC	49	22.4	34.7	26.5	14.3	2.0	36.7	0.0	20.4	--	16.3	16.3	46.9
Dekalb Medical Center, Decatur, GA	52	32.7	11.5	11.5	1.9	0.0	26.9	0.0	17.3	--	7.7	3.8	28.8
University of Louisville Hospital, Louisville, KY	48	22.9	10.4	4.2	8.3	0.0	20.8	0.0	6.3	--	2.1	6.3	18.8
University of South Alabama, Mobile, AL	58	17.2	24.1	17.2	6.9	0.0	37.9	0.0	13.8	--	13.8	12.1	39.7
Mount Sinai Medical Center, Miami Beach, FL	27	18.5	33.3	25.9	22.2	0.0	29.6	0.0	29.6	--	25.9	7.4	48.1

I, intermediate resistance; R, resistant; TMP/SMX, trimethoprim/sulfamethoxazole.

South and Southeast. The lowest overall rate of ceftriaxone resistance was 2.9%; the highest was 48.1%. With erythromycin, overall rates of resistance were 3.8% to 43.8%. With tetracycline, chloramphenicol, and TMP/SMX, the rates were 5.7% to 29.6%, 0.0% to 25.9%, and 11.9% to 63.2%, respectively. In general, the highest rates of resistance with most antimicrobial classes were observed in the same centers.

Among the 34 medical centers, 24 had participated in a similar study 3 years before (4). Because the sampling period, the nature of the patients, and the test methods were the same, resistance rates obtained with selected antimicrobial drugs were compared at the 24 centers for the two study periods (Table 6). In 19 of 24 centers, penicillin resistance rates (intermediate and resistant) increased by at least 2% during the 3-year interval. In six cases, the rate of increase was statistically significant (Table 6). The number of centers in which rates of resistance increased by >2% with other selected antimicrobial drugs over the 3-year period were ceftriaxone 13, erythromycin 21, tetracycline 20, chloramphenicol 12, and TMP/SMX 16. In most cases, resistance rates increased; however, with certain antimicrobial agents (i.e., ceftriaxone, TMP/SMX, and chloramphenicol), resistance rates remained the same or decreased. Three centers (Texas Children's Hospital, UNC Hospital, and the University of South Alabama Medical Center) had statistically significant increased rates of resistance to nearly every class of antimicrobial drug.

Conclusions

A total of 1,601 clinical isolates of *S. pneumoniae* obtained from November 1997 to April 1998 from patients in 34 U.S. medical centers were characterized with respect to the in vitro activity of 23 antimicrobial drugs. The overall rate of penicillin resistance was 29.5% (17.4% with intermediate resistance; 12.1% fully resistant). Penicillin, amoxicillin, and amoxicillin/ clavulanate had essentially equivalent MICs for the pneumococcal isolates examined. Among the cephalosporins tested, the rank order of activity based on a comparison of MICs was ceftriaxone > cefpodoxime = cefuroxime > cefprozil > cefixime > cefaclor = loracarbef > ceftibuten. NCCLS has developed MIC interpretive breakpoints for two of these compounds, ceftriaxone and cefuroxime. On the basis of these breakpoints, overall resistance rates were 14.9% with ceftriaxone (10.9% intermediate, 4.0% resistant) and 23.3% with cefuroxime (1.3% intermediate and 22.0% resistant). Although no NCCLS-approved breakpoints have been developed for cefaclor, loracarbef, and ceftibuten versus *S. pneumoniae*, the high MICs we obtained with these drugs indicate that they would be poor choices for treating pneumococcal infections.

We observed a direct relationship between penicillin activity and the activity of all the other ß-lactam drugs we examined. This relationship, which has been observed by others, results from the principal mechanism of penicillin resistance in *S. pneumoniae*, alterations in penicillin binding proteins (4-6). The same proteins to which penicillin binds are necessary for the expression of the activity of all other ß-lactam antimicrobial drugs (8-11).

Among the macrolides examined, clarithromycin was generally one dilution more active than erythromycin, which in turn was one dilution more active than azithromycin. However, since NCCLS MIC interpretive breakpoints differ for these agents and given the actual distribution of MICs, overall rates of resistance for these three agents were similar (19.0%).

Two previous studies have indicated that macrolide resistance with *S. pneumoniae* exists primarily in one of two forms: strains with altered ribosomal targets due to expression of the *ermAM* gene and strains with an active efflux pump due to expression of the *mefE* gene (13,14). *ErmAM*-positive isolates have constitutive expression of resistance and typically have high levels of resistance to both clindamycin (MICs ≥8 µg/ml) and erythromycin (MICs ≥64 µg/ml). Efflux mutants are susceptible to clindamycin (MICs ≤0.25 µg/ml) and typically have erythromycin MICs 1 to 32 µg/ml.

In a recent survey in Canada, approximately 60% of macrolide-resistant strains of *S. pneumoniae* appeared to be efflux mutants (19). In our study, 214 (70.8%) of 302 macrolide-resistant strains were characterized by the efflux phenotype. When a random sample of 35 of these isolates was examined by PCR, 31 (88.6%) lacked the *ermAM* gene but had the *mefE* gene responsible for efflux. Among the remaining four strains in this group that were characterized at a molecular level, one was *ermAM* positive and *mefE* negative, two were positive for both *ermAM* and *mefE,* and one was negative for all

Table 6. Resistance rate comparison of selected antimicrobial drugs for 24 medical centers, by study period

Medical center	No. of isolates	Study period	Peni-cillin %I	%R	Ceftria-xone %I	%R	Erythro-mycin %I	%R	Tetra-cycline %I	%R	Chloram-phenicol %I	%R	TMP/SMX %I	%R
Children's Hospital & Medical	37	1994-95	21.6	13.5	8.1	8.1	0.0	5.4	0.0	18.9	--	8.1	10.8	37.8
Center, Seattle, WA	50	1997-98	26.0	12.0	10.0	8.0	0.0	30.0[a]	0.0	24.0	--	10.0	8.0	34.0
Denver Health, Denver, CO	62	1994-95	11.3	3.2	1.6	0.0	0.0	3.2	0.0	3.2	--	0.0	6.5	9.7
	26	1997-98	7.7	7.7	11.5	0.0	0.0	7.7	0.0	11.5	--	11.5[a]	3.8	15.4
Good Samaritan Medical	57	1994-95	21.1	19.3	21.1	7.0	0.0	12.3	0.0	14.0	--	5.3	10.5	19.3
Center, Phoenix, AZ	54	1997-98	11.1	29.6	24.1	11.1	0.0	35.2[a]	0.0	20.4	--	5.6	11.1	38.9
Texas Children's Hospital,	63	1994-95	9.5	15.9	9.5	6.3	0.0	22.2	0.0	9.5	--	7.9	7.9	30.2
Houston, TX	48	1997-98	39.6	25.0[a]	25.0	10.4[a]	0.0	43.8[a]	2.1	22.9[a]	--	14.6	20.8	41.7
Parkland Health & Hospital	58	1994-95	8.6	13.8	8.6	5.2	0.0	6.9	0.0	5.2	--	5.2	15.5	12.1
System, Dallas, TX	36	1997-98	19.4	11.1	11.1	2.8	0.0	27.8[a]	0.0	22.2[a]	--	11.1	16.7	22.2
Mayo Clinic, Rochester, MN	35	1994-95	2.9	11.4	5.7	5.7	0.0	8.6	2.9	8.6	--	5.7	8.6	22.9
	48	1997-98	16.7	6.3	6.3	4.2	0.0	20.8	2.1	10.4	--	6.3	10.4	16.7
Children's Hospital,	65	1994-95	20.0	13.8	6.2	6.2	0.0	18.5	0.0	6.2	--	7.7	6.2	29.2
Milwaukee, WI	55	1997-98	12.7	7.3	7.3	3.6	0.0	10.9	1.8	5.5	--	3.6	12.7	14.5
Evanston Hospital,	49	1994-95	10.2	4.1	6.1	2.0	0.0	8.2	0.0	4.1	--	4.1	4.1	16.3
Evanston, IL	35	1997-98	11.4	2.9	2.9	0.0	0.0	14.3	0.0	5.7	--	0.0	5.7	8.6
Rush-Presbyterian St. Luke's	41	1994-95	19.5	14.6	4.9	14.6	0.0	17.1	0.0	7.3	--	12.2	9.8	29.3
Medical Center, Chicago, IL	41	1997-98	14.6	4.9	4.9	2.4	0.0	19.5	0.0	17.1	--	7.3	2.4	19.5
Clarian Health Methodist	63	1994-95	17.5	3.2	6.3	1.6	0.0	7.9	0.0	3.2	--	1.6	14.3	14.3
Hospital, Indianapolis, IN	55	1997-98	18.2	7.3	3.6	0.0	1.8	16.4	0.0	7.3	--	3.6	10.9	12.7
Washington University,	57	1994-95	19.6	5.4	8.8	3.5	0.0	8.9	0.0	7.1	--	5.4	7.1	12.5
St. Louis, MO	55	1997-98	12.7	16.4	14.5	3.6	0.0	12.7	0.0	9.1	--	5.5	18.2	12.7
Henry Ford Health System,	63	1994-95	17.5	1.6	1.6	4.8	0.0	6.3	0.0	4.8	--	3.2	7.9	12.7
Detroit, MI	60	1997-98	21.7	8.3	10.0	3.3	0.0	10.0	0.0	8.3	--	6.7	15.0	10.0
The Cleveland Clinic Foun-	42	1994-95	4.8	14.3	4.8	9.5	0.0	11.9	0.0	9.5	--	7.1	11.9	26.2
dation, Cleveland, OH	60	1997-98	10.0	13.3	11.7	3.3	0.0	20.0	1.7	10.0	--	6.7	11.7	15.0
Temple University Hospital,	47	1994-95	2.1	0.0	2.1	0.0	0.0	2.1	0.0	0.0	--	0.0	4.3	4.3
Philadelphia, PA	42	1997-98	7.1	14.3[a]	4.8	9.5[a]	2.4	9.5	0.0	7.1	--	4.8	2.4	9.5
Geisinger Medical Center,	57	1994-95	10.5	10.5	5.3	5.3	0.0	5.3	1.8	8.8	--	7.0	5.3	10.5
Danville, PA	49	1997-98	30.6	8.2[a]	12.2	0.0	0.0	20.4[a]	0.0	20.4	--	12.2	10.2	14.3
SUNY Health Science Center,	23	1994-95	8.7	0.0	0.0	0.0	0.0	8.7	0.0	4.3	--	0.0	4.3	4.3
Syracuse, NY	50	1997-98	12.0	8.0	8.0	2.0	0.0	8.0	0.0	8.0	--	6.0	12.0	8.0
University of Rochester	58	1994-95	5.2	5.2	5.2	1.7	0.0	6.9	0.0	10.3	--	5.2	5.2	13.8
Medical Ctr., Rochester, NY	50	1997-98	10.0	10.0	10.0	2.0	2.0	10.0	0.0	8.0	--	6.0	8.0	14.0
Columbia Presbyterian Medical	64	1994-95	6.3	6.3	4.7	4.7	0.0	4.7	0.0	3.1	--	0.0	10.9	10.9
Center, New York, NY	53	1997-98	18.9	1.9	3.8	0.0	0.0	3.8	0.0	7.5	--	5.7	5.7	7.5
Hartford Hospital,	61	1994-95	3.31	4.9	1.6	4.9	0.0	3.3	0.0	0.0	--	0.0	0	8.2
Hartford, CT	51	1997-98	17.6	9.8[a]	9.8	0.0	2.0	5.9	0.0	7.8[a]	--	2.0	11.8	17.6
Children's Hospital,	60	1994-95	16.7	6.7	5.0	3.3	0.0	13.3	0.0	3.3	--	0.0	6.7	15.0
Washington, DC	28	1997-98	17.9	17.9	21.4	0.0	0.0	28.6	0.0	17.9[a]	--	3.6	7.1	28.6
University of North Carolina	60	1994-95	21.7	10.0	8.3	3.3	0.0	10.0	0.0	8.3	--	6.7	16.7	25.0
Hospital, Chapel Hill, NC	49	1997-98	22.4	34.7[a]	26.5	14.3[a]	2.0	36.7[a]	0.0	20.4	--	16.3	16.3	46.9[a]
Dekalb Medical Center,	61	1994-95	16.4	19.7	13.1	13.1	0.0	23.0	0.0	11.5	--	9.8	1.6	27.9
Decatur, GA	52	1997-98	32.7	11.5	11.5	1.9	0.0	26.9	0.0	17.3	--	7.7	3.8	28.8
University of South	68	1994-95	13.2	7.4	5.9	2.9	1.5	14.7	0.0	4.4	--	0.0	2.9	25.0
Alabama, Mobile, AL	58	1997-98	17.2	24.1[a]	17.2	6.9[a]	0.0	37.9[a]	0.0	13.8	--	13.88	12.1	39.7[a]
Mount Sinai Medical Center,	17	1994-95	29.4	23.5	5.9	17.6	0.0	5.9	0.0	17.6	--	11.8	23.5	23.5
Miami Beach, FL	27	1997-98	18.5	33.3	25.9	22.2	0.0	29.6	0.0	29.6	--	25.9	7.4	48.1

[a]Statistically significant change in resistance rates (I + R) from 1994-95 to 1997-98; p value:50.05.
I, intermediate; R, resistant; TMP/SMX, trimethoprim/sulfamethoxazole.

macrolide-resistance markers. Based on their phenotype (i.e., erythromycin MICs $\geq$64 µg/ml, with clindamycin MICs $\geq$8 µg/ml), 83 (27.5%) of the 302 macrolide-resistant strains appeared to have constitutive expression of *ermAM*-mediated methylation of ribosomal targets. All 38 randomly selected isolates from this group were *ermAM* positive; 12 were also *mefE* positive by PCR.

Five macrolide-resistant strains were not readily placed into either the efflux or the target modification groups based on phenotype. Two

strains with high-level clindamycin resistance but erythromycin MICs 2 and 32 µg/ml were *ermAM* positive but *mefE* negative. Three strains with erythromycin MICs $\geq$64 had clindamycin MICs 0.5, 1, and 4 µg/ml. One of these strains, which had a clindamycin MIC of 4 and a high erythromycin MIC, appeared to be an efflux mutant since it was *mefE* positive but *ermAM* negative. The mechanism of resistance in the last two isolates in this group is unclear. Both isolates were negative by PCR for *ermAM* and *mefE*, as well as other genetic markers for macrolide and lincosamide resistance in gram-positive bacteria.

Several conclusions can be drawn from these observations. Macrolide resistance in *S. pneumoniae* is nearly always characterized either by efflux or ribosomal target modifications. The phenotype of isolates in both categories is highly predictable. Efflux mutants usually have erythromycin MICs 1 - 32 µg/ml and clindamycin MICs <0.25 µg/ml. Target modified strains are characterized by constitutive expression of resistance and typically have erythromycin MICs $\geq$64 µg/ml and clindamycin MICs $\geq$8 µg/ml. Although rare exceptions to these patterns exist, clindamycin activity, as assessed by an MIC determination, is a reliable indicator of the nature of macrolide resistance.

These results show that therapeutic efficacy can be predicted for patients with pneumococcal infections who are treated with macrolides. Using current NCCLS interpretive criteria, 18% to 19% of clinical isolates of *S. pneumoniae* in the United States would be categorized as resistant. Approximately three-fourths of the resistant strains, however, are efflux mutants and have mid-range resistant macrolide MICs. Patients infected with *mefE* versus *ermAM*-positive strains of *S. pneumoniae* may differ in their response to macrolide therapy. The answer to this important question would define true clinical rates of macrolide resistance. If *mefE* strains respond to therapy with macrolide drugs, then true macrolide resistance rates may not be 18% to 19%, but closer to 4% to 5%, and high-level clindamycin MICs could be used to accurately identify strains highly resistant to macrolide drugs.

Multidrug resistance was noted with 16.0% of 1,601 isolates, a substantial increase over the 9.1% prevalence of multidrug resistant *S. pneumoniae* reported in our 1994-95 study (4). Among multidrug resistant strains, four resistance patterns were the most common: penicillin and TMP/SMX (n = 111); penicillin, macrolide, and chloramphenicol (n = 75); penicillin, macrolide, tetracycline, and TMP/SMX (n = 57); and penicillin, macrolide, tetracycline, TMP/SMX, and chloramphenicol (n = 87). No obvious clustering of multidrug resistant strains was observed in either individual medical centers or geographic regions.

That we found resistance to ß-lactam antimicrobial agents and numerous non-ß-lactam agents in the same organism is of interest. The mechanisms of resistance to the different antimicrobial classes are distinct and do not appear to be linked genotypically. One explanation, therefore, for the increasing prevalence of multidrug resistant strains is clonal spread of organisms resistant to more than one class of antimicrobial drugs. Under such circumstances, regardless of the antimicrobial drug used to select for resistance, multidrug resistant organisms may become more prevalent. Clonal spread warrants further study using appropriate techniques for establishing clonal relationships among multiple geographically distinct isolates of *S. pneumoniae*.

Acknowledgments

Dr. Doern is Director of the Clinical Microbiology Laboratories at the University of Iowa College Hospital and Clinics and Professor in the Department of Pathology in the medical school. His research interests focus on antibiotic resistance among bacterial pathogens. He has been Chair of the Clinical Microbiology Division of the American Society for Microbiology and a member of the Antimicrobial Subcommittee of the National Committee for Clinical Laboratory Standards.

Appendix

Isolates were frozen at -70°C at the University of Iowa College of Medicine until further characterization. After two

subcultures, the identity of isolates was confirmed as *S. pneumoniae* by conventional tests and criteria. MICs were determined in Mueller-Hinton broth supplemented with 3% lysed horse blood, according to the broth microdilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (15). Microdilution trays (final volume 100 µl per well) were inoculated with approximately 5×10^5 colony-forming units (CFU)/ml (final concentration) of test organism and incubated 22 to 24 hours at 35°C in ambient air before MICs were determined.

$S. pneumoniae$ ATCC 49619 and *Haemophilus influenzae* ATCC 49247, ATCC 49766, and ATCC 10211 were used as controls. Calculations of the percentages of isolates resistant to individual agents were based on the most recent MIC interpretive criteria published by NCCLS (16).

Selected isolates were examined for mutations in the quinolone resistance-determining region of the *parC* and *par E* genes encoding the C and E subunits of topoisomerase IV, respectively, and the quinolone resistance-determining region of the *gyrA* gene encoding for the A subunit of DNA gyrase. Polymerase chain reaction (PCR) amplification with subsequent sequencing of PCR products was done by using the method and primers described by Pan et al. (17) and Perichon et al. (18). Other isolates were screened for various macrolide resistance determinants by PCR amplification as described by Shortridge et al. (13). Primers were chosen by using Oligo 5.0 software (NBI, Plymouth, MI) from sequences deposited in Genbank. Strains negative for both *mefE* (efflux mutants) and *ermAM* (ribosome methylase) were characterized further for the presence of other methylase genes (*ermA* and *ermC*), erythromycin esterase (*ereA* and *ereB*), MS efflux (*msrA*) and the gene for lincosamide resistance (*linA*), again as described by Shortridge et al. (13).

References

1. Jorgensen JH, Doern GV, Maher LA, Howell AW, Redding JS. Antimicrobial resistance among respiratory isolates of *Haemophilus influenzae, Moraxella catarrhalis*, and *Streptococcus pneumoniae* in the United States. Antimicrob Agents Chemother 1990;34:2075-80.
2. Spika JS, Acklam RR, Plikaytis BD, Oxtoby MG, the Pneumococcal Surveillance Working Group. Antimicrobial resistance of *Streptococcus pneumoniae* in the United States, 1979-1987. J Infect Dis 1991;163:1273-8.
3. Barry AL, Pfaller MA, Fuchs PC, Packer RR. In vitro activities of 12 orally administered antimicrobial agents against four species of bacterial respiratory pathogens from U.S. medical centers in 1992 and 1993. Antimicrob Agents Chemother 1994;38:2419-25.
4. Doern GV, Brueggemann A, Holley HP Jr, Rauch AM. Antimicrobial resistance of *Streptococcus pneumoniae* recovered from outpatients in the United States during the winter months of 1994 to 1995: results of a 30-center national surveillance study. Antimicrob Agents Chemother 1996;40:1208-13.
5. Doern GV, Pfaller MA, Kugler K, Freeman J, Jones RN. Prevalence of antimicrobial resistance among respiratory tract isolates of *Streptococcus pneumoniae* in North America: 1997 results from the SENTRY Antimicrobial Surveillance Program. Clin Infect Dis 1998;27:764-70.
6. Jones RN, Pfaller MA, Doern GV. Comparative antimicrobial activity of trovafloxacin tested against 3,049 *Streptococcus pneumoniae* isolates from the 1997-1998 respiratory infection season. Diagn Microbiol Infect Dis 1998;32:119-26.
7. Thornsberry C, Brown SD, Yee C, Bouchillon SK, Marler JK, Rich T. Increasing penicillin resistance in *Streptococcus pneumoniae* in the U.S. Infections in Medicine Supplement 1993;93:15-24.
8. Grebe T, Hakenbeck R. Penicillin binding proteins 2b and 2x of *Streptococcus pneumoniae* are primary resistance determinants for different classes of ß-lactam antibiotics. Antimicrob Agents Chemother 1996;40:829-34.
9. Jamin M, Hakenbeck R, Frere JM. Penicillin binding protein 2x as a major contributor to intrinsic ß-lactam resistance of *Streptococcus pneumoniae*. FEBS 1993;331:101-4.
10. Laible G, Hekenback R. Five independent combinations of mutations can result in low-affinity penicillin-binding protein 2x of *Streptococcus pneumoniae*. J Bacteriol 1991;173:6986-90.
11. Markiewicz Z, Tomasz A. Variation in penicillin-binding protein patterns of penicillin-resistant clinical isolates of pneumococci. J Clin Microbiol 1989;27:405-10.
12. Kaplan SL, Mason EO Jr. Management of infections due to antibiotic-resistant *Streptococcus pneumoniae*. Clin Microbiol Rev 1998;11:628-44.
13. Shortridge VD, Flamm RK, Ramer N, Beyer J, Tanaka SK. Novel mechanism of macrolide resistance in *Streptococcus pneumoniae*. Diagn Microbiol Infect Dis 1996;26:73-8.
14. Sutcliffe J, Tait-Kamradt A, Wondrack L. *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. Antimicrob Agents Chemother 1996;40:1817-24.
15. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard M7-A4. Wayne (PA): The Committee; 1997.
16. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Eighth informational supplement, M100-S8. Wayne (PA): The Committee; 1997.
17. Pan X-S, Ambler J, Mehtar S, Fisher LM. Involvement of topoisomerase IV and NDA gyrase as ciprofloxacin targets in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1996;40:2321-6.
18. Perichon B, Tankovic J, Courvalin P. Characterization of a mutation in the *par E* gene that confers fluoroquinolone resistance in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1997;41:1166-7.
19. Johnston NJ, De Azavedo JC, Kellner JD, Low DE. Prevalence and characterization of the mechanisms of macrolide, lincosamide, and streptogramin resistance in isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1998;42:2425-6.

Epidemiologic Studies of *Cyclospora cayetanensis* in Guatemala

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In 1996 and 1997, cyclosporiasis outbreaks in North America were linked to eating Guatemalan raspberries. We conducted a study in health-care facilities and among raspberry farm workers, as well as a case-control study, to assess risk factors for the disease in Guatemala. From April 6, 1997, to March 19, 1998, 126 (2.3%) of 5,552 surveillance specimens tested positive for *Cyclospora*; prevalence peaked in June (6.7%). Infection was most common among children 1.5 to 9 years old and among persons with gastroenteritis. Among 182 raspberry farm workers and family members monitored from April 6 to May 29, six had *Cyclospora* infection. In the case-control analysis, 62 (91%) of 68 persons with *Cyclospora* infection reported drinking untreated water in the 2 weeks before illness, compared with 88 (73%) of 120 controls (odds ratio [OR] 3.8, 95% confidence interval [CI] 1.4, 10.8 by univariate analysis). Other risk factors included water source, type of sewage drainage, ownership of chickens or other fowl, and contact with soil (among children younger than 2 years).

Before 1995, the coccidian parasite *Cyclospora cayetanensis* was primarily reported as a cause of gastroenteritis among children living in poor sanitary conditions (1,2) and adults from industrialized countries who lived or traveled in developing countries (3-5). From 1990 to 1995, three small *Cyclospora* outbreaks were reported in North America, at least one of them epidemiologically associated with drinking water (6-8). However, in the spring and early summer of 1996, the largest ever reported outbreak of cyclosporiasis, affecting more than 1,400 persons in North America (9), was associated with eating fresh raspberries; trace-back data indicated that the source of the berries was Guatemala (9). A year later, despite improvements in water quality and sanitary practices instituted on berry farms after the 1996 outbreak, Guatemalan raspberries were again implicated in cyclosporiasis outbreaks (10).

The source of contamination of the implicated raspberries has not been established. Most likely, fecal contamination of water used for spraying fungicides and other substances directly on fruit was the source (9). Another possibility is that berries were contaminated with sporulated organisms during hand-sorting and packing; however, because *Cyclospora* may require 1 to 2 weeks in the environment to become infectious (2), direct fecal contamination from the hands of an infected worker is less likely. The possibility that berries might have been contaminated by bird droppings before being picked has also been raised (11), although no natural (12) or experimental infection with *C. cayetanensis* has been confirmed in any animal (M. Eberhard, pers. comm.). Studies of *Cyclospora* have been limited by this lack of an animal model; by dependence on microscopy for detection; by the lack of sensitivity of the available methods in water, fruit, and other environmental specimens; and by inability to culture the organism.

Cyclospora in Guatemala was first reported in 1994 (13). Since then, the organism has been

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reported among AIDS patients (14) and in several case series in hospital outpatient departments in Guatemala City (15-17). However, diagnosis of *Cyclospora* infection is not part of routine stool examination in health-care facilities in Guatemala.

In 1997, we initiated a study whose objectives were to provide the first comprehensive description of the epidemiology of *Cyclospora* in Guatemala and explore potential environmental sources of contamination. The study included surveillance among patients in outpatient departments and among raspberry farm workers, a case-control study to identify risk factors for *Cyclospora* infection, and screening of surface water specimens for fecal contamination and for *Cyclospora* oocysts.

Methods

Outpatient Surveillance

In two government health centers (both in the Department of Guatemala) and three hospital outpatient departments (two in the Department of Guatemala and one in the Department of Sacatepequez), we interviewed outpatients whose health-care providers had ordered stool specimens for bacterial pathogen testing or screening for helminth eggs. In each center, we screened up to 25 specimens submitted each day. The study was explained to potential participants and written consent was requested. For each person who agreed to participate, part of the stool specimen was collected, and information was recorded about age, sex, and current diarrhea (defined as 3 or more loose or liquid stools in 24 hours) or other gastrointestinal symptoms. For data analysis, we defined gastroenteritis as diarrhea, other gastrointestinal symptoms, or both. Persons with symptomatic *Cyclospora* infection were treated with trimethoprim-sulfamethoxazole unless they had a history of allergy to sulfa drugs. Asymptomatic persons were not treated, as the risk for side effects was thought greater than the benefits of treating a self-limited asymptomatic infection.

Raspberry Farm Cohorts

On three raspberry farms (two in the Department of Guatemala and one in the Department of Chimaltenango), cohorts of workers and their family members were recruited in early April 1997. All participating farms, except one, had been implicated in the 1996 outbreak and were still exporting raspberries. The farms included at least one from each of the two main areas where raspberries are cultivated. The study was described in detail to participants, and written consent was solicited. A stool specimen was collected every other week from workers (less regularly from family members) and each time a participant reported having gastroenteritis. Persons found to have *Cyclospora* infection were treated with trimethoprim-sulfamethoxazole unless they had a history of allergy to sulfa drugs; they were monitored until their stool specimens were negative. Because of concerns about potential contamination of berries, all workers and their family members with *Cyclospora* infection, whether symptomatic or asymptomatic, were treated. Surveillance was intended to continue throughout the spring and fall berry harvest seasons.

Case-Control Study

From May 7 to September 3, 1997, we recruited participants for the case-control study. Because surveillance on raspberry farms ended earlier than anticipated, only four case-control participants were from the farms; all others were recruited from health-care facilities. All persons with symptomatic *Cyclospora* infection detected through the surveillance system were sought in their homes; however, some could not be located because of incorrect addresses. The purpose of the visit was to offer antibiotic treatment; at the same time, we solicited participation in the case-control study. We also requested stool specimens from family members of case-patients; however, family members were not included in the case-control study, and no rigorous attempt was made to include all family members.

We recruited controls from persons who had a stool specimen screened in any of the surveillance system's health centers or hospitals during the period in which we were enrolling cases. Participants were eligible to be controls if they reported no gastrointestinal symptoms and had negative stool results. We chose the controls to permit a final age distribution similar to that seen for *Cyclospora* cases in the surveillance system and a roughly equal distribution by surveillance site; individual cases and controls were not matched. Thus, each week we rotated

the surveillance site from which we recruited controls, and we attempted to enroll 10 controls per week: one to two children younger than 1.5 years of age, four children 1.5 to 4 years of age, three 5 to 14 years of age, and one to two persons older than 14 years of age.

We administered a questionnaire that focused on potential risk factors for *Cyclospora*, including drinking water source and handling, household sanitation, presence of animals, socioeconomic variables, and consumption of specific fresh fruits, vegetables, and herbs. Several key variables were defined: drinking untreated water was defined as having drunk other than commercially bottled water that had not been boiled, chlorinated, or filtered in the household; water source was divided into high risk (public standpipe, well, spring, or water bought from a vendor) and low risk (in-house piped municipal water or commercially bottled water). Among children <5 years of age, we defined breastfed as having been breastfed at all, whether exclusively or partially, at the time the child provided the specimen.

Laboratory Methods

Stool specimens were processed by using a standard formalin-ethyl acetate concentration procedure and screened by two methods: light-microscopy examination of a modified acid-fast stained smear and UV epifluorescence examination of a wet mount (18). To confirm the identity of the parasite as *C. cayetanensis*, three positive specimens stored in 2.5% dichromate at ambient temperature (approximately 23°C) were examined at regular intervals over a 2-week period, starting from the time the specimen was provided (18). We observed characteristic sporulation in all three.

Analysis of Water Samples

From May 13 to July 17, 1997, we collected a weekly 10-liter water sample from each of three rivers (Villa Lobos, Los Verdes, and Guacalate). Two sampling sites were located in the Department of Guatemala and one in the Department of Sacatepequez; these rivers all contain raw sewage outfalls. Standard methods were used to quantify the level of fecal contamination with *Escherichia coli* (19). The specimens were concentrated by the flocculation method developed for detection of *Cryptosporidium* in water (20), with the

following modifications for logistical reasons: the specimens were left to flocculate overnight; the centrifugation speed was 600 g, the maximum for our centrifuge, rather than 3,000 g; and we used only one washing with Tween. The concentrated sediments were examined by bright-field and UV epifluorescence microscopy for *Cyclospora* oocysts.

Data Analysis

All data were entered in Epi-Info 6.04b. Surveillance data were analyzed in Epi-Info and in Excel spreadsheets. The case-control data were analyzed in SAS 6.12. Potential risk factors were assessed in a series of univariate analyses; unless otherwise specified, the significance of differences was tested by Mantel-Haenszel chi-square test. We evaluated potential interactions among variables through a series of stratified analyses before constructing models that included interaction terms. Factors that were significant at p<0.10 level were then examined in stepwise regression models. The final multivariable model was chosen from alternative models resulting from the stepwise regressions, including interaction terms when indicated.

Results

Hospital and Health-Center Surveillance

From April 6, 1997, to March 19, 1998, we screened 5,552 specimens from health-care facilities. Overall, *Cyclospora* was detected in 126 (2.3%) specimens. The rate of *Cyclospora* detected began to rise (3.8%) in May and peaked at 6.7% in June (Figure). This period corresponds to the first 2 months of the rainy season in Guatemala City. The prevalence remained above 3% in July and August and fell to undetectable levels after mid-November. In January and February, *Cyclospora* was detected in fewer than 1% of specimens.

Cyclospora infection was more frequent among children and among persons with gastroenteritis (Table 1). The prevalence of *Cyclospora* was 3.5% in specimens from children 1.5 to 4 years of age and 3.8% among children 5 to 9 years of age, irrespective of symptom status. Children in these age groups were five times more likely than adults screened in the surveillance system to have *Cyclospora* infection. Persons with gastroenteritis were two to three times more likely than asymptomatic persons of the same age to have *Cyclospora*.
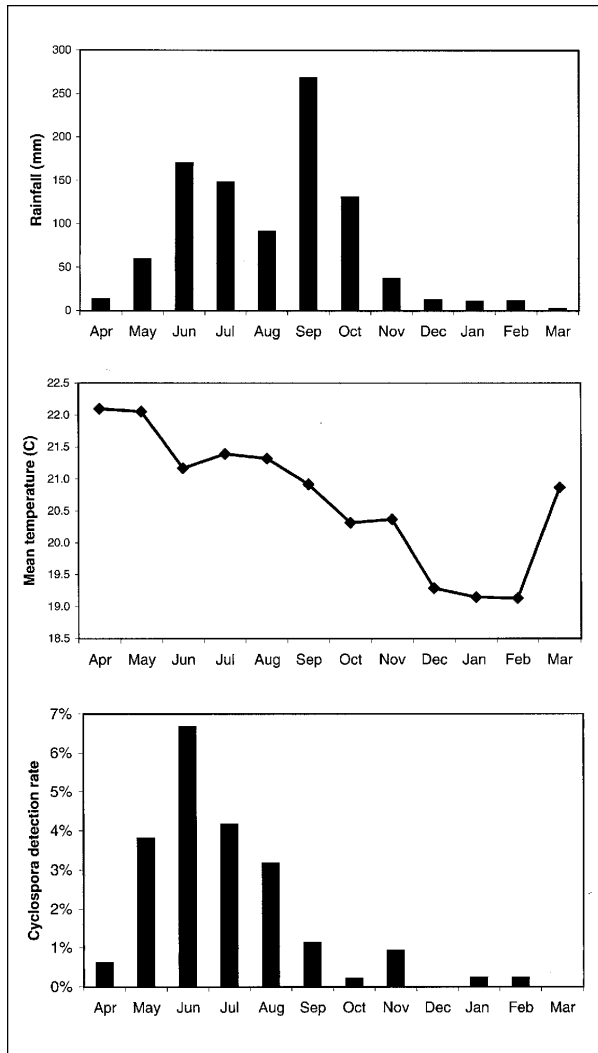
Figure. Surveillance for *Cyclospora cayetanensis* infection in stool specimens from three hospital outpatient departments and two health centers in Guatemala, April 6, 1997, to March 19, 1998. From the bottom, the three graphs demonstrate the *Cyclospora* detection rate, mean temperature in centigrade, and rainfall in mm by month. Median number of specimens per month 444 (324-638).

Cyclospora infection was relatively uncommon among children <18 months of age (1.0%). A substantial proportion of gastroenteritis cases during the early rainy season was associated with *Cyclospora* infection: from May 1 to June 30, 30 (19%) of 160 children 1.5 to 9 years old with gastroenteritis had *Cyclospora* detected in their stool. The overall rate of infection did not differ significantly by sex (44 [2.1%] of 2,067 male versus 57 [1.7%] of 3,266 female patients [p = 0.32]).

Although we did not collect data concerning HIV status, one hospital submitted specimens for screening from a clinic that served the HIV-infected population in Guatemala City. Of 32 specimens from adult patients attending this clinic, 9 (28%) were positive for *Cyclospora*, a much higher rate than that detected among other adults at the same hospital (2 [0.4%] of 448; RR 87, 95% CI 16.2, 847). Seasonality among these patients was the same as that observed for other participants in the surveillance system: eight of the nine *Cyclospora* detections occurred during May to August. These patients were not included in the case-control study.

Raspberry Farm Cohort

The raspberry farm cohort comprised 164 workers and 18 family members of workers who submitted specimens from April 6 to May 29, 1997. All 176 workers on the three farms agreed to participate; however, not all submitted specimens. We collected 269 specimens from workers and 31 from their family members, a median of one specimen per person (1 to 4). Six of 182 persons had an episode of infection with *Cyclospora*. Of the six infections, two occurred in family members, ages 1 and 10 years; both had gastroenteritis. The other four *Cyclospora* infections occurred in farm workers; all but one were asymptomatic. On May 29, 1997, the Guatemalan Berry Exporters Commission required us to suspend surveillance.

Case-Control Study

During the case-control study period, we recruited 69 persons with *Cyclospora* infection and 125 controls. Five controls were excluded because they had gastrointestinal symptoms when their specimens were submitted, leaving 69 cases and 120 controls for the analysis. Of 69 cases, 33 (48%) were in patients < 5 years of age and 26 (38%) were in male patients; among 120 controls, 56 (47%) were <5 years of age and 56 (47%) were male (p = 0.23).

In addition to specimens from the case-control study, we screened stool specimens from 182 family members belonging to 56 families of case-patients. Of these, 14 persons from 11 families tested positive for *Cyclospora*. The detection rate was highest among children 1.5 to 9 years (10 [11.6%] of 86). There were no cases among 14 family members <18 months of age and few among adults >14 years of age (2 [4%] of 57).

Table 1. Prevalence of *Cyclospora* in specimens from outpatients attending three hospitals and two health centers. April 6, 1997, to March 19, 1998

| Age (yrs) | Prevalence by age group | | Prevalence by presence of symptoms | | |
	n[a]/N[b] (%)	RR (95% CI)[c]	n/N (%) with gastroenteritis	n/N (%) without gastroenteritis	RR (95% CI)[d]
<1.5	10/844 (1.2)	1.7 (0.8, 3.8)	7/502 (1.4)	3/330 (0.9)	1.5 (0.4, 5.9)
1.5-4	41/1,160 (3.5)	5.1 (2.8, 9.1)	31/583 (5.3)	10/567 (1.8)	3.0 (1.5, 6.1)
5-9	31/813 (3.8)	5.5 (3.0, 10.1)	22/451 (4.9)	8/354 (2.3)	2.2 (1.0, 4.8)
10-14	4/328 (1.2)	1.8 (0.6, 5.2)	2/147 (1.4)	2/176 (1.1)	1.2 (0.2, 8.4)
>14	15/2150 (0.7)	Referent	8/958 (0.8)	7/1,181 (0.6)	1.4 (0.5, 3.9)
All ages	101/5,295[e] (1.9)	--	70/2,641[e] (2.7)	30/2,609[e] (1.1)	2.3 (1.5, 3.5)

[a]n represents the number positive for *Cyclospora*.
[b]N represents the number of specimens in each category.
[c]Each age group compared with patients >14 years of age.
[d]Specimens associated with gastroenteritis versus those without gastroenteritis.
[e]Apparent inconsistencies in denominator data are due to the following missing data and exclusions. Data for 32 patients of the clinic serving the HIV-infected population were excluded. Age and symptom data were missing for 187 specimens; symptom data only were missing for 45 specimens.

The rate among family members ages 1.5 to 9 years did not differ from that among children of the same age in surveillance data from the same period (64 [9%] of 714; p = 0.42).

Data were available on characteristics of illness in 62 patients in the case-control study (Table 2). The median duration of illness before diagnosis was 15 days in those <5 years of age and 10 days in those ≥5 years of age. Young

Table 2. Characteristics of illness reported by 62 persons with *Cyclospora* infection who participated in the case-control study, Guatemala, May 7- September 3, 1997

| Characteristic | Age group of participants | | |
	<5 years (n = 32)	>5 years (n = 30)	p
Days of illness	15 (3-76)[a]	10 (2-90)[a]	0.05[b]
Stools per day	5 (1-10)[a]	3 (1-20)[a]	0.04[b]
Stool consistency			ns[c]
Liquid/watery	22 (69)	18 (60)	
Semisolid	10 (31)	6 (20)	
Solid	0 (0)	4 (13)	
Blood in stool	7 (22)	1 (3)	0.05
Mucus in stool	29 (91)	11 (37)	<0.001
Fever	24 (75)	9 (30)	<0.001
Vomiting	16 (50)	8 (27)	ns
Abdominal pain	10 (31)	13 (43)	ns
Anorexia	8 (25)	7 (23)	ns
Headache	2 (6)	7 (23)	ns
Treated before visiting health-care facility with			
Any antibiotic	22 (69)	15 (50)	ns
Metronidazole	11 (34)	9 (30)	ns
Cotrimoxazole	1 (3)	4 (13)	ns
Mebendazole or albendazole	7 (22)	2 (7)	ns

[a]Median (range).
[b]By Wilcoxon 2-sample test.
[c]ns = not significant.

children also tended to have a higher median number of stools per day than older patients and were significantly more likely to have fever and mucoid stools. Of the 62 patients, 40 (65%) reported predominantly watery or liquid stools, but 33 (53%) reported that diarrhea was intermittent. Of the 62 for whom data were available, 37 (60%) had been treated with an antibiotic, most often metronidazole, before *Cyclospora* was diagnosed.

A number of variables were associated with risk for *Cyclospora* infection in univariate analyses; all were related to water, sanitation, or presence of animals in the household (Table 3). Persons with *Cyclospora* infection were significantly more likely than controls to report having drunk untreated water in the 2 weeks before illness, having obtained drinking water from a high-risk source, or having swum in or drunk water from a river or spring. In addition, having a septic tank rather than municipal sewage drainage and having had direct contact with soil were associated with an elevated risk for infection. Persons with *Cyclospora* infection were twice as likely as controls to own dogs, chickens, or other fowl; other animals such as cats and pigs were not associated with increased risk for infection. We asked about eating 16 kinds of fresh, uncooked produce in the 2 weeks before illness, including raspberries, blackberries, lettuce, cabbage, mint, and cilantro; none was associated with an elevated risk for *Cyclospora* infection.

Among children <5 years of age, maternal education was protective: 10 of 33 children with

Table 3. Univariate analysis of factors associated with risk for *Cyclospora* infection among 69 cases and 120 controls in Guatemala

| Characteristic | Proportion (n^a/N^b [%]) with characteristic | | OR (95% CI) |
	Cases	Controls	
High-risk water source[c]	18/69 (26)	15/120 (13)	2.5 (1.1, 5.9)
Drank untreated water[d,e]	62/68 (91)	88/120 (73)	3.8 (1.4, 10.8)
Drank river or spring water[e]	12/68 (18)	8/120 (7)	3.0 (1.1, 8.9)
Swam in river or spring[e]	10/68 (15)	6/120 (5)	3.3 (1.0, 11.5)
Contact with soil[e]	54/69 (78)	73/120 (61)	2.3 (1.1, 4.9)
Septic tank vs. municipal drainage	33/69 (49)	39/120 (33)	2.0 (1.0, 3.8)
Any animals in household	53/69 (77)	74/120 (62)	2.1 (1.0, 4.3)
Dog	38/69 (55)	47/120 (39)	1.9 (1.0, 3.7)
Chickens	35/69 (51)	40/120 (33)	2.1 (1.1, 4.0)
Other poultry or birds	22/69 (32)	20/120 (17)	2.3 (1.1, 5.0)
Any poultry or birds	40/69 (58)	49/120 (41)	2.0 (1.1, 3.6)

[a]n represents the number positive for *Cyclospora*.
[b]N represents the number of specimens in each category.
[c]High-risk water sources defined as public standpipe, well, spring, water truck. Low-risk defined as municipal water piped into house or commercial bottled water.
[d]Untreated water defined as water that was not commercially bottled and had not been boiled, chlorinated, or filtered before drinking.
[e]In the last 2 weeks.

cyclosporiasis (compared with 31 of 55 controls) had mothers with 6 or more years of education (OR 0.34, 95% CI 0.12, 0.92). Breastfeeding also had a protective effect, although not of statistical importance: 3 of 33 case patients were breastfed, compared with 13 of 52 controls (OR 0.30, 95% CI 0.05, 1.25).

In stepwise regression analyses, the variables that remained significant were drinking untreated water and, among young children, contact with soil (Table 4). Drinking untreated water was associated with a fourfold increase in risk. For contact with soil, there was a significant interaction with age, carrying a 20-fold increased risk for *Cyclospora* infection among children <2 years of age but not among those 2 years of age or older (p for interaction term = 0.03). The presence of chickens in the

household was associated with a twofold increased risk but did not reach statistical significance in the multivariable model (p = 0.054). The presence of chickens, poultry, or other birds in the household could be substituted for chickens in the model with similar results (OR 1.7; p = 0.09). The results of the model were unchanged when data were adjusted for socioeconomic variables, including educational level (for adult participants and of mother for children <15 years of age), housing type, and ownership of items such as radio, television, bicycle, or motor vehicle.

River Water Analysis

All three rivers had evidence of heavy fecal contamination throughout the period of study, with mean *E. coli* counts of 4.7×10^5, 1.9×10^7, and 4.5×10^6 colonies per 100 cc in the Department of Guatemala sampling sites and the Department of Sacatepequez site, respectively. We detected both sporulated and unsporulated *Cyclospora* oocysts in two of the 30 specimens, one specimen from each of the two rivers in the Department of Guatemala; both positive specimens were collected on May 26, 1997. The number of oocysts seen was small (2 oocysts and 3 oocysts, respectively). However, calculating from the amount of sediment obtained in each specimen (40 and 20 ml), the amount of sediment examined (20 and 40 µl), and the recovery rate of oocysts (5% to 10%; estimated from studies of seeded specimens, M. Alvarez de Mejia, M.B. Lopez,

Table 4. Multivariable logistic regression model of risk factors for *Cyclospora* infection among 68 cases and 120 controls

Characteristic	OR (95% CI)[a]	p
Drank untreated water	4.2 (1.4, 12.5)	0.009
Chickens	1.9 (1.0, 3.7)	0.054
Contact with soil among children <2 years old	19.8 (2.2, 182)	0.008
Contact with soil among persons ≥ 2 years old[b]	1.4 (0.6, 2.9)	ns[c]

[a]Model adjusted for age category (<2 years, ≥2 years).
[b]Odds ratio for persons 2 years or older calculated as (OR for persons <2) *(OR for interaction term for soil contact with age <2). p value for the interaction term = 0.03.
[c]ns = not significant.

unpub. results), we estimate that these findings may represent actual concentrations of 15,000 or more oocysts per 10-liter specimen.

Conclusions

This study advances our understanding both of the epidemiology of *C. cayetanensis* in a disease-endemic area and of the context in which repeated outbreaks of foodborne cyclosporiasis have occurred in North America. We now know that *Cyclospora* is commonly associated with pediatric gastroenteritis in Guatemala, as it is in Peru, Nepal, and other developing countries (1,2,5,21). Moreover, as in Peru and Nepal, the organism displays a marked seasonality. In Guatemala, the prevalence of *Cyclospora* in specimens submitted to health-care facilities increased around the time of the spring raspberry harvest in April-June (9). In June, we recorded weekly detection rates as high as 10%. In our surveillance data from May through June, 19% of cases of pediatric gastroenteritis were associated with *Cyclospora*. We were also able to detect oocysts in surface water specimens, despite the fact that the available methods for detection in water have very low sensitivity (22). By October, the detection rate in human surveillance specimens had dropped below 1%. These data suggest that the association of *Cyclospora* outbreaks with raspberries from the spring but not the fall harvest may be related to the inherent seasonality of the organism.

A major limitation of our study was our inability to test for other diarrheal pathogens. We cannot be certain that every gastroenteritis episode during which we detected *Cyclospora* was due to *Cyclospora*. However, in the aggregate data, we observed a consistent epidemiologic and clinical pattern associated with detections of *Cyclospora*.

Although 1 year of surveillance is insufficient to say with certainty what the pattern of *Cyclospora* infections will be from year to year, the data so far suggest that the seasonality of *Cyclospora* in Guatemala City is similar to that in Kathmandu, another subtropical city at approximately the same altitude (1,500 m) above sea level (3,23). In Guatemala as in Kathmandu, the rainy season starts in May or June, with warmer temperatures in the preceding months; in both places, *Cyclospora* appears around this time. Nevertheless, the organism's seasonality cannot be explained by rainfall alone, since in

Lima an equally marked pattern is seen in the near absence of rain (21). Several years of surveillance in multiple sites with varying climates, as well as a better understanding of the organism's biology in the environment, will be necessary to explain why *Cyclospora* infections apparently fluctuate with the seasons.

Cyclospora has a number of other remarkable characteristics. It tends to cause prolonged diarrhea (2,3,24). Because all our patients were treated once the diagnosis was made, we could not estimate a total duration of illness; nevertheless, among children <5 years of age, the median length of illness before diagnosis was 15 days. Among children with diarrhea in developing countries, the proportion of persistent episodes (>14 days long) is 3% to 23% depending on the study cited. These episodes, however, carry a higher risk for malnutrition and death than shorter episodes (25-27). Perhaps because of the long duration of illness, persons with *Cyclospora* gastroenteritis are likely to receive various antibiotics before the correct diagnosis is made: in our study, 70% of children <5 years of age had received at least one antibiotic, and a third of all our patients had received metronidazole. Prompt diagnosis would avert such inappropriate use of antibiotics.

In North America, patients have been infected with *Cyclospora* through eating fresh raspberries, pesto dishes, and mesclun lettuce (9,28-30). In Guatemala, the main vehicle of infection appears to be untreated water. That water truly is an important vehicle for *Cyclospora* in Guatemala is underlined by the other water-related factors that were associated with infection. Our results support the findings in other studies that contaminated water is a likely source of infection (1,3,31). However, multiple routes of transmission for *Cyclospora* in Guatemala almost certainly exist. Among very young children, soil contact was a strong risk factor; an outbreak investigation in the United States also raised the possibility that soil might be a potential source of infective oocysts (6). Family members of patients had a rate of infection similar to that of persons in the same age group screened in our surveillance system at the same time of year, a finding consistent with the postulated lack of direct person-to-person transmission.

The fact that the presence of chickens or other domestic fowl was a significant risk factor

is intriguing but difficult to interpret. Scientists have failed to establish experimental *C. cayetanensis* infection in any bird; a limited survey of 110 wild birds captured in May 1997 in Guatemala did not demonstrate natural infection, although other coccidia were observed (M. Eberhard, pers. comm.). Although the association remains when the data are adjusted for socioeconomic status, we cannot rule out the possibility that ownership of fowl is a marker for some other unidentified factor. The role of birds in *Cyclospora* transmission merits further investigation.

C. cayetanensis is a pathogen commonly associated with pediatric gastroenteritis in Guatemala, especially from May through August. The seasonality of *Cyclospora* in Guatemala follows a pattern similar to that seen in Kathmandu. Our case-control analysis suggests that contaminated water and, for young children, soil, are likely vehicles of transmission. More sensitive diagnostic tools are urgently needed to help establish how fresh produce becomes contaminated.

Acknowledgments

Dr. Bern is a medical epidemiologist in the Division of Parasitic Diseases, CDC. Her research interests include the epidemiology of the enteric parasites *Cyclospora*, *Cryptosporidium*, and microsporidia.

References

1. Hoge CW, Echeverria P, Rajah R, Jacobs J, Malthouse S, Chapman E, et al. Prevalence of *Cyclospora* species and other enteric pathogens among children less than 5 years of age in Nepal. J Clin Microbiol 1995;33:3058-60.

2. Ortega YR, Sterling CR, Gilman RH, Cama VA, Diaz F. *Cyclospora* species—a new protozoan pathogen of humans. N Engl J Med 1993;328:1308-12.

3. Hoge CW, Shlim DR, Rajah R, Triplett J, Shear M, Rabold JG, et al. Epidemiology of diarrhoeal illness associated with coccidian-like organisms among travellers and foreign residents in Nepal. Lancet 1993;341:1175-9.

4. Soave R. *Cyclospora*: an overview. Clin Infect Dis 1996;23:429-37.

5. Soave R, Herwaldt BL, Relman DA. *Cyclospora*. In: Hughes JM, Conte JE, editors. Infectious disease clinics of North America. Vol 12, No. 1. Philadelphia: W.B. Saunders Company; 1998.

6. Koumans EHA, Katz DJ, Malecki JM, Kumar S, Wahlquist SP, Arrowood MJ, et al. An outbreak of cyclosporiasis in Florida 1995: a harbinger of multistate outbreaks in 1996 and 1997. Am J Trop Med Hyg 1998;59:235-42.

7. Huang P, Weber JT, Sosin DM, Griffin PM, Long EG, Murphy JJ, et al. The first reported outbreak of diarrheal illness associated with *Cyclospora* in the United States. Ann Intern Med 1995;123:409-14.

8. Carter R, Guido F, Jacquette G, Rapoport M. Outbreak of cyclosporiasis associated with drinking water. Proceeding of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1996 Sep 15-18; New Orleans, Louisiana. Washington: American Society for Microbiology, 1996.

9. Herwaldt B, Ackers ML, the Cyclospora Working Group. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. N Engl J Med 1997;336:1548-56.

10. Herwaldt BL, Beach MJ, the Cyclospora Working Group. The return of *Cyclospora* in 1997: another outbreak of cyclosporiasis in North America associated with imported raspberries. Ann Intern Med 1999;130:210-20.

11. Osterholm MT. Cyclosporiasis and raspberries—lessons for the future [editorial]. N Engl J Med 1997;336:1597-8.

12. Ortega YR, Roxas CR, Gilman RH, Miller NJ, Cabrera L, Taquiri C, et al. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. Am J Trop Med Hyg 1997;57:683-6.

13. Pratdesaba RA, Velaquez T, Torres MF. Occurrence of *Isospora belli* and cyanobacterium-like bodies in Guatemala. Ann Trop Med Parasitol 1994;88:449-50.

14. Arathoon E, Velasquez T, Estrada y Martin RM, Mayorga R. *Cyclospora cayetanensis*, un nuevo patógeno causante de diarrea en pacientes infectados por el VIH. Revista del Colegio de Médicos y Cirujanos de Guatemala 1994;4:36-7.

15. Merida SC. Prevalencia de *Cyclospora* en niños menores de 60 meses. Doctor of Medicine Thesis, School of Medicine, Universidad Francisco Marroquin, Guatemala City; 1998, 59 pages.

16. Cuellar NS. Prevalencia de infecciones intestinales causadas por coccidios: *Cryptosporidium* spp, *Cyclospora cayetanensis*, e *Isospora belli* en pacientes con SIDA. Masters Thesis, Faculty of Laboratory Sciences and Pharmacy, Universidad de San Carlos de Guatemala, Guatemala City, 1997, 71 pages.

17. Alvarado KM. *Cyclospora cayetanensis* como agente causal de diarrea en pacientes de la consulta externa del Hospital General San Juan de Dios. Masters Thesis, Faculty of Laboratory Sciences and Pharmacy, Universidad de San Carlos de Guatemala, Guatemala City, 1997, 41 pages.
18. Eberhard ML, Pieniazek NJ, Arrowood MJ. Laboratory diagnosis of *Cyclospora* infections. Arch Pathol Lab Med 1997;121:792-7.
19. Environmental Protection Agency. Test Methods for *Escherichia coli* and enterococci in water by the membrane filter procedure. Manual EPA-600/4-85/076: Environmental Monitoring and Support Laboratory, 1985.
20. Vesey G, Slade JS, Byrne M, Shepherd K, Fricker CR. A new method for the concentration of *Cryptosporidium* oocysts from water. J Appl Bacteriol 1993;75:82-6.
21. Madico G, McDonald J, Gilman RH, Cabrera L, Sterling CR. Epidemiology and treatment of *Cyclospora cayetanensis* infection in Peruvian children. Clin Infect Dis 1997;24:977-81.
22. Sturbaum GD, Ortega YR, Gilman RH, Sterling CR, Cabrera L, Klein DA. Detection of *Cyclospora cayetanensis* in wastewater. Appl Environ Microbiol 1998;64:2284-6.
23. Connor BA, Shlim DR, Scholes JV, Rayburn JL, Reidy J, Rajah R. Pathologic changes in the small bowel in nine patients with diarrhea associated with a coccidia-like body. Ann Intern Med 1993;119:377-82.
24. Baqui AH, Sack RB, Black RE, Haider K, Hossain A, Alim ARMA, et al. Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children under 5 years of age. J Infect Dis 1992;166:792-6.
25. Lima AAM, Fang G, Schorling JB, de Albuquerque L, McAuliffe JF, Mota S, et al. Persistent diarrhea in Northeast Brazil: etiologies and interactions with malnutrition. Acta Paediatr Supple 1992;381:39-44.
26. Black RE. Persistent diarrhea in children of developing countries. Pediatr Infect Dis J 1993;12:751-61.
27. Cruz JR, Bartlett AV, Mendez H, Sibrian R. Epidemiology of persistent diarrhea among Guatemalan rural children. Acta Paediatr Suppl 1992;381:22-6.
28. Centers for Disease Control and Prevention. Update: Outbreaks of cyclosporiasis—United States and Canada, 1997. MMWR Morb Mortal Wkly Rep 1997;46:521-2.
29. Centers for Disease Control and Prevention. Update: Outbreaks of cyclosporiasis—United States, 1997. MMWR Morb Mortal Wkly Rep 1997;46:461-2.
30. Centers for Disease Control and Prevention. Outbreaks of cyclosporiasis—United States, 1997. MMWR Morb Mortal Wkly Rep 1997;46:451-2.
31. Rabold JG, Hoge CW, Shlim DR, Kefford C, Rajah R, Echeverria P. *Cyclospora* outbreak associated with chlorinated drinking water [letter]. Lancet 1994;344:1360.

Serologic Evidence of Human Monocytic and Granulocytic Ehrlichiosis in Israel

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We conducted a retrospective serosurvey of 1,000 persons in Israel who had fever of undetermined cause to look for *Ehrlichia chaffeensis* antibodies. Four of five cases with antibodies reactive to *E. chaffeensis* were diagnosed in the summer, when ticks are more active. All patients had influenzalike symptoms with high fever. None of the cases was fatal. Three serum samples were also seroreactive for antibodies to *E. canis,* and one was also reactive to the human granulocytic ehrlichiosis (HGE) agent. The titer to the HGE agent in this patient was higher than the serum titer to *E. chaffeensis,* and the Western blot analysis also indicated that the HGE agent was the primary cause of infection. We present the first serologic evidence that the agents of human monocytic ehrlichiosis (HME) and HGE are present in Israel. Therefore, human ehrlichiosis should be included in the differential diagnoses for persons in Israel who have been exposed to ticks and have influenzalike symptoms.

Human ehrlichiosis (HME) and human granulocytic ehrlichiosis (HGE), two emerging infectious diseases transmitted by ticks, are caused by *Ehrlichia chaffeensis* and the HGE agent of the *E. phagocytophila* genogroup, respectively. In the United States, HME and HGE were first described in 1987 and 1994, respectively (1). Since then, seroepidemologic studies have shown that these infections are also present in other parts of the world.

The first cases of HME and HGE were reported in Europe in 1991 and 1995, respectively (2,3). Serologic evidence of HGE has been found in Norway and Sweden (4). In South America, a case of *E. canis* infection was reported in Venezuela (5). One clinical case of HME has been reported in Mali, Africa (6). A serosurvey for HME of 756 patients from eight African countries suggested that the disease is rare in Africa (7).

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We describe the first serologic survey in Israel for HME and HGE, which documents the detection of antibodies reactive with HME and HGE agents.

Materials and Methods

Sera

One thousand serum samples from patients in Israel with fever of undetermined cause from 1994 to 1997 were received by the Israel National Reference Laboratory for Rickettsial Diseases. All specimens were serologically negative for Mediterranean spotted fever, murine typhus, and Q fever.

Serology

The sera were tested retrospectively for immunoglobulin (Ig) G antibodies to *E. chaffeensis* and *E. canis* by indirect immunofluorescence antibody (IFA) (8). Briefly, DH82 cells heavily infected with the Israeli strain of *E. canis* (#611) (8) or the Arkansas strain of *E. chaffeensis* were pelleted and resuspended in growth medium.

Five microliters of the suspension were placed in each well of eight-well teflon-coated slides. The slides were dried at room temperature for approximately 30 minutes, fixed in acetone for 15 minutes, and then stored at 40°C. Serum samples were assayed for IgG by preparing and testing serum dilutions in PBS at their cutoff points of 1:64 for *E. chaffeensis* and 1:40 for *E. canis*. Positive sera were subsequently assayed at twofold dilutions. Positive control sera were provided by the Centers for Disease Control and Prevention (CDC).

Serum samples were sent to CDC for confirmation of results of the HME titers and for testing for HGE.

Western Blot Analysis

One patient (#3) sample found positive for HGE was tested by Western blot at Johns Hopkins University, Baltimore, Maryland.

Results

Of the 1,000 sera tested, five were found seropositive with HME (Table). During validation of the sera for HME antibodies, the CDC laboratory also found that one patient (#3) had an antibody titer of 1:2,048 to HGE. This sample was confirmed positive for HGE by Western blot.

None of the patients documented in this study had traveled overseas before their illness. All five cases occurred in persons, three male and two female, who lived on the coastal plain. Two of the three men lived in agricultural settlements. The average age of patients was 34.2 years (8 to 77 years). Four of the five patients were ill during summer.

The disease lasted up to 14 days. None of the cases was fatal. None of the patients reported being bitten by a tick. All patients had fever from 38.5°C to 40.2°C. Clinical signs were inconsistent: macular rash was present in only three patients and lymphomegaly in two. Four patients were leukopenic, and two were also thrombocytopenic. No changes in liver enzymes were detected in any of the patients.

The antibody titers to *E. chaffeensis* were 1:128 to 1:1,024. Similar results were obtained by CDC. Three of the five sera were also seropositive for *E. canis* antibodies; however, their titers were lower than those to *E. chaffeensis*.

Table. Clinical and serologic data for patients in Israel with antibodies to *Ehrlichia chaffeensis* and the human granulocytic ehrlichiosis agent

Clinical and serologic data	Patients				
	1	2	3	4	5
Date	7/94	7/94	8/95	2/97	7/97
Sex	F	F	M	M	M
Age (years)	77	8	22	12	52
IFA titer (HME)	1:128	1:256	1:1,024	1:256	1:256
IFA titer *E. canis*	1:80	1:80	1:640	<1:40	<1:40
IFA titer (HGE)	<1:64	<1:64	1:2,048[a]	<1:64	<1:64
Body temperature (°C)	40	39.2	40.2	40	38.5
Symptoms:					
Vomiting	-	+	-	-	
Headache		+		++	
Chills	+		+		
Macular rash	+			+	+
Lymphomegaly		+	+		
Neck pain	-	+	-	-	-
Duration (days)	7	>5	12	14	14
Tetracycline therapy	+	+		+	-
Total leukocyte count/µl[b]	3,700	4,000	3,500	4,000	16,700
Total platelet count/µl[c]	86,000	272,000	95,000	177,000	318,000

[a]Western blot analysis of the serum proved positive for HGE.
[b]Normal range for total leukocyte count 4,000-10,000/µl.
HGE, human granulocytic ehrlichiosis; HME, human monocytic ehrlichiosis; IFA, immunofluorescence assay.
[c]Normal range for total platelet count 150,000-450,000/µl.

Conclusions

We retrospectively looked for *E. chaffeensis* antibodies in human patients with fever of undetermined cause. Four cases of HME were found, as well as a possible case of HGE with cross-reacting antibodies to HME.

Four of the five cases were diagnosed in summer, during peak tick activity. Patients' ages were 8 to 77 years. Symptoms were nonspecific, as has been described (1). All patients had influenzalike symptoms with high fever. Leukopenia was seen in four patients and thrombocytopenia in two; both these hematologic changes are typical of HME.

In our study three sera positive for *E. chaffeensis* were also seropositive for *E. canis*, unlike the African study in which all *E. chaffeensis*-positive sera were seronegative to *E. canis* in spite of the known strong cross-reactivity between the strains (7). The reason for this lack of cross-reactivity is unknown; however, reactivity in *E. chaffeensis* patients to *E. canis* antigens may develop only after prolonged exposure to the *Ehrlichia*, allowing expression of common antigens to be revealed.

In one seropositive *E. chaffeensis* case (#3), the titer to the HGE agent was higher than to *E. chaffeensis* and Western blot analysis for HGE was positive, which indicates that the HGE agent was the primary cause of infection. Serologic reactions with *E. chaffeensis* have been demonstrated after HGE infection (9). Cross-reaction between the two species of *Erhlichia* has been found in a small proportion of all HGE patients tested, which suggests that the causative *Ehrlichiae* share antigenic determinants.

Several tick species of the genus *Ixodes* are found in Israel, including the *I. ricinus*, which is the vector of the disease in Europe, and *I. redikorzevi,* which often bites humans (10,11). *Rhipicephalus sanguineus,* which is abundant in Israel, is a potential vector of HME agent. Coinfection by a number of tickborne diseases is not uncommon, and persons may be infected by both HME and HGE agents simultaneously.

A recent serosurvey of jackals in Israel tested against *E. canis, E. chaffeensis,* and *E. phagocytophila* genogroup antigens has shown that some jackals were seroreactive only to the *E. phagocytophila* genogroup antigen (12). The latter group of *Ehrlichia* consists of *E. equi, E. phagocytophila,* and the HGE agent (13). A close serologic and genetic relationship has been shown to exist among these three members, suggesting that they may be strains of a single species (1). The finding in jackals adds further evidence of one or more of the *E. phagocytophila* genogroup of *Ehrlichiae* in Israel.

In conclusion, we have presented the first serologic evidence that the agents of HME and HGE are present in Israel. Human ehrlichiosis should therefore be included in the differential diagnoses for persons in Israel who have been exposed to ticks and have influenzalike symptoms.

References

1. Walker DH, Babour AG, Oliver JH, Lane RS, Dumler JS, Dennis DT, et al. Emerging bacterial zoonotic and vector-borne diseases. JAMA 1996;275:463-9.
2. Morais JD, Dawson JE, Greene C, Filipe AR, Galhardas LC, Bacellar F. First European case of ehrlichiosis [letter]. Lancet 1991;338:633-4.
3. Brouqui P, Dumler JS, Leinhard R, Brossard M, Raoult D. Human granulocytic ehrlichiosis in Europe. Lancet 1995;346:782-3.
4. Bakken JS, Krueth J, Tilden RL, Dumler JS, Kristiansen BE. Serological evidence of human granulocytic ehrlichiosis in Norway. Eur J Clin Microbiol Infect Dis 1996;15:829-32.
5. Arraga-Alvarado C, Montero-Ojeda M, Bernardoni A, Parra 0. Human ehrlichiosis: report of the first case in Venezuela. Invest Clin 1996;37:35-49.
6. Uhaa IJ, MacLean JD, Greene CR, Fishbein DB. A case of human ehrlichiosis acquired in Mali: clinical and laboratory findings. Am J Trop Med Hyg 1992;46:161-4.
7. Brouqui P, Lecam C, Kelly PJ, Laurens R, Tounkara A, Sawadogo S, et al. Serologic evidence for human ehrlichiosis in Africa. Eur J Epidemiol 1994;10:695-8.
8. Keysary A, Waner T, Rozner M, Dawson JE, Zass R, Warner CK, et al. The first isolation, in vitro propagation, and genetic characterization of *Ehrlichia canis* in Israel. Vet Parasitol 1996;62:331-40.
9. Dumler JS, Brouqui P. Human granulocytic ehrlichiosis. In: Anderson B, Friedman H, Bendinelli M, editors. Rickettsial infection and immunity. New York and London: Plenum Press; 1997. p. 149-61.
10. Reed KD, Mitchell PD, Persing DH, Cameron V. Transmission of human granulocytic ehrlichiosis. JAMA 1995;273:23.
11. Theodor O, Costa M. In: Ectoparasites. A survey of the parasites of wild mammals and birds in Israel. Part 1. Jerusalem: The Israel Academy of Sciences and Humanities; 1997. p. 92-103.

12. Waner T, Beneth G, Strenger C, Keysary A, King R, Harrus S. Antibodies reactive with *Ehrlichia canis, Ehrlichia phagocytophila* genogroup antigens and the spotted fever group antigens, in free-ranging jackals (*Canis aureus syriacus)* from Israel. Vet Parasitol 1999;82:121-8.

13. Dumler JS, Asanovich KM, Bakken JS, Richter P, Kimsey R, Madigan JE. Serologic cross-reactions among *Ehrlichia equi, Ehrlichia phagocytophila,* and human granulocytic ehrlichia. J Clin Microbiol 1995;33:1098-103.

Research

Supplementing Tuberculosis Surveillance with Automated Data from Health Maintenance Organizations

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Data collected by health maintenance organizations (HMOs), which provide care for an increasing number of persons with tuberculosis (TB), may be used to complement traditional TB surveillance. We evaluated the ability of HMO-based surveillance to contribute to overall TB reporting through the use of routinely collected automated data for approximately 350,000 HMO members. During approximately 1.5 million person-years, 45 incident cases were identified in either HMO or public health department records. Eight (18%) confirmed cases had not been identified by the public health department. The most useful screening criterion (sensitivity of 89% and predictive value positive of 30%) was dispensing of two or more TB drugs. Pharmacy dispensing information routinely collected by many HMOs appears to be a useful adjunct to traditional TB surveillance, particularly for identifying cases without positive microbiologic results that may be missed by traditional public health surveillance methods.

As more persons move into managed health-care organizations, traditional tuberculosis (TB) surveillance methods, which rely heavily on information collected and channeled through the public health system, may need to be supplemented. Accurate, complete surveillance information is important for identification and follow-up of persons with TB, as well as for accurate assessment of the impact of TB on public health, the effectiveness of control activities, and the planning and prioritizing of interventions.

Automated data routinely collected by managed care organizations may complement TB surveillance obtained through reporting to local and state health departments. In this study, we evaluated the use of pharmacy dispensing information and other inpatient and ambulatory-patient data routinely collected by managed care organizations for identifying TB cases.

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Methods

Study Population

The study population consisted of approximately 350,000 persons with pharmacy coverage who received their care at one of the 14 Harvard Pilgrim Health Care centers with automated full-text medical records for ambulatory patients and 100,000 persons with pharmacy coverage at 17 practices without such records within Massachusetts from January 1, 1992, to June 30, 1996. Automated pharmacy and billing data, however, were available for the entire study population.

Identification of TB Cases from HMO Records

Ambulatory care, hospital, and emergency room claims for the entire study population were screened for any of 60 International Classification of Diseases,, 9th Revision Clinical Modification diagnosis codes or current procedures terminology codes suggestive of TB. Automated

pharmacy records were searched for dispensing of any of 10 antituberculosis medications during the study period (Table 1). The automated ambulatory-patient record, available for approximately 250,000 persons within our study population, has been described in detail (1). The automated medical record system uses standardized forms that are completed for every patient encounter at specific Harvard Pilgrim Health Care centers, including telephone calls, office visits, urgent care visits, and hospitalizations. For each encounter, the provider either writes in or selects from a list of all coded diagnoses, tests, procedures, and prescriptions and enters additional comments as free text. The automated ambulatory-patient records were also screened

Table 1. Components of the health maintenance organization-based screening criteria for tuberculosis (TB)

Code type	Code	Description of code
Antituberculosis drugs		
Pharmacy dispensing		Isoniazid
Pharmacy dispensing		Ethambutol
Pharmacy dispensing		Rifampin
Pharmacy dispensing		Pyrazinamide
Pharmacy dispensing		Streptomycin
Pharmacy dispensing		Para-aminosalicyclic acid (PAS)
Pharmacy dispensing		Kanamycin
Pharmacy dispensing		Capreomycin
Pharmacy dispensing		Cycloserine
Pharmacy dispensing		Ethionamide
Microbiology codes		
CPT[a]	87015	Concentration (any type) for parasites, ova, or tubercle bacillus (TB, AFB)
CPT	87116	Culture, tubercle, or other acid-fast bacilli; any source, isolation only
CPT	87117	Culture, tubercle, or other acid-fast bacilli; concentration plus isolation
CPT	87118	Culture, mycobacteria, definite identification of each organism
CPT	87190	Sensitivity studies, antibiotic; tubercle bacillus (TB, AFB), each drug
CPT	87206	Smear, primary source, with interpretation; fluorescent or acid-fast stain for bacteria, fungi, or cell types
ICD-9[b] procedure	90.4	Microscopy examination of sputum
ICD-9 procedure	90.41	Bacterial smear
ICD-9 procedure	90.42	Culture
ICD-9 procedure	90.43	Culture and sensitivity
ICD-9 procedure	90.49	Other microscopic examination
COSTAR[c]	TB234	AFB smear
COSTAR	TB850	AFB culture and sensitivity
Radiology codes		
CPT	71010	Chest, single view, frontal
CPT	71020	Chest, two views, frontal and lateral
CPT	71021	Chest with apical lordotic procedure
CPT	71030	Chest, complete, minimum of four views
CPT	71250	CT, thorax, without contrast
CPT	71260	CT, thorax, with contrast
CPT	71270	CT, thorax, without contrast, followed by contrast
CPT	71550	MRI[d] chest
CPT	71555	MRI chest (excluding myocardium)
ICD-9 procedure	87.44	Chest X-ray
COSTAR	TR027	Chest, PA[e] only
COSTAR	TR028	Chest X-ray
COSTAR	TR029	Chest, PA, and last with fluoroscopy
COSTAR	TR032	Chest, fluoroscopy
COSTAR	TR178	MRI-chest
COSTAR	TR184	CAT[f] scan-chest
COSTAR	TR236	Chest, PA, and lateral
COSTAR	TR237	Chest-PA, lateral, both obliques
COSTAR	TR238	Chest-four views
COSTAR	TR240	Chest-special views

[a]CPT, current procedures terminology.
[b]ICD9, International Classification of Diseases, 9th revision.
[c]COSTAR, coding system used for the automated ambulatory-patient medical records (10).
[d]MRI, magnetic resonance imaging.
[e]PA, posteroanterior.
[f]CAT, computer-assisted tomography.
[g]PPD, purified protein derivative of tuberculin.

Table 1, cont'd. Components of the health maintenance organization-based screening criteria for tuberculosis (TB)

Code type	Code	Description of code
PPD[g] status		
COSTAR	DG249	Positive PPD
COSTAR	DA129	Tuberculin conversion
Diagnosis codes for TB		
COSTAR	DR185	TB
COSTAR	DG250	Pulmonary TB
COSTAR	DG251	Active TB
ICD-9 diagnosis	010.0	Primary TB infection
	010.1	
	010.8	
ICD-9 diagnosis	011.0	Pulmonary TB
	011.1	
	011.2	
	011.3	
	011.5	
	011.6	
	011.8	
	011.9,	
	011.90-011.96	
ICD-9 diagnosis	012.0	Other respiratory TB
	012.1	
	012.2	
ICD-9 diagnosis	013.0	TB of meninges and central nervous system
	013.1	
	013.2	
	013.3	
	013.4	
	013.5	
ICD-9 diagnosis	015.0	TB of bones and joints
	015.7	
	015.8	
	015.9	
ICD-9 diagnosis	016.0	TB of genitourinary system
	016.3	
ICD-9 diagnosis	017.2	TB of peripheral lymph nodes
ICD-9 diagnosis	018.0	Miliary TB
	018.8	
	018.9	
ICD-9 diagnosis	795.3	Sputum positive only
Bronchoscopy and biopsy		
ICD-9 procedure	33.22-33.24	Diagnostic procedures on lung and bronchus
	33.26-33.28	Biopsy of lymphatic structure
	40.11	

[a]CPT, current procedures terminology.
[b]ICD9, International Classification of Diseases, 9th revision.
[c]COSTAR, coding system used for the automated ambulatory-patient medical records (10).
[d]MRI, magnetic resonance imaging.
[e]PA, posteroanterior.
[f]CAT, computer-assisted tomography.
[g]PPD, purified protein derivative of tuberculin.

for any one of 17 coded diagnoses, tests, and procedures suggestive of TB (Table 1).

Twelve combinations of screening codes suggestive of active TB were used for automated ambulatory-patient records, and five combinations of screening codes were used for other records (Table 2). To limit the number of persons meeting screening criteria, we focused on combinations of codes likely to have the highest yield of TB cases. Cases that met any of these screening criteria were assessed further.

Full-text ambulatory-patient medical records were reviewed for all persons identified by screening criteria who had automated ambulatory records. For individuals identified through screening who did not have automated ambulatory records, a modified version of the Centers for Disease Control and Prevention's

Table 2. Performance of health maintenance organization-based screening criteria for tuberculosis (TB)

Screening criteria	No. meeting screening criteria	No. TB cases detected using screening criteria	No. TB cases unknown to public health dept.	Sensitivity (95% CI)	Positive predictive value (95% CI)
All patients (45 incident TB cases)					
Two or more anti-TB drugs[a]	133	40	7	89 (76,96)	30 (22, 39)
Two or more anti-TB drugs[a] dispensed on the same date	108	39	7	87 (73,95)	36 (27,50)
Three or more anti-TB drugs[a]	76	38	7	84 (71,94)	50 (38,62)
Only patients with automated medical records (41 incident TB cases)					
One or more anti-TB drugs,[a] a microbiology code,[b] and a radiology code[c]	132	21	2	51 (35, 67)	16 (10, 23)
At least one anti-TB drug[a] and a CPT[c] code for mycobacterial culture/stain	106	17	2	42 (26,58)	16 (10,24)
Diagnosis code[d] for tuberculosis, a microbiology code,[b] and a radiology code[c]	49	16	0	39 (24,56)	33 (20,48)
Diagnosis code[d] for positive PPD,[e] a microbiology code,[b] and a radiology code[c]	157	8	1	20 (9,35)	5 (2,10)
At least one anti-TB drug[a] and an ICD-9 diagnosis code for tuberculosis	14	7	1	17 (7,32)	50 (23,77)
ICD-9 procedure code for bronchoscopy, a microbiology code,[b] and a radiology code[c]	15	1	0	2 (0.1,13)	7 (0.2,32)
Diagnosis code[d] for active tuberculosis	4	1	0	2 (0.1,13)	25 (1,81)
Diagnosis code[d] for pulmonary tuberculosis	75	0	0	0	0
Diagnosis code[d] for tuberculin conversion, a microbiology code,[b] and a radiology code[c]	1	0	0	0	0
Only patients without automated medical records (4 incident TB cases)					
ICD-9 diagnosis code for tuberculosis	251	4	2	100 (40, 100)	2 (0.4, 40)
A CPT code relating to mycobacterial culture/stain or a radiology code	92	2	1	50 (7, 93)	2 (0.3, 8)

[a]Pharmacy dispensing data; antituberculosis drugs include isoniazid, rifampin, pyrazinamide, ethambutol, streptomycin, capreomycin, kanamycin, ethionamide, para-aminosalicylic acid, and cycloserine.
[b]Microbiology codes include COSTAR (coding system for automated ambulatory-patient records [10]) or ICD-9CM (International Classification of Diseases, 9th Revision Clinical Modification) procedure codes for acid fast bacilli smear, culture and sensitivities and microscopy examination of sputum.
[c]Radiology codes include current procedures terminology (CPT), COSTAR, or ICD-9 procedure codes for chest radiograph, thoracic computer assisted tomography (CT), or thoracic magnetic resonance imaging (MRI).
[d]Ambulatory codes were obtained from automated ambulatory-patient records in the staff model division and from claims in the network and group model division.
[e]PPD, purified protein derivative.

(CDC) Report of Verified Case of Tuberculosis form was sent to the primary-care physicians. The form is routinely used to report to CDC individual TB case information, including clinical characteristics and laboratory results. Our modified form included the question "While under your care, did this patient have suspected and/or confirmed ACTIVE tuberculosis?" If "Yes" was checked, the full-text medical records of the person were reviewed. In addition, the medical records of a random sample of 10% of the patients with questionnaires returned by providers were reviewed to validate the use of data obtained from questionnaire results. A case of TB was defined according to the CDC surveillance definition (2). A culture-positive case is defined as isolation of *Mycobacterium tuberculosis* from a clinical specimen. A smear-positive case is defined as demonstration of acid-fast bacilli (AFB) in a specimen if either a culture was not obtained or results were unknown. In the absence of laboratory evidence of disease, a clinical case is one that meets the following criteria: a positive tuberculin skin test, a completed diagnostic work-up, clinical evidence and signs and symptoms compatible with TB, an abnormal and unstable (worsening or improving) chest radiograph if intrathoracic disease is present, and treatment with two or more antituberculosis drugs. All cases without a positive culture for *M. tuberculosis* that were not known to the public health department were verified by review with the Massachusetts State Tuberculosis Control Officer, using all available primary patient data from the ambulatory-patient medical record, public health records, and hospital records.

Identification of TB Cases from Public Health Department Records

Reporting of confirmed or clinically suspected TB cases to the Massachusetts Department of Public Health by health-care providers, laboratories, boards of health, or administrators of hospitals is mandatory. In addition, the Massachusetts State Laboratory Institute performs susceptibility testing on most *M. tuberculosis* isolates in Massachusetts and provides the public health department with direct access to microbiology information about virtually all persons in Massachusetts with culture-positive *M. tuberculosis*. All verified cases are entered into the public health TB registry.

The entire HMO population was matched to the public health TB registry by using limited patient identifiers (first two letters of last name, first two letters of first name, month and year of birth, and sex) to maintain patient confidentiality. Potential matches were confirmed by using full identifiers. This method for matching registries with minimal disclosure of individual identities is described elsewhere (3).

Analysis

The sensitivity, defined as the proportion of TB cases detected by either HMO-based screening criteria or routine public health surveillance, was determined by comparison with any verified TB case identified through public health or HMO records. Positive predictive value was defined as the proportion of persons with verified TB meeting screening criteria. Exact binomial confidence intervals were calculated for sensitivity and positive predictive value (4). The performance of the different screening rules for detecting TB was compared.

Results

In approximately 1.5 million person-years, 768 persons met at least one of the HMO-based screening criteria, with a positive screening criteria rate of 0.4 per 10,000 person-years among persons with automated ambulatory-patient records and 0.7 per 10,000 person-years among those without such records. Thirty-nine (9%) incident TB cases were identified among the 415 persons with automated ambulatory-patient records who met screening criteria, and 4 (1%) incident TB cases were identified among the 353 persons without automated ambulatory records who met screening criteria. The response rate to the provider questionnaire was 100%, as was the agreement rate between classification of TB cases based on provider questionnaire results and on-site medical record review.

Thirty-five (81%) of the 43 incident TB cases detected by HMO-based screening had been identified previously by the public health department. Of these 35 cases, 32 were culture-positive, and three met the clinical case definition. Two additional TB cases, both of which were culture-positive, were known to the public health department but did not meet HMO-based screening criteria. These two patients

received treatment and medication from state-funded TB clinics. Thus, 45 cases were identified through either HMO-based screening or public health department records. All 45 cases met the CDC surveillance definition. Eight (18%) of these cases were unknown to the public health system. Most cases (41 of 45) were diagnosed at one of the HMO centers with automated ambulatory-patient records, a proportion consistent with the concentration of urban regions within their catchment areas. The rates were approximately 11.7 TB cases per 100,000 population among HMO members with automated ambulatory-patient records and four TB cases per 100,000 population among those without such records.

The sensitivity of each of the screening criteria was 0% to 100%, and the positive predictive value was 0% to 52% (Table 2). Screening criteria based on pharmacy dispensing information had the best combinations of sensitivity and positive predictive value. Two or more dispensed antituberculosis drugs, combining the results for persons with and without automated ambulatory-patient records, had an overall sensitivity of 89% (95% confidence interval [CI] = 76%, 96%) and positive predictive value of 30% (95% CI = 22%, 39%). Three or more antituberculosis drugs had an overall sensitivity of 84% (95% CI = 71%, 94%) and positive predictive value of 50% (95% CI = 38%, 62%), and two or more antituberculosis drugs dispensed on the same date had an overall sensitivity of 87% (95% CI = 73%, 95%) and positive predictive value of 36% (95% CI = 27%, 50%). The differences between the performance of two or more dispensed antituberculosis drugs among persons with automated ambulatory-patient records (sensitivity = 90%, positive predictive value = 34%) and persons without automated records (sensitivity = 75%, positive predictive value = 12%) were not statistically significant, although the small number of TB cases in each group limits the power to detect a difference.

Among the 71 persons with automated ambulatory-patient records who received two or more antituberculosis drugs but did not have incident TB, 9 (13%) had active TB diagnosed outside the study period, 23 (32%) were treated for other mycobacterial infections, 11 (15%) received more than one drug during TB prophylaxis, 2 (3%) received drugs for multiple unrelated conditions (e.g., rifampin for eradication of *Staphylococcus aureus*; ethambu-

tol for *M. avium* complex prophylaxis), and the remaining 26 (37%) were suspected of having active TB without subsequent confirmation (Table 3).

Of the 118 persons with automated ambulatory-patient records who met screening criteria involving a diagnosis code for TB but did not have incident TB, 57 received the diagnosis code as an indication of routine prenatal screening for TB, 12 had a previous history of TB, 31 were suspected of having active TB without subsequent confirmation, and 18 had the diagnosis code documented in their HMO ambulatory medical record for no apparent reason (Table 3).

Of the eight patients whose cases had not been identified by the public health department, seven were culture-negative and met the TB clinical case definition, and one did not have a microbiology culture and met the smear-positive TB case definition. Three of the patients had AFB smear-positive pathology specimens; of these, two had negative cultures for *M. tuberculosis,* and one did not have a culture performed. Of the eight cases, one involved pulmonary TB, and the remaining seven were extrapulmonary. One patient was 2 years old at the time of diagnosis; the remaining seven were 18 years of age or older. All cases were confirmed by review with the Massachusetts State Tuberculosis Control Officer. Of these eight cases, seven were detected by the two or more antituberculosis drug screening criterion.

Conclusions

Since the establishment of a national surveillance system for TB in 1953, TB surveillance has depended on laboratories, public health clinics, and reporting by private practitioners. Several retrospective studies performed by local TB programs suggest that TB cases may be underreported (5-7). Although ascertainment of culture-positive cases is likely to be nearly complete, since laboratories are required by law in most states to report isolation of *M. tuberculosis* to the state health department, surveillance for cases lacking positive cultures depends largely on reporting by health-care providers or referrals to public health clinics for treatment. Underreporting of TB cases without positive cultures may contribute to incomplete surveillance. A study assessing the completeness of TB case reporting

Table 3. Reasons for meeting screening criteria among individuals without incident tuberculosis (TB) who had automated ambulatory-patient records

Reasons why non-TB cases met screening criteria	Screening criteria that include a TB diagnosis code or multiple anti-TB drugs				
	Two or more anti-TB drugs[a]	Diagnosis code[b] for active TB	Diagnosis code[b] for pulmonary TB	Diagnosis code[b] for TB, a microbiology code,[c] and a radiology code[d]	At least one anti-TB drug[a] and an ICD-9 diagnosis code for TB
Active TB diagnosed outside study window	9 (13%)	0	0	0	0
Suspected active TB	26 (37%)	2 (67%)	4 (5%)	19 (58%)	6 (86%)
TB prophylaxis	11 (15%)	0	0	0	0
Prenatal TB screening	0	0	57 (76%)	0	0
Prior history of TB	0	0	5 (7%)	7 (21%)	0
Other mycobacterial infections	23 (32%)	0	0	0	0
Treatment of other conditions	2 (3%)	0	0	0	0
No documentation of reason in HMO medical record	0	1 (33%)	9 (12%)	7 (21%)	1 (14%)
Total no. without incident active TB	71	3	75	33	7

[a]Pharmacy dispensing data; antituberculosis drugs include isoniazid, rifampin, pyrazinamide, ethambutol, streptomycin, capreomycin, kanamycin, ethionamide, para-aminosalicyclic acid, and cycloserine.
[b]Ambulatory codes were obtained from automated ambulatory records in the staff model division and from claims in the network and group model division.
[c]Microbiology codes include COSTAR (coding system for automated ambulatory-patient records [10]) or ICD-9CM (International Classification of Diseases, 9th Revision Clinical Modification) procedure codes for acid fast bacilli smear, culture and sensitivities and microscopy examination of sputum.
[d]Radiology codes include current procedures terminology (CPT), COSTAR, or ICD-9 procedure codes for chest radiograph, thoracic computer- assisted tomography (CT), or thoracic magnetic resonance imaging (MRI).

in Puerto Rico (6) found that 19.5% of patients with TB were not reported, partly because of underreporting of cases without positive cultures for *M. tuberculosis*.

The recent shift into managed care of populations at high risk for TB, including Medicaid and Medicare recipients, has raised additional concern about the continued completeness of reporting. However, HMOs routinely collect information that can be used to identify persons likely to have TB. McCray et al. (6) noted that, according to pharmacy prescription data in Maryland, the cases of 19% of patients receiving two or more antituberculosis drugs had not been reported to the public health department; however, the patients' medical records were not reviewed to verify a diagnosis of active TB. Maggini et al. (8) evaluated the use of Italy's National Health Service pharmacy dispensing information to identify TB cases in the province of Rome and found that pharmacy screening detected seven times more new TB cases than routine passive surveillance. Hripcsak et al. (9) evaluated a number of screening rules based on automated information available at an urban medical center in New York City and found that inpatient use of antituberculosis drugs had a sensitivity of 68% and a positive predictive value of <1% for detecting TB cases based on their health department's TB registry. These investigators did not, however, have access to records of antituberculosis drugs received by ambulatory patients and did not specifically evaluate the use of more than one antituberculosis drug as a screening criterion. No previous study has compared the utility of pharmacy data with that of other automated administrative or health-care data.

Of the screening criteria evaluated in our study, dispensing of two or more antituberculosis drugs was the most useful, with an overall sensitivity of 89%. The most common reasons for

dispensing of two or more antituberculosis drugs to persons without TB were the empiric use of antituberculosis drugs for suspected active TB (37%) and the use of antituberculosis drugs for treatment of mycobacterial infections other than TB (32%). In addition, 15% of patients without TB received more than one drug for TB prophylaxis, which can occur, for example, when isoniazid is switched to another antituberculosis drug because of adverse drug reactions. A possible strategy for improving the positive predictive value of screening criteria based on pharmacy dispensing information is the use of more rigorous criteria, such as dispensing of three or more antituberculosis drugs (positive predictive value = 50%), or restricting the timing of drug dispensing, such as requiring that two or more antituberculosis drugs be dispensed on the same date (positive predictive value = 36%). The improvement in positive predictive value for these more rigorous criteria, however, must be weighed against loss of sensitivity in identifying TB cases. For our HMO study population, requiring three or more antituberculosis drugs missed two TB cases, and requiring that two or more drugs be dispensed on the same date missed one case detected by the less stringent criterion. The choice of the screening criterion with the most useful balance between sensitivity and specificity depends in part on the surveillance strategy used.

Surveillance based on HMO pharmacy dispensing information can be used to identify HMO enrollees most likely to have active TB, so that efforts can be focused on additional evaluation of these persons. As with traditional TB surveillance methods, surveillance based on pharmacy dispensing information requires information from the patients' medical records to verify whether the TB case definition is satisfied. Using this surveillance strategy, screening for two or more antituberculosis drugs would require reviewing the medical records of approximately three patients to identify each case of incident active TB. We feel that the positive predictive value of 30% is sufficient to make this surveillance screening method practical if it can be applied in other managed care settings.

The positive predictive values of screening criteria that include TB diagnosis codes are limited by a number of factors. TB diagnosis codes, for example, were frequently used for patients with suspected active TB during the weeks required for diagnostic work-up or observation for clinical response to therapy. These codes were also frequently used to indicate that routine TB skin testing had been performed rather than to indicate the presence of active disease or prior history of TB.

The difference in the TB case rates between HMO members with automated ambulatory-patient records (approximately 11.7 TB cases per 100,000 population) and members without such records (approximately four TB cases per 100,000 population) in our study could either reflect a true difference in the underlying risk for TB in the two populations or case ascertainment bias resulting from differences in the methods used to identify TB cases. The former explanation is more likely for several reasons. First, the HMO health centers with automated ambulatory-patient records serve a largely urban population concentrated in the Boston area, while the HMO-affiliated practices without such records serve a largely suburban population. The difference in the rates found in our study mirrors the difference in the 1992 to 1998 TB case rate averages reported by the Massachusetts Department of Public Health for the city of Boston (17.7 TB cases per 100,000 population) compared with the rest of the state of Massachusetts (4.1 TB cases per 100,000 population). Second, the match between the health department's TB registry and the HMO membership list did not identify any TB patients who had not previously been detected through screening criteria and record review based on our modified RVCT results among HMO members without automated ambulatory patient records. This argues against inadequate case finding resulting in apparent lower TB case rates in this group.

A substantial number of TB cases in our study were unknown to the public health department (18% of cases among our HMO study population). This proportion is comparable with the fraction described in the studies cited above. Underreporting of these cases compromises the usefulness of TB surveillance. Screening for dispensing of antituberculosis drugs may be a particularly useful method for identifying cases without positive cultures for *M. tuberculosis* that might otherwise be missed by routine surveillance methods dependent on laboratory- and provider-based reporting.

The positive predictive value of screening criteria based on the dispensing of antituberculosis drugs may also be limited to some degree in clinical settings where many patients receive these medications for other indications, including other mycobacterial infections (e.g., in cases of HIV infection). Strategies that could be applied in such settings include excluding those persons also receiving medications frequently used for treatment of *M. avium* complex infections (e.g., clarithromycin). During our study period, however, more than 1,000 known HIV-infected patients were treated in HMO centers with automated ambulatory-patient records, of whom only 23 (Table 3) had false-positive cases identified by the two or more TB drug criterion. In addition, widespread implementation of new CDC recommendations for use of multidrug therapy for TB prophylaxis may require modification of the screening criteria. One possible strategy would be to require that antituberculosis drugs be dispensed over a minimum time interval (e.g., >4 months).

Although TB surveillance based on pharmacy dispensing information depends upon availability of automated pharmacy data, such data are available for most of the U.S. population, including most Medicaid and Medicare recipients. Our results indicate that pharmacy dispensing information routinely collected by many HMOs has high sensitivity and reasonable positive predictive value and is particularly useful for identifying TB cases without positive cultures, which may be missed by traditional public health surveillance.

References

1. Platt R. Studies of prescription drugs at Harvard Community Health Plan. In: Strom B, editor. Pharmacoepidemiology. 2d ed. New York: John Wiley and Sons; 1994. p. 278-87.
2. Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance. MMWR Morb Mortal Wkly Rep 1997;46(RR-10):40-1.
3. Subramanyan G, Yokoe D, Sharnprapai S, Tang Y, Platt R. An algorithm to match registries with minimal disclosure of individual identities. Public Health Reports 1999;114:91-3.
4. Stata statistics and data analysis [computer program]. Version 5.0. College Station (TX): Stata Corporation; 1997.
5. Marier R. The reporting of communicable diseases. Am J Epidemiol 1977;105:587-90.
6. McCray E, Weinbaum CM, Braden CR, Onorato IM. The epidemiology of tuberculosis in the United States. Clin Chest Med 1997;18:99-113.
7. Driver C, Braden CR, Nieves R, Navarro AM, Rullan JV, Valway SE, et al. Completeness of tuberculosis case reporting, San Juan and Caguas Regions, Puerto Rico, 1992. Public Health Rep 1996;111:157-61.
8. Maggini M, Salmaso S, Alegiani SS, Caffari B, Raschetti R. Epidemiological use of drug prescriptions as markers of disease frequency: An Italian experience. J Clin Epidemiol 1991;44:1299-307.
9. Hripcsak G, Knirsch C, Jain N, Pablos-Mendez A. Automated tuberculosis detection. JAMIA 1997;4:376-81.
10. Winickoff RN, Barnett GO, Morgan M, Coltin KL. Quality assurance in a prepaid group practice. Journal of Ambulatory Care Management 1979;2:19-28.

Using Automated Pharmacy Records to Assess the Management of Tuberculosis

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We used automated pharmacy dispensing data to characterize tuberculosis (TB) management for 45 health maintenance organization (HMO) members. Pharmacy records distinguished patients treated in HMOs from those treated elsewhere. For cases treated in HMOs, they provided useful information about appropriateness of prescribed regimens and adherence to therapy.

Health-care coverage, especially from health maintenance organizations (HMOs), often includes pharmacy benefits. Pharmacy dispensing records can identify cases of tuberculosis (TB) unknown to the public health system (1). In this article, we examine the utility of automated pharmacy dispensing data in assessing the quality of management of active TB and patients' compliance with recommended therapy.

Methods

We used automated pharmacy dispensing records to characterize therapy in 45 cases of active TB diagnosed from January 1, 1992, through June 30, 1996, at Harvard Pilgrim Health Care, a mixed model HMO in New England. These were all known cases of TB in a sample of 350,000 HMO members (1,2); all met the Centers for Disease Control and Prevention's (CDC) surveillance case definition (3). Cases were drawn from the 90% of HMO members with pharmacy benefits.

We identified all dispensings of isoniazid, rifampin, pyrazinamide, ethambutol, streptomycin, ethionamide, kanamycin, cycloserine, capreomycin, and para-aminosalicylic acid (PAS). We also reviewed the full medical records. Empiric regimens, i.e., those dispensed before susceptibility results were known, and final treatment regimens were graded for consistency with

American Thoracic Society (ATS) and CDC guidelines in effect at the time of diagnosis (4,5). Two measures of the adequacy of therapy were calculated. 1) The standard regimen dispensed is a percentage calculated by comparing the cumulative dose of each drug dispensed with the total recommended. Each drug received equal weight to a maximum of 100% per drug, as noted in the following formula for a three-drug regimen: percent standard regimen = $([D_1/SR_1] + [D_2/SR_2] + [D_3/SR_3]) \times (100/3)$, where D_x is the cumulative dose dispensed of drug X and SR_x is the recommended total dose. Patients with a score $\geq 80\%$ were considered to have received an appropriate amount of antituberculosis medications (6). 2) The days without medication (identical to the "MED-OUT" adherence index validated for other medications) (7), for isoniazid or another drug required for the entire duration of treatment, is a percentage calculated by dividing the total number of days without medication (based on medication refill intervals and quantities dispensed) by the number of days between the first and last dispensing. The last refill does not influence this calculation. All the preceding calculations included all medicine dispensed to a patient, from all pharmacies required to report dispensing to the HMO to be reimbursed.

Results

Medical records indicated that 27 (60%) of 45 TB cases were treated solely by HMO providers (Table 1); nearly all remaining patients received


Table 1. Characteristics of tuberculosis cases

	Tuberculosis cases treated in HMO, n = 27 (60%)	Tuberculosis cases treated outside HMO, n = 18 (40%)
Mean age	39	40
Male	16 (59%)	7 (39%)
Foreign born	22 (81%)	15 (83%)
Pulmonary disease, with or without extrapulmonary involvement	12 (44%)	9 (50%)
Adequate prescribed regimen by treating physician	26 (96%)	17 (94%)
Antituberculosis medications dispensed through HMO pharmacies	26 (96%)	15 (83%)
Duration (in days) of antituberculosis medication dispensing by HMO (median, interquartile range)	189 (148-291)	1 (0-32)
Standard regimen dispensed by HMO (median, interquartile range)	99% (86%-100%)	24% (17%-40%)

their non-HMO care in public health-funded TB programs. Thirty-seven (82%) cases received empiric regimens through pharmacies reimbursed by the HMO. In 34 (92%) instances, the empiric regimen dispensed was appropriate; for the remainder, empiric regimens contained too few drugs. Twenty-six (96%) of the 27 solely HMO-treated cases were prescribed a final antituberculosis regimen that was adequate in agents used, doses prescribed, and duration of treatment.

In 15 of the 18 cases treated at least partially outside the HMO, patients received some antituberculosis medication from pharmacies reimbursed by the HMO. In 14 of these cases, patients received medications only once or twice. A cutoff value of 70 days of drug dispensing through HMO-reimbursed pharmacies differentiated HMO-treated cases from cases treated in other settings. Among HMO-treated cases, 26 (96%) of 27 patients received medications for 70 days, compared with 1 (6%) of 18 who were at least partially treated outside the HMO (Figure 1) (RR = 17, 95% confidence
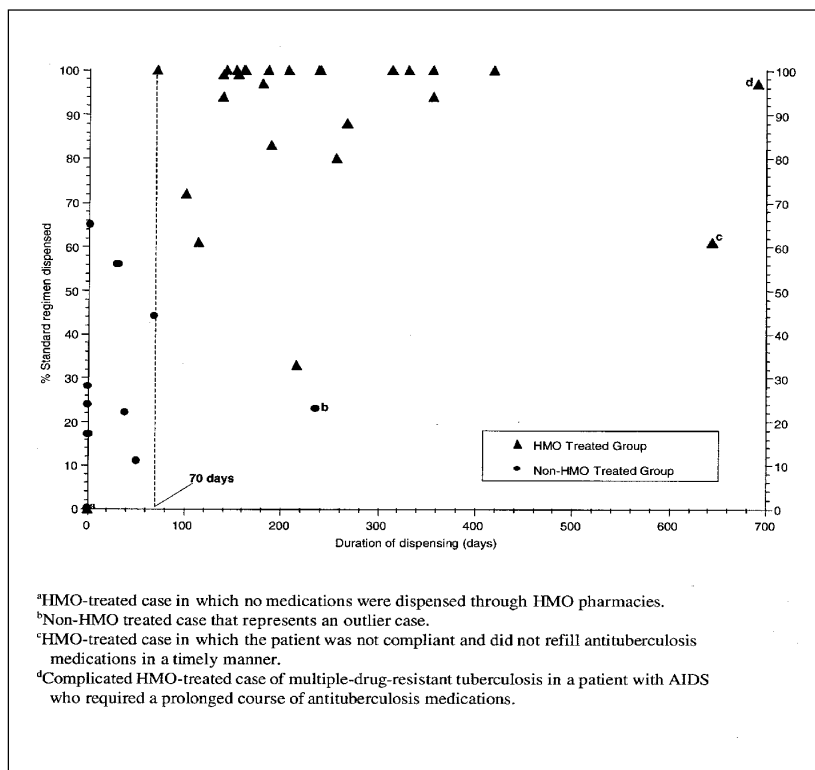
[a]HMO-treated case in which no medications were dispensed through HMO pharmacies.
[b]Non-HMO treated case that represents an outlier case.
[c]HMO-treated case in which the patient was not compliant and did not refill antituberculosis medications in a timely manner.
[d]Complicated HMO-treated case of multiple-drug-resistant tuberculosis in a patient with AIDS who required a prolonged course of antituberculosis medications.

Figure 1. Pharmacy dispensing profiles of tuberculosis (TB) cases treated in the health maintenance organization (HMO) and outside the HMO. Standard regimen (percentage) and duration of dispensing of antituberculosis medication dispensed for TB cases. A cutoff value of ≥70 days of medication dispensed from HMO-reimbursed pharmacies, as assessed from automated pharmacy records, differentiated HMO-treated cases from cases at least partially treated outside the HMO.

interval [CI] 3 to 117, p <0.0001) (8). In HMO cases, median duration of dispensing from HMO pharmacies was 189 days (interquartile range: 148 days to 291 days) and the median standard regimen dispensed score was 99% (interquartile range: 86% to 100%). In cases outside the HMO, median duration of dispensing through HMO-reimbursed pharmacies was 1 day (interquartile range: 0 days to 32 days), and the median standard regimen dispensed score was 24% (interquartile range: 17% to 40%). Figure 2 shows the relationship between the appropriateness of the amount of medications dispensed and the timeliness of medication refills. In 4 (15%) of 26 HMO-treated cases, standard regimen dispensed scores were <80%, and days without medication scores were >30%. In only one of these four undertreated cases did the treating physician document nonadherence. Two other patients who received 100% of a standard regimen with unremarkable refill intervals were noted as noncompliant in physicians' medical records.

Conclusions

Automated pharmacy data provided useful information both about physicians' intended management of TB and about patients' adherence to prescribed therapy. The ability to monitor these aspects of TB care efficiently is particularly important when care is decentralized or a substantial proportion of patients receive care from more than one provider. We were able to determine that in nearly all cases appropriate initial or empiric regimens were prescribed, and that in most cases managed by HMO providers full ATS/CDC-recommended regimens were dispensed. This approach was thus more informative and efficient than prior study methods that assessed prescribed regimens by reviewing patients' medical records (9). Many HMOs and other insurers routinely monitor pharmacy dispensing records for various reasons. While the initial cost of creating a routine monitoring report varies, the marginal cost of running it periodically is usually negligible. This allows 100% surveillance of therapy, compared with manual record review, which is more expensive even when only a sample of records is reviewed. Additional investigation would determine whether this method will be helpful for other infectious diseases, such as pelvic inflammatory disease.

In using a pharmacy-based system to monitor adherence to therapy, it is important to identify cases treated solely within a delivery system, since automated pharmacy information is reliably complete only for these persons. Restricting to patients with at least 70 days of therapy accomplishes this, since it excludes those who receive empiric regimens within one health system and then complete their care at another. Most care delivered by non-HMO providers was

[a]In this case, no antituberculosis medications were dispensed through HMO pharmacies.
[b]In this case, the treating physician noted that the patient was nonadherent to prescribed medications.
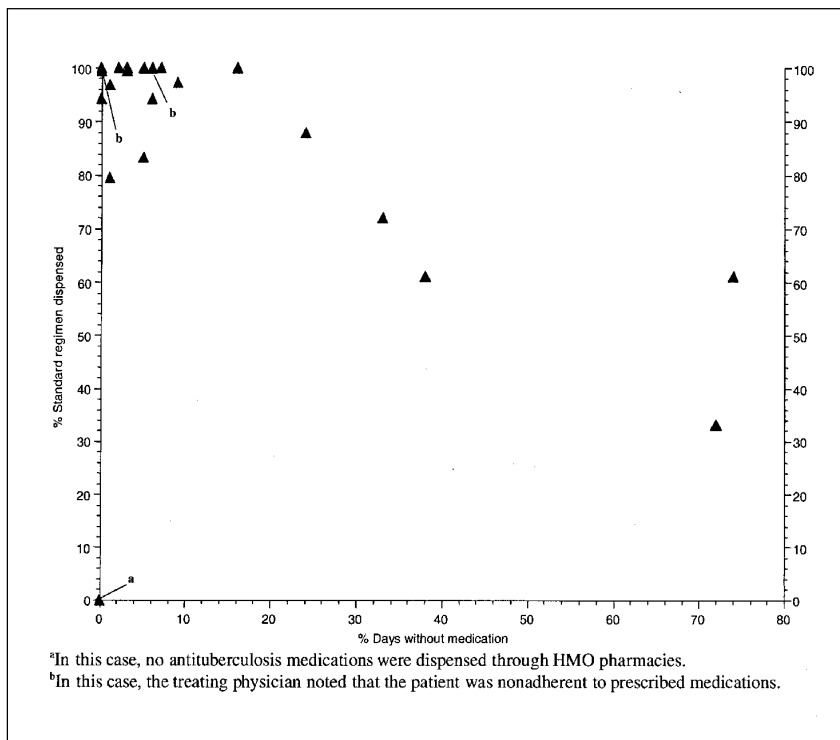
Figure 2. Appropriateness of the amount of antituberculosis medications dispensed and the timeliness of medication refills. Percentage of standard regimen dispensed is plotted against percentage of days without antituberculosis medication for tuberculosis cases treated in the health maintenance organization.

provided by public health clinics. Potential physician incentives for transfer of care included closer monitoring of therapy by experts in TB treatment, particularly in difficult-to-manage cases. Patients' incentives included free medications provided by the department of public health. Although all patients described here had pharmacy benefits, the cost to the patients of copayments for a standard treatment regimen would have been $75 to $200.

Monitoring automated pharmacy records of patients treated for TB was an efficient adjunct for monitoring adherence but did not entirely replace providers' assessments documented in the medical records. It also provided no advantage in settings that used directly observed therapy, which is not routinely used in Massachusetts.

Pharmacy records may be used to contribute to management of TB in two ways: in real time, to identify suboptimal regimens and noncompliant patients and periodically, to assess overall appropriateness of care. In real time, one might monitor the dispensing of antituberculosis medications for confirmed cases of TB, intervening if necessary with physicians to ensure that appropriate regimens are used and with patients to minimize gaps in dispensing. Identifying noncompliant patients in a regular and timely manner could allow for interventions (e.g., directly observed therapy) to improve adherence to the treatment regimen. Such oversight could be coordinated with or overseen by public health agencies. Coordination between delivery systems and public health agencies is expected to become an important element of TB control (10). Periodic assessment of dispensing records can also provide a simple, efficient measure of the overall appropriateness of TB care in a wide array of settings. These measures would allow targeting of resources to improve organizations' management of TB. If these findings are confirmed in other settings, routine monitoring of dispensing of antituberculosis medications may be useful as an adjunct to other methods of assessing and ensuring appropriate therapy.

Dr. Subramanyan was a research fellow at the Channing Laboratory, Brigham and Women's Hospital, Boston, Massachusetts, and a student at Harvard Medical School when he performed this work. He is currently a resident at the University of California, San Francisco.

References
1. Yokoe DS, Subramanyan GS, Nardell E, Sharnprapai S, McCray E, Platt R. Supplementing tuberculosis surveillance with automated data from health maintenance organizations. Emerg Infect Dis 1999;5:779-87.
2. Subramanyan GS, Yokoe DS, Sharnprapai S, Tang Y, Platt R. An algorithm to match registries with minimal disclosure of individual identities. Public Health Rep 1999;114:91-3.
3. Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance. MMWR Morb Mortal Wkly Rep 1997;46:40-1.
4. Bass JB Jr, Farer LS, Hopewell PC, O'Brien R, Jacobs RF, Ruben F, et al. Treatment of tuberculosis and tuberculosis infection in adults and children. Am J Respir Crit Care Med 1994;149:1359-74.
5. American Thoracic Society. Treatment of tuberculosis and tuberculosis infection in adults and children. American Review of Respiratory Disease 1986;134:355-63.
6. Fox W. Whither short-course chemotherapy? British Journal of Diseases of the Chest 1981;75:331-57.
7. Steiner JF, Koepsell TD, Fihn SD, Inui TS. A general method of compliance assessment using centralized pharmacy records: description and validation. Med Care 1988;26:814-23.
8. EpiInfo [computer program]. Version 6.04b. Atlanta (GA): Centers for Disease Control and Prevention; 1997.
9. Migliori GB, Spanevello A, Ambrosetti M, Neri M. Surveillance of tuberculosis treatment prescriptions in Italy. The Varese TB Study Group. Monaldi Arch Chest Dis 1998;53:37-42.
10. Halverson PK, Mays GP, Miller CA, Kaluzny AD, Richards TB. Managed care and the public health challenge of TB. Public Health Rep 1997;112:22-8.

Hantavirus Reservoir Hosts Associated with Peridomestic Habitats in Argentina

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Five species of sigmodontine rodents have been identified in Argentina as the putative reservoirs of six circulating hantavirus genotypes. Two species of Oligoryzomys are associated with the genotypes causing hantavirus pulmonary syndrome, *Oligoryzomys flavescens* for Lechiguanas and *O. longicaudatus* for Andes and Oran genotypes. Reports of human cases of hantavirus pulmonary syndrome prompted rodent trapping (2,299 rodents of 32 species during 27,780 trap nights) at potential exposure sites in three disease-endemic areas. Antibody reactive to Sin Nombre virus was found in six species, including the known hantavirus reservoir species. Risk for peridomestic exposure to host species that carry recognized human pathogens was high in all three major disease-endemic areas.

Hantaviruses, a genus in the family Bunyaviridae, are rodentborne pathogens producing chronic persistent infections in their reservoir hosts. Although the exact mechanism of transmission from rodents to humans is unknown, strong evidence suggests that these viruses are infectious by aerosols. Inhalation of aerosolized virus from rodent excreta is thought to be the main route of transmission to humans (1).

Although hantaviruses have been reported in the Americas since the 1980s (2,3), before 1993 human illnesses caused by hantaviruses, grouped under the name of hemorrhagic fever with renal syndrome, were thought to be limited to Europe and Asia. After hantavirus pulmonary syndrome (HPS) was described as a clinical form of hantavirus illnesses in the New World, outbreaks of HPS as well as isolated cases were recognized in many parts of the Americas. In Argentina, where cases of HPS were identified retrospectively as early as the 1980s (4), three geographically and ecologically distinct HPS-endemic areas have been recognized (5): the northern zone, a subtropical area bordering the Bermejo River; the central zone, a region of humid plains and temperate climate; and the southern zone, a cold, forested region bordering the Andean range (Figure).

The common rodents in populated areas of Argentina belong to two groups of the family Muridae. The most common rodents in natural, as well as disturbed habitats outside urban and peridomestic areas, are numerous species of the

Figure. Sites of rodent trapping and human cases in three hantavirus pulmonary syndrome-endemic zones in Argentina.

1 – Pergamino
2 – Morse
3 – Florencio Varela
4 – Ranchos
5 – Las Flores
6 – Castelli
7 – Dolores
8 – Crotto
9 – Olavarría
10 – Chillar
11 – Coronel Vidal
12 – Mar del Plata
13 – Rosario
14 – Villa Constitución
15 – Islas Lechiguanas
16 – El Huecú – Ranquilón
17 – Trolope
18 – El Hoyo
19 – Bariloche
20 – El Bolsón
21 – Epuyén
22 – Esquel
23 – El Corcovado
24 – Orán
25 – Libertador Gral. San Martín
26 – La Mendieta

Murid subfamily, Sigmodontinae (the New World rats and mice) (6). All hantaviruses known to cause HPS are associated with sigmodontine rodents. The common rodents in towns, cities, and peridomestic (in and around homes) environments are three introduced species of the subfamily Murinae: *Rattus rattus* (black rat), *R. norvegicus* (Norway rat), and *Mus musculus* (house mouse) (6).

In South America, hantaviruses are associated with several species of indigenous sigmodontine rodents. In Argentina, seven viral genotypes have been described: Bermejo and Oran in the northern zone; Lechiguanas, Hu39694, Maciel, and Pergamino in the central zone; and Andes in the southern zone (7,8). Andes, Lechiguanas, Hu39694, and Oran have been associated with human disease, and the putative reservoirs of three of these genotypes are two species of *Oligoryzomys*: *O. longicaudatus* from southern Argentina for Andes, *O. longicaudatus* from northern Argentina for Oran, and *O. flavescens* for Lechiguanas. *O. longicaudatus* (reservoir of Oran and Andes

genotypes) may represent two species (8). The putative reservoir for the Bermejo genotype, not yet associated with human disease, is reported to be *O. chacoensis*. The reservoir for Hu39694 is unknown, although its close genetic similarity to Andes, Oran, and Bermejo suggests that it may be another *Oligoryzomys* species from central Argentina. In the central zone, two genotypes not yet associated with HPS were identified from other sigmodontine species: Maciel, from *Necromys benefactus* (previously designated *Bolomys obscurus*), and Pergamino, from *Akodon azarae* (8).

Since 1996, follow-up investigations have been conducted when HPS cases in Argentina were confirmed. As of January 20, 1999, 210 cases of HPS had been confirmed in Argentina (Ministerio de Salud y Acción Social). This investigation includes rodent studies to identify areas in which HPS poses a high risk and to determine the spatial distribution of rodent reservoir populations in relation to the suspected sites of exposure for persons with HPS.

Identification of HPS Cases and Study Areas

Confirmed cases of HPS were defined as having the following characteristics: 1) a compatible clinical illness and 2) laboratory evidence of acute hantavirus infection, such as a positive enzyme-linked immunosorbent assay (ELISA) hantavirus immunoglobulin (Ig) M or a fourfold rise in ELISA IgG; a positive reverse transcription-polymerase chain reaction (RT-PCR) for hantavirus RNA; or positive immunohistochemistry for hantavirus antigen. When an HPS case was confirmed, small mammals were trapped in collaboration with the local health authorities at the patient's home or work sites and neighboring areas (Figure).

Selection and Classification of Potential Exposure Sites

The potential exposure sites were chosen by selecting all places where patients had been living or working or had visited during the 6 weeks before onset of symptoms. Rodents were trapped in all these sites, which were classified into six categories: domestic and peridomestic urban, domestic and peridomestic rural, other urban, and other rural. Peridomestic urban and rural categories were all sites in the immediate vicinity of homes or buildings, including yards, parks, driveways, adjoining lands, outbuildings,

vegetable gardens, and fence lines. The peridomestic rural category includes ponds, natural or planted woodlots, weeds, sugar cane or plantain plantations, and corn stubble in the immediate vicinity of the house. All other trapping sites distant from the previously mentioned settings were considered other urban or other rural. Other urban includes sites from the outskirts of towns and natural and artificial corridors that could allow the access of sigmodontine rodents to urban areas, such as railroad rights-of-way and roadsides inside the perimeter of the town. In other rural sites rodents were captured in open fields, where the representative habitats of each area were sampled, including natural and modified land, such as cultivated areas and weeds.

Small-Mammal Trapping and Processing

In the southern and central zones, rodents were trapped as soon as HPS case reports were received. In the remote northern zone, three expeditions were organized to trap rodents at sites frequented by six persons with HPS reported in previous months, and only rarely was trapping conducted inside houses. The three expeditions took place in July 1995, October 1996, and May 1998; rodents were trapped at 18 sampling sites.

From August 1994 to April 1998, 46 sampling sites were selected in the central zone. In the southern zone, we included 51 sampling sites from November 1996 to April 1998 (Table 1).

Each site was sampled with Sherman (8 x 9 x 23 cm) and Tomahawk (14 x 14 x 40 cm) live-capture traps. The number of traps depended on the area available for trap placement at each site. Animals were trapped and sampled according to established safety guidelines (9) and were anesthetized with Isoflurane (Abbott Laboratories) before blood was drawn from the retroorbital sinus. Carcasses were tentatively identified in the field and kept in a

Table 1. Relative density (as indicated by trap success[a]) for frequently captured rodent species in three hantavirus pulmonary syndrome-endemic zones in Argentina

Zone/trap nights	Species[b]	Site type/no.						
		DU[c]/1	PU/1	DR/2	PR/10	OU/1	OR/3	All sites/18
Northern	Av	0	1.0	0	1.3	0.6	2.4	1.4
	Cc	0.7	1.0	0	0.9	1.2	1.3	1.0
	Och	0	1.0	0	0.8	0	0.1	0.7
	Ol	0	0	0	0.8	0	0.3	0.6
	As	0	0	2.6	0.5	0	0.1	0.5
	Mm	0.7	0	0	<0.1	0	0.1	0.1
	Rr	0	0	41.0	0.2	1.2	0.1	0.5
Trap nights		136	100	39	4,069	164	739	5,247
		DU/10	PU/8	DR/5	PR/14	OU/3	OR/6	All sites/46
Central	Aa	0	9.5	0	3.1	0	13.9	4.7
	Of	0	1.1	0.4	4.6	0	4.2	3.8
	Cm	0	0.4	0	0.5	0	3.5	0.8
	Cl	0	0.1	0.4	0.4	0	0.3	0.4
	Hb	0	0	0	0	0	1.7	0.2
	Mm	1.7	5.9	0.4	1.2	6.0	0.1	1.5
	Rr	0	0.1	0	0.1	0	<0.1	0.1
Trap nights		829	939	260	7,900	116	1,494	11,538
		DU/7	PU/10	DR/5	PR/9	OU/8	OR/12	All sites/51
Southern	Ol	1.6	0.2	0	6.1	0.8	5.4	3.2
	Al	0	0.5	0	0.9	0.8	3.5	1.6
	Ao	0	<0.1	0	1.0	<0.1	0.3	0.3
	Mm	0.4	0.5	0	0.9	0	0.2	0.3
Trap nights		512	1,650	251	1,731	3,101	3,750	10,995

[a]Number of captures per 100 trap nights, where a trap night is one trap for one night.
[b]Av, Akodon varius; Cc, Calomys callosus; Och, Oligoryzomys chacoensis; Ol, Oligoryzomys longicaudatus; As, Akodon spegazzinii; Mm, Mus musculus; Rr, Rattus rattus; Aa: Akodon azarae; Of, Oligoryzomys flavescens; Cm, Calomys musculinus; Cl, Calomys laucha; Hb, Holochilus brasiliensis; Al, Abrothrix longipilis; Ao, Abrothrix olivaceus.
[c]DU, domestic urban; PU, peridomestic urban; DR, domestic rural; PR, peridomestic rural; OU, other urban; OR, other rural.

solution of 10% formalin for confirmation of identification at the Museum of Natural Sciences "Bernardino Rivadavia," Buenos Aires.

Structure of Small-Mammal Communities

During 26,458 Sherman and 1,322 Tomahawk trap-nights, 2,299 small mammals belonging to two orders (Rodentia and Didelphimorphia) and three families (Muridae, Caviidae, and Didelphidae) were captured. These animals belonged to 32 species, with the murid subfamily Sigmodontinae representing 86.3% of the total sample.

The introduced murine rodents *R. rattus* and *M. musculus,* as well as *Cavia aperea* (Caviidae), were captured in all three areas. Sigmodontine rodents were represented by different species in the three regions.

Distribution of Species by Site of Capture

In all three regions, *M. musculus* was found in domestic urban sites (Table 1). In two of the three areas, we also observed rodents inside urban homes; this is the first documented

occurrence of sigmodontine species entering homes in Argentina.

We also found sigmodontine rodents inside rural homes: one *Calomys laucha* and one *O. flavescens* in the central zone and one *Akodon spegazzinii* in the northern zone. Sigmodontine rodents, including the reservoirs for Lechiguanas and Andes viruses, were also captured in the peridomestic urban sites, especially in the central and southern zones. In peridomestic rural habitats next to open fields, captures of sigmodontines were expected. The trap success values for hantavirus reservoir species in peridomestic rural sites were similar or higher than those in open fields represented by other rural sites. The relative proportion of rodent species among site categories includes all species antibody positive and the species that were numerically dominant but antibody negative in each zone. The category "others" includes species that were less representative in each zone; the high values observed in PU and OU sites in the northern zone were due to the low number of

Table 2. Relative proportion[a] of rodent species in each site category, by site

Zone	Species[b]	Site type/total no. captured						p[c]		
		DU/2	PU/5	DR/18	PR/227	OU/10	OR/58	PRvsOR		
Northern	*Av*	0	20.0	0	23.8	10.0	31.0	NS		
	Cc	50.0	20.0	0	17.2	20.0	17.2	NS		
	Och	0	20.0	0	14.5	0	1.7	*		
	Ol	0	0	0	14.1	0	3.4	*		
	As	0	0	5.6	9.7	0	1.7	NS		
	Mm	50.0	0	0	1.3	0	1.7	NS		
	Rr	0	0	88.9	4.0	20.0	1.7	NS		
	Others	0	40.0	5.6	15.4	50.0	41.4	*		
		DU/14	PU/162	DR/3	PR/805	OU/7	OR/389	PUvsPR	PRvsOR	PUvsOR
Central	*Aa*	0	54.9	0	30.3	0	53.2	*	*	NS
	Of	0	6.2	33.3	45.3	0	16.2	*	*	*
	Cm	0	2.5	0	5.1	0	13.6	NS	*	*
	Cl	0	0.6	33.3	4.3	0	1.3	*	*	NS
	Hb	0	0	0	0	0	6.4	ND	*	*
	Mm	100	33.9	33.3	11.5	100	0.5	*	*	*
	Rr	0	0.6	0	1.1	0	0.3	NS	NS	NS
	Others	0	1.2	0	2.2	0	8.5	NS	*	*
		DU/10	PU/21	DR/0	PR/161	OU/55	OR/355	PRvsOU	PRvsOR	OUvsOR
Southern	*Ol*	80.0	14.3	0	65.8	47.3	57.5	*	NS	NS
	Al	0	38.1	0	9.3	45.4	36.6	*	*	NS
	Ao	0	4.8	0	10.6	1.8	3.1	NS	*	NS
	Mm	20.0	38.1	0	9.3	0	2.0	*	*	NS
	Others	0	4.8	0	5.0	5.4	0.8	NS	*	*

[a]Calculated as the percentage of total captures in a given site category represented by each species.
[b]*Av, Akodon varius; Cc, Calomys callosus; Och, Oligoryzomys chacoensis; Ol, Oligoryzomys longicaudatus; As, Akodon spegazzinii; Mm, Mus musculus; Rr, Rattus rattus: Aa, Akodon azarae; Of, Oligoryzomys flavescens; Cm, Calomys musculinus; Cl, Calomys laucha; Hb, Holochilus brasiliensis; Al, Abrothrix longipilis; Ao, Abrothrix olivaceus.*
[c]Chi-square test for comparison of two proportions in two independent samples. Epi Info version 6.04.
*p <0.05; NS, p >0.05; ND, not done. Comparisons were made and are shown only for cases where sample size was sufficient for statistical comparisons.

captures and in OR to the high diversity of species captured (Table 2). The relative proportion was compared by chi-square test with Epi Info version 6.04. Only site categories with ≥30 captures could be tested. An increase in the relative proportion of *O. flavescens* (host of the genotype Lechiguanas, associated with human disease) in the central zone and *O. longicaudatus* (putative reservoir of the genotype Orán, also associated with human disease) in the northern zone was seen in peridomestic rural settings in comparison with other rural. *O. longicaudatus* (proposed reservoir for Andes virus) was captured in similar relative proportions in both peridomestic and other rural sites. In all cases, these findings emphasize the risk linked to peridomestic settings.

Hantavirus Infection in Rodents

We tested 2,159 (93.9%) rodents in IgG ELISA by using Sin Nombre virus antigen (CDC, SPR293). We used a recombinant nucleocapsid protein as antigen applied to the solid phase of a microtiter plate. Hantavirus-specific IgG in test samples of rodent whole blood was allowed to bind to the antigen. A mixture of two conjugates (anti-*Peromyscus leucopus* and anti-*Rattus norvegicus*, Kirkegaard and Perry) was used to detect immune globulins from various murid rodent phyla. This was followed by 2,2'-azino-di(3-ethybenthiazonline sulfonate) substrate (Kirkegaard and Perry Laboratories, Inc.) and read with a Bio-Tek Microplate autoreader at 405 and 450 nm. A titer ≥1:400 was considered positive (10).

Of 330 rodents tested in the north, 5 (1.5%) were positive (Table 3). In the central zone, we found 35 (2.6%) positives among 1,326 rodents, associated with eight HPS cases. In the south, 27 (5.4%) of 503 rodents tested had positive results. In the northern zone, the presence of infected *O. longicaudatus* was associated with HPS cases in peridomestic rural habitats. The importance of detecting infected *O. chacoensis* and *Akodon varius* associated with an HPS case in peridomestic urban and rural sites cannot be assessed until data on the viral genotypes of the rodents and the case patients are available.

In the central zone, apart from *O. flavescens,* already shown to be associated with HPS,

Table 3. Antibody distribution in rodents, by province, species, and site category

Site zone[a]	Province	Immunoglobulin G antibody[b] species (pos/tested) (%)	Site category[c]
Northern	Jujuy	*Oligoryzomys chacoensis* 1/12 (8.3)	PU
	Salta	*O. chacoensis* 1/27 (3.7)	PR
		Akodon varius 1/26 (3.8)	PR
		O. longicaudatus 2/26 (7.7)	PR
		Other species 0/239 (0.0)	
Central	Buenos Aires	*O. flavescens* 8/170 (4.7)	PR-OR
		A. azarae 15/549 (2.7)	PU-PR-OR
	Entre Rios	*O. flavescens* 11/243 (4.5)	PR
		H. brasiliensis 1/30 (3.3)	PR
		Other species 0/334 (0.0)	
Southern	Rio Negro	*O. longicaudatus* 18/195 (9.2)	PR-OR
	Chubut	*O. longicaudatus* 5/40 (12.5)	PR-OR
	Neuquén	*O. longicaudatus* 4/88 (4.5)	PR
		Other species 0/180 (0.0)	

[a]Animals tested and found negative, by zone and species.
Northern: *Akodon varius* (48), *A. boliviensis* (1), *A. albiventer* (1), *A. spegazzinii* (26), *Akodon* sp. (10), *Calomys callosus* (53), *C. laucha* (2), *Calomys* sp. (1), *Cavia aperes* (3), *Eligmodontia moreni* (6), *Galea musteloides* (2), *Graomys griseoflavus* (2), *Holochilus brasiliensis* (1), *H. chacarius* (4), *Mus musculus* (6), *Oligoryzomys flavescens* (1), *O. longicaudatus* (8), *Oligoryzomys* sp. (10), *Oxymycterus paramensis* (5), *Phillotys osilae* (2), *Rattus rattus* (28), *Rattus* sp. (2), *Thylamys elegans* (5), and unidentified (13). Total 240.
Central: *Calomys musculinus* (94), *C. laucha* (40), *Necromys benefactus* (2), *Oxymicterus rufus* (9), *Mus musculus* (157), *Rattus rattus* (8), *R. norvegicus* (1), *Cavia aperes* (1), *Monodelphia dimidiata* (3), and unidentified (19). Total 334.
Southern: *Abrotrix longipilis* (138), *A. oliveceus* (25), *Mus musculus* (12), *Rattus rattus* (1), *Thylamys elegans* (1), and unidentified (3). Total 180.
[b]Enzyme-linked immunosorbent assay using Sin Nombre virus antigen.
[c]PU, peridomestic urban; PR, peridomestic rural; OR, other rural. Seropositive animals were found only in these three site categories.

another species found infected was *A. azarae*, the putative reservoir of the Pergamino genotype, which has not yet been associated with human disease. Spatial and temporal association between an HPS case and an infected *A. azarae* does not confirm this species as the source of infection. Further genetic studies are under way to determine if Pergamino virus was responsible for the HPS cases.

In the southern zone, human cases were associated with *O. longicaudatus* captured in peridomestic and other rural settings (Table 3). In the three zones, in all other site categories, no seropositive animals were found. Nevertheless, because of small sample sizes, any conclusions concerning lack of infection in these site categories are tentative.

Conclusions

Infected hantavirus reservoir hosts (as evidenced by antibody positivity) were found within peridomestic environments in all three HPS-endemic zones in Argentina. Reservoir species were captured inside urban houses in two of the three endemic zones. Although host species were not captured in homes in the northern zone, sampling was not sufficient to exclude the possibility that they enter homes occasionally.

The presence of hantavirus reservoir species in peridomestic environments indicates risk for human inhabitants. The primary measure for reducing the risk is preventing access of rodents to homes (11). The efficacy of proposed and currently used exclusion methods in Argentina needs to be evaluated (12).

Sigmodontine rodents, including known hantavirus reservoir species, were frequently captured in the rural and small-town peridomestic environments we studied. At many of the case sites, the level of hygiene was suboptimal. The widespread presence of such conditions underscores the importance of local habitat management to prevent wild (sigmodontine) rodents from entering domestic areas in towns, villages, and urban centers and of health education for the local population to reduce the risk for hantavirus infection.

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References

1. McKee KT Jr, LeDuc JW, Peters CJ. Hantaviruses. In: Belshe RB, editor. Textbook of human virology. St. Louis (MO): Mosby Year Book; 1991.
2. LeDuc JW, Smith GA, Childs JE, Pinheiro FP, Maiztegui JI, Niklasson B et al. Global survey of antibody to Hantaan-related viruses among peridomestic rodents. Bull WHO 1986;64:139-44.
3. Weissenbacher MC, Cura E, Segura E, Hortal M, Back LJ, Yong K, et al. Serological evidence of human hantavirus infection in Argentina, Bolivia and Uruguay. Medicina (Buenos Aires) 1996;56:17-22.
4. Parisi M, Enria D, Pini N, Sabattini MS. Detección retrospectiva de infecciones clínicas por hantavirus en la Argentina. Medicina (Buenos Aires) 1996;56:1-13.
5. Levis SC, Briggiler AM, Cacase M, Peters CJ, Ksiazek TG, Cortés J, et al. Emergence of hantavirus pulmonary syndrome in Argentina. Proceedings from the 44th Annual Meeting; 1995 Nov 17-21; San Antonio, Texas. American Society of Tropical Medicine and Hygiene; 1995. p. 233.
6. Redford K, Eisenberg J. Mammals of the neotropics. The southern cone. Vol 2. The University of Chicago Press; 1992.
7. Lopez N, Padula R, Rossi C, Lázaro ME, Franze-Fernandez MT. Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. Virology 1996;220:223-6.
8. Levis S, Morzunov S, Rowe J, Enria D, Pini N, Calderón G, et al. Genetic diversity and epidemiology of hantaviruses in Argentina. J Infect Dis 1998;177:529-38.
9. Mills J, Childs J, Ksiazek T, Peters CJ, Velleca W. Métodos para trampeo y muestreo de pequeños mamíferos para estudios virológicos. Washington: Organización Panamericana de la Salud; 1998. Report #OPS/HPS/HCT98.104.
10. Ksiazek T, Peters CJ, Rollin PE, Zaki S, Nichol S, Spiropoulou C, et al. Identification of a new North American hantavirus that causes acute pulmonary insufficiency. Am J Trop Med Hyg 1995;52:117-23.
11. Centers for Diseases Control and Prevention. Hantavirus infection-southwestern United States: interim recommendations for risk reduction. MMWR Morb Mortal Wkly 1993;42(RR-11):1-13.
12. Glass G, Johnson J, Hodenbach G, Disalvo C, Peters CJ, Childs J, et al. Experimental evaluation of rodent exclusion methods to reduce hantavirus transmission to humans in rural housing. Am J Trop Med Hyg 1997;56:359-64.

Large, Persistent Epidemic of Adenovirus Type 4-Associated Acute Respiratory Disease in U.S. Army Trainees

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In May 1997, a large, persistent epidemic of adenovirus type 4-associated acute respiratory disease began at Fort Jackson, South Carolina, the largest army basic training center. The epidemic lasted until December and declined when vaccine administration resumed. More than 1,000 male and female trainees were hospitalized; 66.1% of those hospitalized had an adenovirus type 4 isolate.

Nonvaccine interventions have proven unreliable in the control of adenovirus-associated acute respiratory disease (1). Before live, oral, enteric-coated adenovirus types 4 and 7 vaccines were introduced in 1971, adenovirus-associated acute respiratory disease produced high attack rates and excessive illness in soldiers during service-entry basic combat training (2). The subsequent policy to vaccinate all male trainees during basic combat training drastically reduced adenovirus type 4- and type 7-associated acute respiratory disease (3).

In 1996, the sole manufacturer of the vaccines ceased production (4). To conserve the remaining vaccine lots, the army restricted use of adenovirus vaccines to the period of September 1 through March 31, the peak season for acute respiratory disease. Administration of adenovirus vaccines to military trainees was suspended on March 31, 1997, at all army basic training centers. To monitor the impact of this modified policy, intensive, laboratory-based surveillance was initiated in late April 1997 at Fort Jackson, South Carolina, the army's largest basic combat training center. An epidemic of adenovirus type 4-associated acute respiratory disease occurred among male and female soldiers in basic combat training at Fort Jackson after the vaccination was suspended. The first case appeared in late May, and the outbreak lasted until December, after vaccination was resumed in November.

The Outbreak

During May through December 1997, a monthly average of 6,847 soldiers from all geographic regions of the United States and its protectorates were engaged in the 8-week basic combat training program at Fort Jackson. During this period, 38.2% of the trainees were women. The mean age of soldiers enrolled in this study was 19.7 years.

All trainees who report to sick call with fever of 100.5° F or higher are admitted to a minimal-care hospital ward at the Fort Jackson Army Hospital for observation and self-care. Soldiers from this group who had an oral temperature of 100.5° F or higher, plus at least one sign or symptom of an upper respiratory infection, were enrolled in the surveillance program. Approximately 80% of soldiers hospitalized with acute respiratory disease were enrolled since patients admitted on weekends were not included. A pharyngeal swab was taken from each trainee

and immediately placed into virus transport medium (Viromed Laboratories, Inc., Minneapolis, MN). Specimens were stored at 2°C to 8°C for an average of 72 hours before overnight express shipment on wet ice to the laboratory for virus isolation.

Virus isolations and identifications were performed by the Department of Pathology Laboratory at Dwight David Eisenhower Army Medical Center. Adenoviruses were isolated in human lung carcinoma (A-549) cells (Viromed Laboratories, Inc., Minneapolis, MN). The serotype of isolated adenoviruses was determined by virus neutralization with type-specific antisera (Centers for Disease Control and Prevention, Atlanta, GA); the Reed-Muench method was used for calculating the LD_{50} titer (5). Each culture was also tested for influenza A and B; parainfluenza 1, 2, and 3; herpes simplex virus; and enteroviruses by cell culture, enzyme immunoassay, and fluorescent antibody staining.

The first isolate of adenovirus type 4 came from a patient who was hospitalized on May 22, 1997, approximately 7 weeks after administration of the adenovirus vaccines ended. During May through December 1997, 1,018 basic trainees whose illness met the case definition were examined. Of these, 673 (66.1%) were positive for adenovirus type 4. No other respiratory disease agent was identified as an important cause of illness in this epidemic. The monthly case distribution was calculated by sex for all hospitalized acute respiratory disease patients (Figure 1) and for adenovirus type 4-

positive patients (Figure 2). Of the total acute respiratory disease cases, 35.3% (only slightly less than their representation in the total trainee population of 38.2%) were in women. Similarly, 31.2% of all adenovirus type 4 isolates were from women. During May, June, and July, the monthly rates of acute respiratory disease admissions were higher for women than for men (Figure 1). These higher rates were not due to adenovirus type 4 (Figure 2) or any other agent tested for in this study.

The percentage of cases from which adenovirus type 4 was isolated increased as the epidemic progressed. At its peak, almost all patients had an adenovirus type 4 isolate (Figure 3). Isolation rates of more than 90% were seen in both male and female patients toward the end of the outbreak. At the peak of this epidemic,
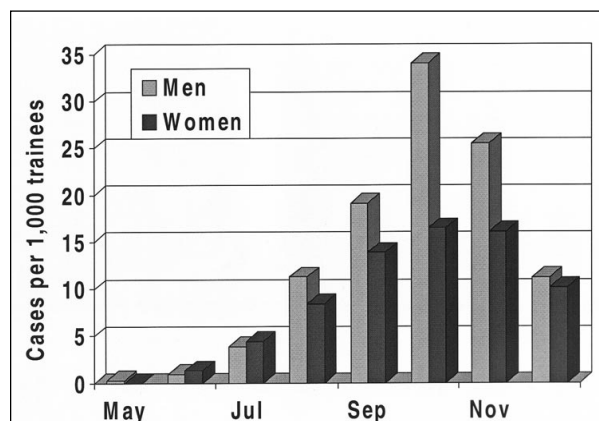
Figure 2. Patients with adenovirus type 4-associated acute respiratory disease, by sex, May–December 1997, Fort Jackson, South Carolina.
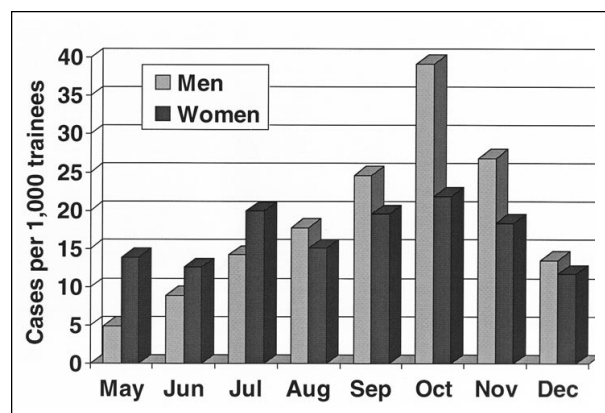
Figure 1. Patients with acute respiratory disease, by sex, May–December 1997, Fort Jackson, South Carolina.
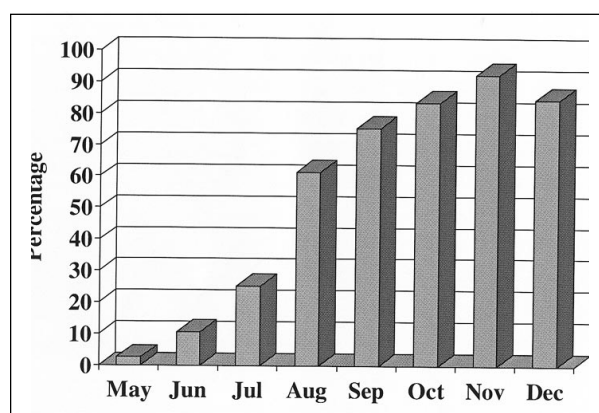
Figure 3. Percentage of total patients with acute respiratory disease who also had an adenovirus type 4 isolate, May–December, 1997, Fort Jackson, South Carolina.

approximately 70 soldiers per week were hospitalized at Fort Jackson Army Hospital. This corresponded to a weekly hospitalization rate for the entire post of approximately 1.0 admission per 100 soldiers. However, cases were not uniformly distributed throughout the trainee population but tended to occur in clusters. The outbreak intensified until November 1997, when the adenovirus vaccines were reintroduced. (Vaccination was to resume on September 1, 1997, according to the modified army policy. However, the vaccines did not reach Fort Jackson until November.) Resumption of adenovirus immunizations was associated with a rapid decline in both new cases of acute respiratory disease and isolations of adenovirus type 4. The last isolate of adenovirus type 4 was seen in December 1997. No additional isolates were reported during January through April 1998, except for a single isolate in a vaccinated trainee in February 1998. Administration of adenovirus types 4 and 7 vaccines continued through March 31, 1998.

Conclusions

Traditionally, adenovirus vaccines were administered to male trainees entering basic combat training between October and April, but outbreaks due to types 4 and 7 occurred in the absence of vaccines. The year-round use of the two vaccines beginning in 1984 resulted in the elimination of outbreaks due to both the type 4 and type 7 viruses (3).

In 1994, administration of the vaccines was temporarily interrupted, and a limited outbreak occurred among male and female basic trainees at Fort Jackson (6). This outbreak, which began in 1995 approximately 5 to 6 weeks after the administration of the adenovirus vaccines was reinstated, was centered in one military unit that had not received the vaccines; the outbreak lasted only a few weeks, probably because the pool of susceptible soldiers was small. Concern about the threat of adenoviruses was validated by serologic data, which revealed that the adenoviral susceptibility of personnel entering the military in the 1990s was similar to that of personnel who entered in the 1970s (4).

The temporal relationship of this epidemic to suspension of adenovirus vaccination in March 1997 demonstrates the effectiveness of type-specific vaccine in controlling and preventing military epidemics. Trainees began and ended the 8-week basic combat training program each week, so it would take approximately 2 months for all trainees who received the vaccines to complete basic combat training and leave Fort Jackson. The first case of adenovirus type 4-associated acute respiratory disease occurred in May, approximately 7 weeks after vaccination was suspended. The epidemic continued until December 1997, when it was interrupted by the administration of adenovirus types 4 and 7 vaccines to all new soldiers and to all soldiers (men and women) already enrolled in basic combat training at Fort Jackson. The army hospital managed the acute respiratory disease patient workload during the 1997 epidemic, but the unexpected inpatient census proved stressful for staff and facilities.

When men and women had separate basic combat training programs, adenovirus-associated acute respiratory disease outbreaks were never documented in women, and the adenovirus vaccines were not administered to women (2,4). When the gender-integrated program started at Fort Jackson approximately 5 years ago, the decision was made to continue providing the vaccine only to men. In the limited adenovirus type 4-associated acute respiratory disease outbreak at Fort Jackson in 1995, women were hospitalized and had adenovirus type 4 isolated (6). During this large 1997 epidemic, women and men in the integrated basic combat training program were similarly affected by adenovirus type 4-associated acute respiratory disease. Early in this outbreak, admission rates for acute respiratory disease were higher in women. However, an etiologic agent was not identified. Acute respiratory disease in women in integrated basic combat training programs cannot be assumed to be the same as that observed in men and must be studied further.

Historically, adenovirus types 4 and 7 have been the major causes of acute respiratory disease in the military (2). We cannot state why type 4 caused this outbreak rather than type 7. However, documented military outbreaks demonstrate that both vaccines are necessary, since prevention of type 4-associated acute respiratory disease alone will result in the emergence of type 7-associated disease (2).

This epidemic demonstrates that adenovirus type 4 is a major threat to both male and female soldiers in basic combat training. Additionally, it confirms that large outbreaks due to adenoviruses

should be expected in unvaccinated basic combat trainees.

Acknowledgment

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References

1. Foy HM. Adenoviruses. In: Evans AS, Kaslow RA, editors. Viral infections in humans. 4th ed. New York: Plenum Press; 1997. p. 119-38.
2. Gaydos CA, Gaydos JC. Adenovirus vaccines in the U.S. military. Mil Med 1995;160:300-4.
3. Brundage JF, Gunzenhauser JD, Longfield JN, Rubertone MV, Ludwig SL, Rubin FA, et al. Epidemiology and control of respiratory diseases with emphasis on group A beta-hemolytic streptococcus: a decade of U.S. Army experience. Pediatrics 1996;97:964-70.
4. Ludwig SL, Brundage JF, Kelley PW, Nang R, Towle C, Schnurr DP, et al. Prevalence of antibodies to adenovirus serotypes 4 and 7 among unimmunized U.S. Army trainees: results of a retrospective nationwide seroprevalence survey. J Infect Dis 1998;178:1776-8.
5. Hawkes RA. General principles underlying laboratory diagnosis of viral infections. In: Lennette EH, Schmidt NJ, editors. Diagnostic procedures for viral, rickettsial, and chlamydial infections. 5th ed. Washington: American Public Health Association; 1979. p. 32-5.
6. Barraza EM, Ludwig SL, Gaydos JC, Brundage JF. Reemergence of adenovirus type 4 acute respiratory disease in military trainees: report of an outbreak during a lapse in vaccination. J Infect Dis 1999;179:1531-3.

Changes in Antimicrobial Resistance among *Salmonella enterica* Serovar Typhimurium Isolates from Humans and Cattle in the Northwestern United States, 1982–1997

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We compared antimicrobial resistance patterns of *Salmonella enterica* serovar Typhimurium (ST) of isolates from humans (n = 715) and cattle (n = 378) in the Pacific Northwest from 1982 through 1997. The major changes in antimicrobial resistance can be attributed to the widespread clonal dissemination of multidrug-resistant definitive phage type 104 ST.

Enteritidis is the most frequent *Salmonella* serovar in all regions of the United States, except the Pacific Northwest where Typhimurium is the most frequent. In 1996, 31.1% of all *Salmonella* isolates from human sources in Washington, Oregon, and Idaho were serovar Typhimurium, while 14.6% were serovar Enteritidis (1). Typhimurium is also one of the most common *Salmonella* serovars from animal sources in the United States (2).

Use of antimicrobial drugs in food animals may lead to resistant strains of pathogens, which may be transmitted to humans through food (3,4). Although there is evidence that this transmission occurs, the contribution of antimicrobial use in food animals to resistance in bacteria infecting humans is the subject of debate (5-8).

We compared antimicrobial resistance patterns of *Salmonella enterica* serovar Typhimurium (ST) from human and cattle sources over a 15-year period and examined how these patterns relate to antimicrobial use in livestock and humans.

The Study

We used all ST (including *S.* Typhimurium var Copenhagen) isolates from clinical bovine samples submitted to the Washington Animal Disease Diagnostic Laboratory from 1986 through 1997 and from cattle herds tested by the Field Disease Investigation Unit during salmonellosis outbreaks over the same period (n = 378). For herds sampled repeatedly over time, all but the first ST isolate (per year) were excluded. Antimicrobial resistance data were also available for ST isolates from cattle for 1982 through 1986 from clinical submissions to the Washington Animal Disease Diagnostic Laboratory. Isolates from human clinical specimens (n = 715) were obtained from the Washington State Department of Health Public Health Laboratory for 1989, 1994, 1996, and 1997, and from the Idaho Division of Health, Bureau of Laboratories for 1997.

Isolates from other laboratories were subcultured onto solid brain heart infusion agar. All isolates were maintained in a -70° C bank freezer in brain heart infusion broth containing 25% to 30% buffered glycerol. Isolates were streaked for isolation on sheep blood agar prior to susceptibility testing. Susceptibility testing for the antimicrobial drugs listed in Table 1 was done by a disk diffusion method (9) on

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Dispatches

Table 1. Resistance to individual antibiotics among *Salmonella* Typhimurium isolates from cattle and humans

A. From cattle

Antimicrobial drug	Number (%) resistant by years			
	1982-1985[a] n = 49	1986-1990 n = 116	1991-1994 n = 90	1995-1997 n = 123
Ampicillin	39 (79.6)	99 (85.3)	72 (80.0)	113 (91.9)
Chloramphenicol[a]	2 (4.1)	2 (1.7)	56 (62.2)	90 (73.2)
Gentamicin	1 (2.0)	6 (5.2)	14 (15.6)	5 (4.1)
Kanamycin[a]	NA	103 (90.4)	49 (54.4)	50 (40.7)
Streptomycin	43 (87.8)	109 (94.0)	78 (86.7)	116 (94.3)
Tetracycline	43 (87.8)	101 (87.1)	77 (85.6)	115 (93.5)
Trimethoprim	NA	1 (0.9)	11 (12.2)	6 (4.9)
Trimethoprim-sulfamethoxazole	7 (14.3)	1 (0.9)	11 (12.2)	9 (7.3)
Triple sulfa	39 (79.6)	108 (93.1)	75 (83.3)	117 (95.1)

B. From humans

Antimicrobial drug	Number (%) resistant by year			
	1989 n = 90	1994 n = 189	1996 n = 187	1997 n = 249
Ampicillin[a]	22 (24.4)	107 (56.6)	109 (58.3)	164 (65.9)
Chloramphenicol[a]	3 (3.3)	84 (44.4)	92 (49.2)	116 (46.6)
Gentamicin	2 (2.2)	5 (2.7)	5 (2.7)	2 (0.8)
Kanamycin	25 (27.8)	44 (23.3)	34 (18.2)	47 (18.9)
Streptomycin[a]	42 (46.7)	112 (59.3)	109 (58.3)	174 (69.9)
Tetracycline[a]	36 (40.0)	101 (53.4)	109 (58.3)	158 (63.5)
Trimethoprim	0	7 (5.1)	6 (3.1)	5 (2.0)
Trimethoprim-sulfamethoxazole	0	6 (3.7)	6 (3.2)	5 (2.0)
Triple sulfa[a]	36 (40.0)	123 (65.1)	143 (76.5)	231 (92.8)

[a]Chi-square test for trend, p value <0.001.

NA, data not available.

Mueller-Hinton agar prepared according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (10,11). Ciprofloxacin susceptibility was tested on a subset of isolates systematically selected to include five per species per year.

Data for the analysis were divided into periods 1982-1986, 1987-1990, 1991-1994, and 1995-1997. These periods were chosen to compare isolates from cattle with isolates from humans for the years for which isolates from humans were available. Each isolate was classified as resistant or susceptible to each antimicrobial drug tested by the threshold zone size for resistance, as recommended by NCCLS (10,11). The proportions of isolates resistant to individual drugs and having each antimicrobial resistance pattern were computed by species and period. Significance testing of differences in proportions was done with Epi Info (12) using the chi-square test and the chi-square test for trend.

Marked changes in resistance to chloram-phenicol were observed for isolates from both cattle and humans (Tables 1, 2). Before 1991, fewer than 5% of isolates from cattle and only 3% of the 1989 isolates from humans were resistant to chloramphenicol; by the mid-1990s, more than 70% (90 of 123) of isolates from cattle (p <0.01) and almost 50% (92 of 187) of isolates from humans (p <0.01) were resistant to chloramphenicol. Most (79%) isolates from cattle were resistant to ampicillin, streptomycin, tetracycline, and sulfonamides throughout the study period. Among isolates from humans, the proportion resistant to these drugs was significantly lower in 1989 than in 1997 (p <0.01). The proportion of isolates from cattle resistant to kanamycin was significantly less (p <0.01) in 1990 to 1994 than in 1986 to 1990. All isolates tested were susceptible to ciprofloxacin, and average zone sizes showed no evidence of decline during the period (data not shown).

Among isolates from both cattle and humans, the ACSSuT resistance pattern was the most

Table 2. Antimicrobial resistance patterns for *Salmonella* Typhimurium isolates from cattle and humans

A. Cattle

	Number (%)			
	1982-1985[a]	1986-1990	1991-1994	1995-1997
	n = 49	n = 114	n = 90	n = 123
ACSSuT	1 (2.0)	1 (0.9)	18 (20.0)	55 (44.7)
ACKSSuT		0	25 (27.8)	24 (19.5)
ASSuT	24 (49.0)	0	5 (5.6)	6 (4.9)
AKSSuT		83 (72.8)	4 (4.4)	14 (11.4)
Su	1 (2.0)	1 (0.9)	0	0
All Others	22 (45.0)	24 (21.1)	29 (32.2)	20 (16.2)
Suscept[b]	1 (2.0)	5 (4.4)	9 (10.0)	4 (3.3)

B. Humans

	Number (%)		
	1989	1994	1996-1997
	n = 90	n = 189	n = 436
ACSSuT	2 (2.2)	44 (23.3)	156 (35.8)
ACKSSuT	0	26 (13.8)	35 (8.0)
ASSuT	0	14 (7.4)	24 (5.5)
AKSSuT	19 (21.1)	2 (1.1)	33 (7.6)
Su	1 (1.1)	9 (4.8)	80 (18.4)
All Others	25 (27.8)	33 (17.5)	47 (10.8)
Suscept[b]	43 (47.8)	61 (32.3)	61 (14.0)

[a]Data on kanamycin susceptibility not available for 1982-1985.
[b]Susceptible to all antimicrobial drugs tested.
A, ampicillin; C, chloramphenicol; T, tetracycline; G, gentamicin; K, kanamycin; S, streptomycin; Su, sulfonamide; Tmp, trimethoprim.

frequent and increased in frequency over the study period (Table 2). Before 1991, ACSSuT accounted for fewer than 4 (2%) of 255 of isolates from both species, and by the mid-1990s it accounted for more than 55 (40%) of 123 of isolates from cattle and more than 156 (35%) of 436 of isolates from humans. Isolates with the ACSSuT resistance pattern together with ACKSSuT-resistant isolates accounted for 79 (64%) of 123 isolates from cattle and 191 (44%) of 436 isolates from humans by the mid-1990s. As previously reported, 57 (95%) of 60 of these isolates were phage typed and found to be DT104 with a single pulsed-field gel electrophoresis pattern (13).

Isolates susceptible to all drugs tested were more common from human than cattle sources (166 [23.2%] of 715 vs. 19 [5.0%] of 378, p <0.01). The proportion of isolates from humans susceptible to all drugs tested decreased substantially from 1989 to 1997 (chi-square test for trend, p <0.01), while that of isolates from cattle was 10% or less for all periods studied (Table 2).

Conclusions

Antimicrobial resistance has been commonly observed in human and bovine ST isolates since the earliest days of antimicrobial use. This study provides a longitudinal perspective on resistance in ST from cattle and humans in a region and allows insight as to the mechanism of changes in antimicrobial resistance. The greatest changes were in chloramphenicol and kanamycin resistance in isolates from cattle and ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline resistance in isolates from humans. Changes in resistance in isolates from both species were primarily due to the sharply increased occurrence of isolates displaying the ACSSuT resistance pattern, a reliable marker for multidrug-resistant definitive type 104 ST (MR-DT104) (14). This has been shown to be so in the Pacific Northwest through a subset of isolates from this study (13).

MR-DT104 was first detected almost simultaneously in several geographic areas, including the United Kingdom (15), the United States (13), and Canada (16). Molecular genetic studies indicate that the same gene cassette accounts for multiple resistance in isolates from these and other diverse geographic areas (17,18). This study not only provides supporting evidence that MR-DT104 from different regions are clonal in origin, but refutes the notion that the multiple antimicrobial resistance of this clone was due to acquisition of new resistance genes by indigenous ST in each region.

It is possible that local antimicrobial selection pressure played an important role in dissemination of MR-DT104 through cattle populations into the human population. However, several observations argue against this hypothesis. First, the rise in the percentage of resistance to chloramphenicol in isolates from cattle occurred after the withdrawal of the drug for use in food animals in the mid-1980s (19) and before the 1996 approval of florfenicol (a chloramphenicol analog that shares resistance loci with chloramphenicol in MR-DT104 [20]) for therapeutic use in cattle. Second, before the dissemination of MR-DT104, most isolates from cattle were resistant to ampicillin, streptomycin, sulfonamides, and tetracycline. It is not evident how, in the absence of chloramphenicol use, antimicrobial selection pressure would favor R-type ACSSuT over ASSuT, although it is possible

that an unmeasured resistance factor favored the dissemination of MR-DT104 over ASSuT strains. Third, early in its global dissemination, MR-DT104 was isolated from several species of wildlife, which are not exposed to substantial amounts of antimicrobial drugs (13). Finally, reports of broad dissemination of *Salmonella* clones susceptible to antimicrobial drugs commonly used in livestock provide evidence that agent factors other than antimicrobial resistance are necessary for broad dissemination (21,22).

Nevertheless, some selection pressure that likely involved antimicrobial use must explain the high prevalence of antimicrobial resistance among ST. There is strong evidence that livestock are the main reservoir for human salmonellosis in industrialized countries (23); however, it would be an error to assume that the emergence of a globally disseminated clone can be attributed to antimicrobial use in livestock. A human reservoir exists for nontyphoidal *Salmonella*, including serovar Typhimurium, in developing countries (24,25), and there is strong evidence that antimicrobial use in humans has not only driven the emergence of multidrug-resistant clones in these regions but has resulted in an increasingly high prevalence of multiple resistance (26-29). Dissemination of multidrug-resistant *Salmonella* from developing countries, through human traffic, is well documented (30,31) and seems a more likely mode of international transport than the far more limited international livestock traffic.

Multidrug-resistant clones capable of global dissemination can emerge as a result of antimicrobial selection pressure in either livestock or humans; simply restricting antimicrobial use in livestock populations cannot prevent broad dissemination. The problem of globally distributed multidrug-resistant bacterial clones can be compared to the nosocomial scenario: prudent antimicrobial use is a sensible step, but the main effort must go toward preventing dissemination if the program is to be effective.

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References
1. Centers for Disease Control and Prevention. U.S. Department of Health and Human Services. Salmonella surveillance. Annual Tabulation Summary 1996.
2. Ferris KE, Miller DA. Salmonella serotypes from animals and related sources reported during July 1996-June 1997. Proceedings of the 101st Annual Meeting of the United States Animal Health Association; 1997 Oct 18-24; Louisville, Kentucky. 1997. p. 423-43.
3. Oosterom J. Epidemiological studies and proposed preventive measures in the fight against human salmonellosis. Int J Food Microbiol 1991;12:41-51.
4. Khachatourians GG. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. CMAJ 1998;159:1129-36.
5. Piddock LJV. Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy? J Antimicrob Chemother 1996;38:1-2.
6. Van den Bogaard AE. Antimicrobial resistance—relation to human and animal exposure to antibiotics. J Antimicrob Chemother 1997;40:453-4.
7. Vernon R. Ciprofloxacin-resistant *Salmonella typhimurium* DT104 [letter]. Vet Rec 1998;142:287.
8. Institute of Medicine. Antimicrobial resistance: Issues and options. Workshop report, Forum on Emerging Infections. Washington: National Academy Press; 1998.
9. Bauer AW, Kirby MMW, Sherris JC, Turck M. Antibiotic susceptibility testing by a standard single disk method. Am J Clin Pathol 1966;45:493-6.
10. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. 5th ed. Approved standard; M2-A5, Vol. 13, No. 24. Villanova (PA): The Committee; 1993.
11. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. 5th informational supplement; M100-S5, Vol. 14, No. 16. Villanova (PA): The Committee; 1994.
12. Dean AG, Dean JA, Coulombier D, Brendel KA, Smith DC, Burton AH, et al. Epi Info, Version 6: A word-processing, database, and statistics program for public health on IBM-compatible microcomputers. Atlanta (GA:) Centers for Disease Control and Prevention; 1995.
13. Besser TE, Gay CC, Gay JM, Hancock DD, Rice D, Pritchett LC, et al. Salmonellosis associated with *S. typhimurium* DT104 in the USA. Vet Rec 1997;140:75.
14. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. N Engl J Med 1998;338:1333-8.

15. Threlfall EJ, Frost JA, Ward LR, Rowe B. Epidemic in cattle and humans of *Salmonella typhimurium* DT104 with chromosomally integrated multiple drug resistance. Vet Rec 1994;134:577.

16. Poppe C, Smart N, Khakhria R, Johnson W, Spika J, Prescott J. *Salmonella typhimurium* DT104: a virulent and drug-resistant pathogen. Can Vet J 1998;39:559-64.

17. Ridley A, Threlfall EJ. Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonella typhimurium* DT104. Microb Drug Resist 1998;4:113-8.

18. Casin I, Breuil J, Brisabois A, Moury F, Grimont F, Collatz E. Multidrug-resistant human and animal *Salmonella typhimurium* isolates in France belong predominantly to a DT104 clone with the chromosome- and integron-encoded ß-lactamase PSE-1. J Infect Dis 1999;179:1173-82.

19. Knapp WA Jr. Report of the Committee on Pharmaceuticals, Pesticides, and Related Toxicology. Proceedings of the 88th Annual Meeting of the United States Animal Health Association. 1984 Oct 21-26; Fort Worth, Texas.

20. Bolton LF, Kelley LC, Lee MD, Fedorka-Cray PJ, Maurer JJ. Detection of multidrug-resistant *Salmonella enterica* serotype *typhimurium* DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. J Clin Microbiol 1999;37:1348-51.

21. Khakhria R, Bezanson G, Duck D, Lior H. The epidemic spread of *Salmonella typhimurium* phage type 10 in Canada (1970-1979). Can J Microbiol 1983;29:1583-8.

22. Passaro DJ, Reporter R, Mascola L, Kilman L, Malcolm GB, Rolka H, et al. Epidemic *Salmonella enteritidis* infection in Los Angeles County, California—the predominance of phage type 4. West J Med 1996;165:126-30.

23. Hancock DD, Lynn TV, Besser TE, Wikse SE. Feasibility of preharvest food safety control. Compendium on Continuing Education for the Practicing Veterinarian 1997;19:S200-7.

24. Gracey M, Iveson JB, Sunoto, Suharyono. Human salmonella carriers in a tropical urban environment. Trans R Soc Trop Med Hyg 1980;74:479-82.

25. Vahaboglu H, Dodanli S, Eroglu C, Öztürk R, Soyletir G, Yildirim I, et al. Characterization of multiple-antibiotic-resistant *Salmonella typhimurium* strains: molecular epidemiology of PER-1-producing isolates and evidence for nosocomial plasmid exchange by a clone. J Clin Microbiol 1996;34:2942-6.

26. Kariuki S, Gilks C, Corkill J, Kimari J, Benea AP, Hart CA. Multi-drug resistant non-typhi salmonellae in Kenya. J Antimicrob Chemother 1996;38:425-34.

27. Agarwal KC, Garg RK, Panhotra BR, Verma AD, Ayyagari A, Mahanta J. Drug resistance in Salmonellae isolated at Chandigarh (India) during 1972-1978. Antonie Van Leeuwenhoek 1980;46:383-90.

28. Sharma KB, Bhat MB, Pasricha A, Vaze S. Multiple antibiotic resistance among salmonellae in India. J Antimicrob Chemother 1979;5:15.

29. Ling J, Chau PY, Rowe B. Salmonella serotypes and incidence of multiply-resistant salmonellae isolated from diarrhoeal patients in Hong Kong from 1973-1982. Epidemiol Infect 1987;99:295-306.

30. Centers for Disease Control. Multiresistant *Salmonella* and other infections in adopted infants from India. MMWR Morb Mortal Wkly Rep 1982;31:285-7.

31. Seyfarth AM, Wegener HC, Frimodt-Møller. Antimicrobial resistance in *Salmonella enterica* subspecies *enterica* serovar *typhimurium* from humans and production animals. J Antimicrob Chemother 1997;40:67-75.

Toxic Shock Syndrome in the United States: Surveillance Update, 1979-1996[1]

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Menstrual toxic shock syndrome (TSS) emerged as a public health threat to women of reproductive age in 1979-80. We reviewed surveillance data for the period 1979 to 1996, when 5,296 cases were reported, and discuss changes in the epidemiologic features of TSS.

Toxic shock syndrome (TSS) emerged as a result of changes in industry and personal behavior but responded to rapid public health action, including active surveillance (1). This illness received national attention in 1980 when unexplained febrile illness associated with shock, multiorgan dysfunction, and high death rates was reported in healthy young women from several states (2,3). This clinical syndrome had been described sporadically since the 1920s (4). The dramatic increase in the number of cases in 1979-80 spurred epidemiologic, clinical, and laboratory studies that resulted in better understanding of the association between high-absorbency tampons and TSS (5). These studies led to recommendations that substantially decreased the risk for TSS (6,7).

TSS became a nationally notifiable disease in 1980 (8). After the initial epidemic, the number of reported cases decreased significantly. In 1986, active surveillance was conducted in many areas in the United States (total population 34 million) to confirm that trend (9). The cumulative incidence (0.5 per 100,000 population) confirmed the substantial decrease in the incidence of menstrual TSS observed in the passive surveillance system. Incidence rates decreased from 6 to 12 per 100,000 among women 12 to 49 years of age (10,11) in 1980 to 1 per 100,000 among women 15 to 44 years of age in 1986. These data

also demonstrated that passive surveillance accurately described the demographic characteristics and case-fatality ratio of TSS cases (12,13).

Ongoing surveillance in the United States allows us to estimate the current incidence of TSS and monitor whether new menstrual or vaginal products affect the risk for disease. Other products, including high-absorbency disposable diapers, have raised similar questions regarding TSS in children. To address these questions and describe the current epidemiologic characteristics and recent temporal trends of the syndrome, we reviewed data from the ongoing national surveillance for TSS from 1979 through 1996, focusing on three periods: 1979 to 1980 (the epidemic years), 1981 to 1986 (a period of increased TSS awareness, culminating in active surveillance in 1986), and 1987 to 1996.

The Study

The national TSS surveillance system has been described (8). Cases of TSS are reported to the Centers for Disease Control and Prevention (CDC) by state health departments in standardized case reports that include information on demographic and clinical characteristics, hospitalization status, outcome, laboratory data, products used during menses, and recurrence of menstruation-associated cases.

We used the surveillance case definition for TSS revised in 1981, which requires five clinical criteria: fever, hypotension, rash, desquamation, and abnormalities in three or more organ systems (8). A definite case fulfilled all five

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criteria (unless the patient died before desquamation), and a probable case fulfilled four of the five criteria. For the purposes of this analysis and because information on desquamation was often unavailable because of death or early discharge, we included all TSS reports meeting either definition. A similar change regarding exclusion of desquamation was adopted in a retrospective study (14).

A TSS case was considered menstrual if onset of symptoms occurred within 3 days of the beginning or end of menses; all other cases were considered nonmenstrual and were classified into three categories: surgical wounds, postpartum or postabortion, and other. Information on recurrent episodes of TSS was collected only from women with menstrual TSS, by asking them about a similar previous illness during menstruation. Data were analyzed by using SAS 6.1 (SAS, Cary, NC). The chi-square test was used to test for statistical significance.

From 1979 to 1996, 5,296 TSS cases were reported; 1,035 of these were reported from 1987 to 1996 (Figure). Overall, 93% of all TSS cases
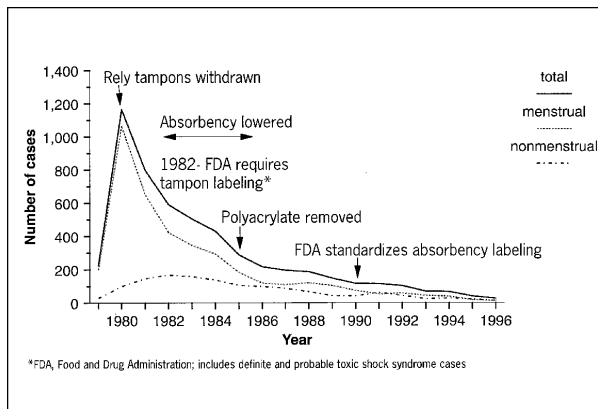
Figure. Toxic shock syndrome cases,* menstrual vs. nonmenstrual, United States, 1979–1996.

reported from 1979 to 1996 were among women. Although the proportion of menstrual cases decreased (Table), TSS affected mostly women. The median age was 22 years (3 days to 87 years); 91% were white. No significant seasonal variation was noted. For cases in which the source of the report was known (n = 2,118), 64% were reported by infection control practitioners and 28% by physicians.

Menstrual TSS accounted for 74% of TSS cases during 1979 to 1996 (n = 5,296); this proportion, however, declined from 91% during 1979 and 1980 to 71% during 1981 to 1986 and 59% during 1987 to 1996. The median age of patients with menstrual TSS was 21 years, but 41% (n = 1,591) of menstrual TSS cases occurred in female patients 13 to 19 years of age. No significant change in the age distribution of menstrual cases was noted between the three periods (median age: 21 years, 1979 to 1980; 20 years, 1981 to 1986; 25 years, 1987 to 1996). Most (98%) patients with menstrual TSS cases for which the menstrual product was known (n = 3,457) reported tampon use, a proportion that did not change; 89% used tampons only, while 5.0% used both tampons and pads and 3% used tampons and minipads. The level of tampon absorbency was reported in only 41% (n = 1,385) of cases: 28% of patients used regular tampons, while 71% used super-absorbency tampons. *Staphylococcus aureus* was isolated from 90% of women with menstrual TSS who had vaginal cultures performed (n = 2,536). Of all the women with menstrual TSS, 1,606 (30%) responded to the question regarding recurrent disease, and 10% reported a previous illness similar to their TSS episode.

Nonmenstrual cases also occurred mostly in women (73%) and whites (87%). During 1987 to 1996, the proportion of all TSS cases that were

Table. Demographic characteristics, outcome, and proportion of menstrual cases of toxic shock syndrome, United States, 1979–1996

| Characteristic | No. (%)a by years | | | |
	1979-80 (n = 1,392)	1981-86 (n = 2,835)	1987-96 (n = 1,069)	Total cases (n = 5,296)
Female	1,365 (98)	2,594 (92)	958 (90)	4,917 (93)
Median age (yrs), (range)	21 (1-70)	22 (1-87)	25 (3d-82)	22 (3d-87)
White race	1,067 (77)	2,564 (90)	967 (90)	4,598 (91)
Menstrual cases	1,264 (91)	2,021 (71)	636 (59)	3,921 (74)
Deaths (Case-fatality ratio [%])	77 (6)	95 (3.5)	36 (3.5)	208 (4)

aCalculations were done by using as denominator the number of persons for whom the information on the specific characteristic was available.

nonmenstrual averaged 41% (30% to 55%). Of nonmenstrual cases, 18.3% were reported after surgical procedures, 11.5% were postpartum or postabortion, and 23.1% had nonsurgical cutaneous lesions. The proportion of all nonmenstrual cases reported after surgical procedures increased from 14% during 1979 to 1986 to 27% during 1987 to 1996 (p <0.05). Among female nonmenstrual case-patients, 12% reported using barrier contraceptives (sponges and diaphragms); this proportion was, however, significantly less (p <0.05) in 1987 to 1996 (6%) than in 1979 to 1986 (14%). Fifty cases of TSS in children $\leq$ 5 years of age were reported during the 17-year period; more than half of these occurred in children $\leq$ 2 years of age, and most (61.7%) were associated with nonsurgical cutaneous lesions. The percentage of TSS associated with nonsurgical cutaneous lesions (23.1%) was higher among younger patients (overall case-fatality ratio 4% [n = 2 deaths]) than among patients with other nonmenstrual cases.

Hospitalization status was known for 2,930 patients; 98% were hospitalized. The overall TSS case-fatality ratio was 4.1% (3% for menstrual cases, 5% for nonmenstrual cases) and was significantly higher among nonmenstrual cases (p <0.005). Among menstrual cases, the case-fatality ratio decreased significantly with time (from 5.5% in 1979 and 1980, to 2.8% in 1981 to 1986, to 1.8% in 1987 to 1996, chi-square for linear trend [p = 0.0001]). This trend was not observed among nonmenstrual cases (for the three time periods: 8.5%, 5.3%, and 6%, respectively).

Conclusions

Our review of recent passive surveillance data confirms the declining trend previously noted by active surveillance in 1986. Changes observed in the epidemiologic characteristics of TSS include an increase in the proportion of nonmenstrual cases and the difference in the risk for death between menstrual and nonmenstrual cases.

A number of factors could account for the observed decline, including the decrease in tampon absorbency, the standardized labeling required by the U.S. Food and Drug Administration; greater awareness of TSS among women; and the proliferation of educational materials for women, including tampon package inserts (12). However, at least 40% of menstrual TSS cases

continue to affect women 13 to 19 years old, an age group not as likely to be aware of the risk for TSS and for whom further education may be needed.

Over the last few years, two changes have occurred in tampon use and composition: 1) All-cotton tampons have been introduced and marketed as an alternative product; in vitro studies have not, however, found any differences in the effects of these new tampons on the production of toxic shock syndrome toxin-1 (TSST-1) or its adsorption (15). 2) Tampons marketed specifically for overnight use have also been introduced. TSST-1 toxin production is not the only indicator of the potential risk for TSS. Previous case-control studies found that exclusive use of tampons was associated with a higher risk for TSS than use in conjunction with pads (13). Continued surveillance will monitor the effect of these changes on TSS occurrence. However, because the syndrome is rare, only large changes in the use of higher absorbency products would be likely to come to public health attention.

One of the important changes in the epidemiology of TSS is the increasing proportion of nonmenstrual cases, in particular, cases reported after surgical procedures. The factors contributing to this increase may include changes in delivery of surgical health-care services, with an increase in both outpatient procedures and the use of prosthetic devices. Hospitalizations caused by infections from prosthetic devices and postoperative infections increased significantly from 1980 to 1994 in the United States (16). In addition, the case-fatality ratio of nonmenstrual TSS cases did not decline, although the case-fatality ratio of menstrual TSS did. This difference in death rates could be due to several factors: nonmenstrual TSS may occur in less healthy (e.g., older) persons; diagnostic and reporting biases may result in more sensitive detection of severe cases of nonmenstrual TSS; decreased awareness of nonmenstrual TSS among health-care professionals may lead to increased deaths because of late treatment; and the nonspecific signs and symptoms of postoperative TSS may delay diagnosis (17). Further studies are needed to validate the difference in deaths between the two types of TSS and to clarify the risk factors for nonmenstrual TSS. Recent molecular studies of the gene that carries toxic shock toxin (18) may contribute to a better understanding of the emergence of TSS and

transmission of toxin-producing strains of *S. aureus* and could improve prevention. Physicians, in particular surgeons, and other health-care professionals may need further education about the risks for nonmenstrual TSS.

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References

1. U.S. Public Health Services. Addressing emerging infectious disease threats. A prevention strategy for the United States. Atlanta: Centers for Disease Control and Prevention; 1994.
2. Todd JK, Kapral FA, Fishaut M, Welch TR. Toxic shock syndrome associated with phage group 1 staphylococci. Lancet 1978;2:1116-8.
3. Centers for Disease Control and Prevention. Toxic-shock syndrome—United States. MMWR Morb Mortal Wkly Rep 1980;29:229-30.
4. Broome CV. Epidemiology of toxic shock syndrome in the United States: Overview. Reviews of Infect Diseases 1989:2 Suppl 1:S14-21.
5. Shands KN, Schmid GP, Dan BB, Blum D, Guidotti RJ, Hargrett NT, et al. Toxic-shock syndrome in menstruating women: association with tampon use and *Staphylococcus aureus* and clinical features in 52 cases. N Engl J Med 1980:303:1436-42.
6. Centers for Disease Control and Prevention. Toxic shock syndrome—United States. MMWR Morb Mortal Wkly Rep 1980:297–9.
7. Centers for Disease Control and Prevention. Follow-up on toxic-shock syndrome. MMWR Morb Mortal Wkly Rep 1980;29:441-5.
8. Reingold AL, Hargrett NT, Shands KN, Dan BB, Schmid GP, Strickland BY, et al. Toxic shock syndrome surveillance in the United States, 1980 to 1981. Ann Intern Med 1982;96 (part 2):875-80.
9. Gaventa S, Reingold AL, Hightower AW, Broome CV, Schwartz B, Hoppe C, et al. Active surveillance for toxic shock syndrome in the United States. 1986. Reviews of Infectious Diseases 1989:2 Suppl 1:S28-34.
10. Davis JP, Chesney PJ, Wand PJ, LaVenture M. Toxic-shock syndrome: epidemiologic features, recurrence, risk factors and prevention. N Engl J Med 1980:303:1429-35.
11. Latham RH, Kehrberg MW, Jacobson JA. Toxic shock syndrome in Utah: a case-control and surveillance study. Ann Intern Med 1982:96:906-8.
12. Schuchat A, Broome CV. Toxic shock syndrome and tampons. Epidemiol Rev 1991:13:99-112.
13. Centers for Disease Control and Prevention. Reduced incidence of menstrual toxic shock syndrome—United States, 1980-1990. MMWR Morb Mortal Wkly Rep 1990;39:421-4.
14. Petitti DB, Reingold AL. Update through 1985 on the incidence of toxic shock syndrome among members of a prepaid health plan. Reviews of Infectious Diseases 1989;11 Suppl 10:S22-7.
15. Schlievert PM. Comparison of cotton and cotton/rayon tampons for effect on production of toxic shock syndrome toxin. J Infect Dis 1995;172:1112-4.
16. Simonsen L, Conn LA, Pinner RW, Teutsch S. Trends in infectious disease hospitalizations in the United States, 1980-1994. Arch Intern Med 1998;158:1923-8.
17. Hughes D, Stapleton J. Postoperative toxic shock syndrome. Iowa Med 1991;81:55-8.
18. Lindsay J, Ruzin A, Ross HF, Kurepina N, Novick RP. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. Mol Microbiol 1998;29:527-43.

New *Rickettsiae* in Ticks Collected in Territories of the Former Soviet Union

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Dermacentor nuttallii from Siberia, *Rhipicephalus sanguineus* from Crimea, and *Rh. pumilio* from the Astrakhan region were infected with *Rickettsia sibirica* (12%), *R. conorii* (8%), and the Astrakhan fever agent (3%), respectively. Three new *Rickettsiae* of the *R. massiliae* genogroup were identified in ticks by 16S rDNA, *gltA,* and *ompA* sequencing.

Rickettsiae are obligate intracellular gram-negative bacteria associated with arthropod vectors, ticks, mites, and insects that, while feeding, can transmit *Rickettsiae* to animals and humans. The rickettsioses have characteristic clinical features, including fever, headache, maculopapular eruption, and sometimes eschar formation (primary lesion). The number of representatives of the genus *Rickettsia* has increased over the last decades as a result of improved cell culture isolation and agent identification techniques (1). Sequence comparison of gene coding for citrate synthase (*gltA*) (2), rOmpA outer membrane protein (*ompA*) (3), and 16S rRNA (4) has become the most reliable method of identifying *Rickettsiae* (5-8). We describe polymerase chain reaction (PCR) amplification and sequence determination to identify *Rickettsiae* in naturally infected ixodid ticks in three regions of Russia endemic for tickborne rickettsioses.

Rhipicephalus pumilio ticks (65 adults) were collected in 1996 from dogs in the Astrakhan region. *Dermacentor nuttallii* ticks (101 adults) were collected in 1994 in the village of Verhnyi Kouus, the Altay Mountains, Siberia. In 1997, *Rh. sanguineus* ticks (2 adults and 35 nymphs) were collected in the town of Saki, Crimea

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region, from dogs whose owners had serologic evidence of Mediterranean spotted fever (Figure 1). The ticks were kept at room temperature before being washed in iodized alcohol (10 minutes) just before testing, rinsed in distilled water, and dried on sterile filter paper. DNA was extracted from ticks by using the QIAmp Tissue Kit (QIAGEN, Hilden, Germany). Rickettsial DNA was detected by PCR with primers specific for *Rickettsiae*: RpCS.877p-RpCS.1273r, which amplify a 396-bp fragment of *gltA* (2), and Rr190.70p-190-701 (3), which amplify a fragment of *ompA* from 629 to 632 bp. For all positive ticks, 587 to 590 bp of *ompA* were sequenced by using the ABI PRISM Dye
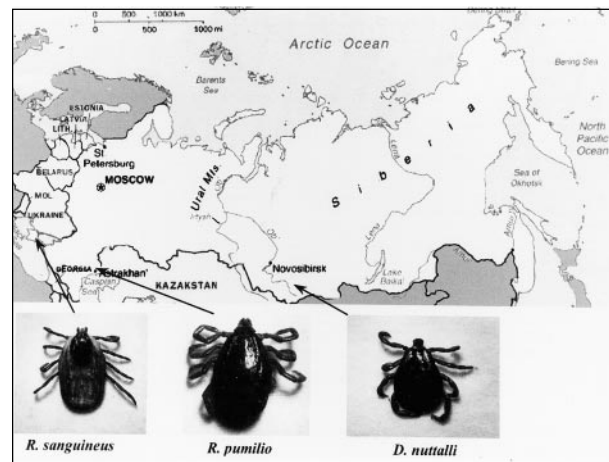
Figure 1. Areas from which ticks in the study were collected.

Terminator Cycle Sequencing Kit with Amplitap Polymerase FS (PE Applied Biosystems, Warrington WA1 4SR, UK). Sequences were analyzed with the Applied Biosystem 377 automatic sequencing system. For newly detected genotypes, sequences of 16S rRNA encoding gene, *gltA,* and *ompA* were determined as previously described (2-4) (see Figure 2 for GenBank codes).

We detected two different *Rickettsiae* in *Rh. pumilio* (Astrakhan fever agent and RpA4 genotype); two *Rickettisiae* from *D. nutallii* in Siberia (*Rickettsia sibirica* and DnS14 and DnS 28 genotypes); and *R. conorii* from *Rh. sanguineous* ticks in Crimea (Table).

Our results confirm previous data of high epidemic activity of the Altay focus for North Asian tick typhus and the crucial role of *D. nuttallii* as a reservoir of *R. sibirica* infection (9). Our results are also consistent with those of a study in 1991 based on hemolymph testing (10), in which 3.2% of ticks from the Astrakhan region were demonstrated to be infected with *Rickettsiae*.

An outbreak of Mediterranean spotted fever due to infection with *R. conorii* occurred in Crimea from 1947 to 1957. Only sporadic cases of the disease were reported (11) until 1995, when the incidence of Mediterranean spotted fever increased in central Crimea, with 40 cases in 1996 and more than 70 in 1997. Most cases occurred in the summer, when the *Rh. sanguineus* nymphs (principal vectors of *R. conorii*) (1) were active. Our results, showing that 8% of the *Rh. sanguineus* studied contained *R. conorii* DNA, provide further evidence of the Mediterranean spotted fever outbreak in the region. To date, only the *R. conorii* strain M-1, isolated in the territories of the former Soviet Union (the Black Sea coast of Georgia), has been genetically characterized. This strain is geneti-

cally distinct from the other strains of *R. conorii*, i.e., Indian tick typhus and the Moroccan and Malish strains (3). Our detection of the *R. conorii* strain identical to the Malish strain is the first evidence of the genetic heterogeneity of *R. conorii* in the region.

The *ompA* sequences obtained from PCR-amplified products were different from those described for the known *Rickettsiae* for one DNA sample extracted from *Rh. pumilio* from the Astrakhan region (RpA4) and four DNA samples from *D. nuttallii* collected in Siberia (DnS14, DnS28, DnS79, DnS94). The sequences for the samples from *D. nuttalii* (DnS28, DnS79, and DnS94) were identical but differed from those of DnS14 and *Rh. pumilio* RpA4/2.

The three new rickettsial agents were closely related and branched with members of the *R. massiliae* group, together with *R. rhipicephali*, Bar 29, *R. aeschlimannii*, and *R. montanensis* (Figure 2). Comparison of the sequences obtained by using the program BLAST demonstrated that they also differed from those of the Cadiz agent characterized from *Ixodes ricinus* in Spain (6), those of the *Cooleyi* genotype characterized from *I. scapularis* (5), MOA and WB-8-2 isolated from *Amblyomma americanum* and *I. scapularis,* respectively (8), and *R. peacockii* (7) in the United States.

The pathogenicity of the members of the *R. massiliae* group is unknown, and their main reservoirs are regarded as ticks of the genus *Rhipicephalus* for *R. massiliae* and Bar 29. *R. aeschlimannii* has been isolated from *Hyalomma marginatum* and *R. montanensis* from ticks of the genus *Dermacentor*. *R. rhipicephali* has been demonstrated in ticks of the genus *Dermacentor* and in *Rh. sanguineus* (1). The similarity of *gltA, ompA,* and 16S rRNA gene sequences indicates that these three new

Table. Ticks infected in regions of the former Soviet Union

| Tick species	Location	No. positive ticks/total examined	% infected ticks	Rickettsial species
Rhipicephalus pumilio	Astrakhan region	2/65	3	Astrakhan fever agent
Rh. pumilio	Astrakhan region	1/65	1.5	RpA4 genotype
Dermacentor nutallii	Siberia	12/101	12	*Rickettsia sibirica*
D. nutallii	Siberia	4/101	4	DnS14 and DnS28 genotypes
Rh. sanguineus	Crimea	3/37	8	*R. conorii* (Malish strain)
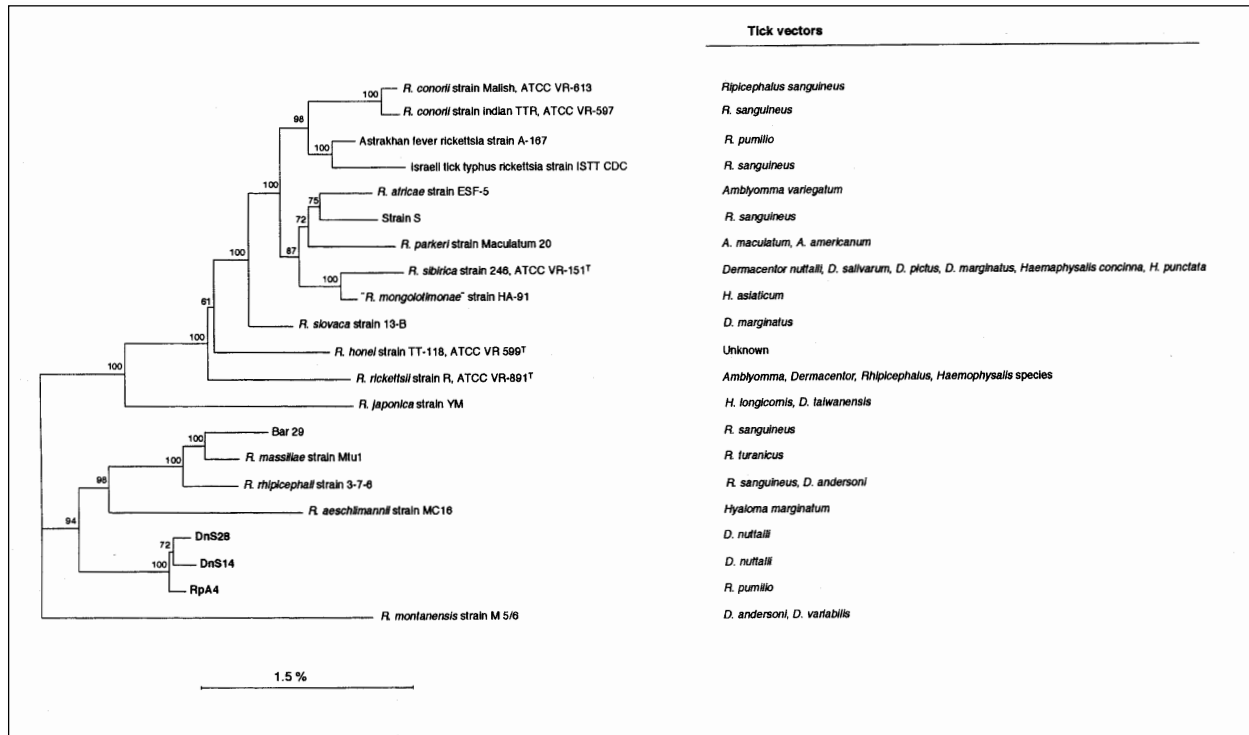
Figure 2.[1] Phylogenetic tree of *Rickettsiae* inferred from comparison of the *ompA* sequences. The known tick vectors for the bacteria presented on the dendrogram are indicated on the right. The *ompA* sequences were aligned by the multisequence alignment program CLUSTAL in the BISANCE software package. Phylogenetic relationships were inferred by using version 3.4 of the PHYLIP software package. Evolutionary distance matrices, generated by DNADIST, were determined by the Kimura method. Matrices were used to construct dendrograms by the neighbor-joining method. Data were also examined by using parsimony analysis (DNAPARS in PHYLIP), and bootstrap analyses were performed to investigate the stability of the trees obtained. Bootstrap values were obtained for a consensus tree based on 100 randomly generated trees by using SEQBOOT and CONSENSE in the same package. The percentage of similarity between strains was determined by using the PC/GENE software package.

[1]The received sequences of the new rickettsial agents have been deposited in GenBank. Sequences of *ompA* were deposited as two parts under accession numbers: DnS28, AF120018 and AF120019; DnS14, AF120021, and AF120020; RpA4, AF120022, and AF120023. The 16S rRNA gene and *gltA* sequences have been deposited in GenBank under accession numbers DnS28, *gltA* - AF120027, 16S rRNA gene – AF120024; DnS14, *gltA* – AF120028, 16S rRNA gene – AF120025; RpA4, *gltA* - AF120029, 16S rRNA gene – AF120026.

agents are close to each other (from 99.7% to 99.9%) and could constitute a new rickettsial species.

In the United States, various tickborne *Rickettsiae* occur in areas endemic for *R. rickettsii,* the agent of Rocky Mountain spotted fever (7). Similarly, in Mediterranean countries, several recently described *Rickettsiae* have been found in ticks of the *Rh. sanguineus* complex in the regions endemic for Mediterranean spotted fever caused by *R. conorii* (6). The effects of the presence of different *Rickettsiae* on the prevalence of infection rates of ticks with individual *Rickettsiae* and on the epidemiology of infections in humans have yet to be determined. *R. sibirica* and the Astrakhan fever agent are prevalent in Siberia and the Astrakhan region, respectively, but the pathogenicity of the new rickettsial genotypes has yet to be investigated.

References

1. Raoult D, Roux V. Rickettsioses as paradigms of new or emerging infectious diseases. Clin Microbiol Rev 1997;10:694-719.
2. Roux V, Rydkina E, Eremeeva M, Raoult D. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the *Rickettsiae*. Int J Syst Bacteriol 1997;47:252-61.
3. Fournier P-E, Roux V, Raoult D. Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein rOmpA. Int J Syst Bacteriol 1998;48:839-49.
4. Roux V, Raoult D. Phylogenetic analysis of the genus *Rickettsia* by 16S rDNA sequencing. Res Microbiol 1995;146:385-96.
5. Billings AN, Teltow GJ, Weaver SC, Walker DH. Molecular characterization of a novel *Rickettsia* species from *Ixodes scapularis* in Texas. Emerg Infect Dis 1998;4:305-9.
6. Marquez FJ, Muniain MA, Soriguer RC, Izquierdo G, Rodriguez-Baño J, Borobio M. Genotypic identification of an undescribed spotted fever group *Rickettsia* in *Ixodes ricinus* from southwestern Spain. Am J Trop Med Hyg 1998;58:570-7.
7. Niebylski ML, Shrumpf ME, Burgdorfer W, Ficher ER, Gage KL, Schwan TG. *Rickettsia peacockii* sp. nov., a new species infecting wood ticks, *Dermacentor andersoni*, in Western Montana. Int J Syst Bacteriol 1997;47:446-52.
8. Weller SJ, Baldridge GD, Munderloh UG, Noda H, Simser J, Kurtti T. Phylogenetic placement of *Rickettsiae* from ticks *Amblyomma americanum* and *Ixodes scapularis*. J Clin Microbiol 1998;36:1305-17.
9. Rudakov NV. Tick-borne rickettsiosis in Russia (epidemiology and current conditions of natural foci). In: Kazar J, Toman R, editors. Rickettsiae and rickettsial diseases. Proceedings of the 5th International Symposium; 1996; Veda, Bratislava. p. 216-20.
10. Eremeeva ME, Beati L, Makarova VA, Fetisova NF, Tarasevich IV, Balayeva NM, et al. Astrakhan fever rickettsiae: antigenic and genotypic analysis of isolates obtained from human and *Rhipicephalus pumilio* ticks. Am J Trop Med Hyg 1994;51:697-706.
11. Kulagin SM, Tarasevich IV, Rubakin PE, Nikitin AM, Krupina ZN. On eradication of Marseilles fever (in Russian). J Microbiol Epidemiol Immunobiol 1960;8:117-21.

Computer-Generated Dot Maps as an Epidemiologic Tool: Investigating an Outbreak of Toxoplasmosis

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We used computer-generated dot maps to examine the spatial distribution of 94 *Toxoplasma gondii* infections associated with an outbreak in British Columbia, Canada. The incidence among patients served by one water distribution system was 3.52 times that of patients served by other sources. Acute *T. gondii* infection among 3,812 pregnant women was associated with the incriminated distribution system.

Epidemiologists have traditionally used maps to examine the spatial distribution of disease incidence. Computer-generated dot maps have facilitated identification of case clusters (1), formulation of hypotheses about the source of infection or spatially distributed risk factors (2,3), and analysis of data. Because most populations are served by identifiable water systems, waterborne disease outbreaks lend themselves to being plotted on dot maps (1,4-6). We describe an automated address-matching and base map system in a geographic information system structure used to assess information related to an outbreak of toxoplasmosis associated with a municipal water system.

The Capital Regional District (population 321,585) is located on Vancouver Island, British Columbia, Canada. The district includes the City of Victoria, surrounding municipalities and districts, and the Gulf Islands.

In March 1995, an outbreak of toxoplasmosis was suspected when 15 residents of this district were identified as having acute infection with *Toxoplasma gondii*. Investigation of these and subsequent cases confirmed an outbreak but

identified no common food, beverage, or event source. A hand-drawn dot map of the initial 47 cases showed clustering in the Greater Victoria area. The municipal water supply system was considered a possible explanation for this spatial distribution. To examine this hypothesis, computer-based geographic mapping was used to study the distribution of all outbreak-related acute cases and data collected from a population-based serologic screening program to detect *T. gondii* infection in women who were or had been pregnant (7).

Classification of Cases and the Water Distribution Systems

Patients were classified as having acute, equivocal, or nonacute cases or as never infected on the basis of serologic tests performed at the Provincial Laboratory (British Columbia Centre for Disease Control) and the Toxoplasma Serology Laboratory, Research Institute, Palo Alto Medical Foundation (8-11). Cases were further classified on the basis of clinical symptoms, outbreak relatedness, patient's residence, and pregnancy status (pregnant, non-pregnant) (7).

At the time of the outbreak, the Greater Victoria Water District operated two disinfection plants supplying unfiltered, chloraminated

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surface water to approximately 292,000 residents of the Capital Regional District. The higher-pressure Japan Gulch distribution system supplied water from the Japan Gulch Reservoir to 73,000 residents, as well as a one-way transfer of water into the other distribution system. The Humpback distribution system supplied 219,000 residents (12). District residents were further classified as receiving high, intermediate, or no exposure to water from the Humpback Reservoir.

Geographic Mapping

Geographic mapping analysis was performed by using MapInfo Version 3.0 for Windows (MapInfo Corporation, Troy, NY, 1992-94). MapInfo, which enables data containing geographic information to be placed on a map, was used for geocoding (i.e., placing markers into the database, like pins onto a map). Street address data for each record in the datafile were matched against an electronic street map of the Capital Regional District. Geographic coordinates were taken from the electronic street map of the district, which is based on the Transportation Centerline Network of the British Columbia Ministry of Transportation and Highways (13). Lotus Approach 3.0 for Windows (Applied Software Corporation, subsidiary of Lotus Development Corporation) was used to prepare and validate the data before mapping. On the basis of information from the Greater Victoria Water District, a street-level scale map was drawn in MapInfo to delineate the geographic area in the Capital Regional District that receives municipal drinking water from the Humpback Reservoir.

Geographic Distribution of Acute Cases

All 94 persons with outbreak-related acute cases who lived in the Capital Regional District were grouped by geographic area as being served by the Humpback Reservoir or other water sources. Incidence rates were calculated by using estimated population figures (1994). Information on residential addresses was obtained from laboratory requisitions, physicians' offices, hospital records, the Medical Services Plan of British Columbia, and direct communication with patients. For each case, the residential street address at the time the first specimen was drawn for testing for *T. gondii* antibodies was used to produce a computer-generated dot map.

Of the 94 persons with outbreak-related acute cases who lived in the Capital Regional District, 83 (88%) lived in the area served by the Humpback Reservoir. The incidence rate of acute infection among persons residing in the area served by the Humpback Reservoir was more than three times that for areas served by other sources (RR = 3.53; 95% confidence interval [CI]: 1.88-6.63; p = 0.0003) (Figure 1).
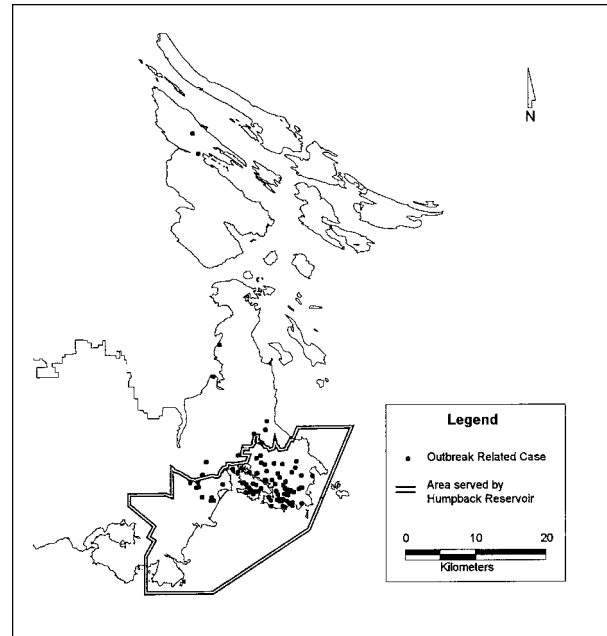
Figure 1. Geographic distribution of outbreak-related acute cases of toxoplasmosis in the Capital Regional District, Vancouver Island, British Columbia, 1995 (n = 94).

Geographic Distribution of Women Screened during Pregnancy

Data from a population-based screening program were used to determine whether residents served by the Humpback Reservoir were more likely to have acute infection with *T. gondii*. Serologic screening was offered to an estimated 4,500 women living in the capital regional district who were pregnant between October 1, 1994, and April 30, 1995. To offer screening to as many of these women as possible, information regarding the screening program was extensively distributed to women, physicians, and the public.

Serologic results were available from the Provincial Laboratory database at the British Columbia Centre for Disease Control. Residen-

tial street addresses were obtained by linking the Provincial Laboratory database with the Medical Services Plan database, using the unique personal health number assigned by the Province. A computer datafile containing the serologic results of screened pregnant women and their addresses was provided to the Capital Regional District Health Department. The data were then prepared and validated, geocoded and mapped, and statistically analyzed.

Three dot maps were generated by MapInfo, showing the geographic distribution of the screened population according to their laboratory classification of never infected (i.e., no serologic evidence of immunoglobulin [Ig] G or IgM antibody to *T. gondii*), nonacute (i.e., typically IgG but not IgM antibody to *T. gondii*), and acute (i.e., serologic evidence of acute infection by a battery of tests) (7-11). Several data subsets were generated on the basis of two variables: 1) residence in the area served by the Humpback Reservoir and 2) municipality of residence. Odds ratios were then calculated by StatCalc in Epi Info (version 6.03) to test the hypothesis that living in the area served by the Humpback Reservoir was associated with infection with *T. gondii*.

Of 3,982 laboratory records for screened women, 3,962 records were available for coding. The 3,812 women with successfully coded addresses comprise 85% of the estimated 4,500 women eligible for screening. Of these women, 36 (0.9%) were classified according to their laboratory results as having acute infection, 216 (5.7%) as having a nonacute infection, 3,558 (93.3%) as never having been infected, and 2 (0.1%) as having equivocal cases. The age distribution of the women (15 to 45 years [mean 29 years]) did not differ by infection status (women with equivocal status were excluded). Women acutely infected with *T. gondii* were more than three times as likely as uninfected women to live in the area served by the Humpback distribution system (odds ratio [OR] 3.05, exact 95% CI: 1.08-11.91). In contrast, the geographic distribution of women with serologic evidence of nonacute infection with *T. gondii* was not associated with the municipal water distribution system (OR 0.91, exact 95% CI: 0.67-1.25).

Computer-generated dot maps (Figures 2, 3, 4) provided visual information, as well as data for
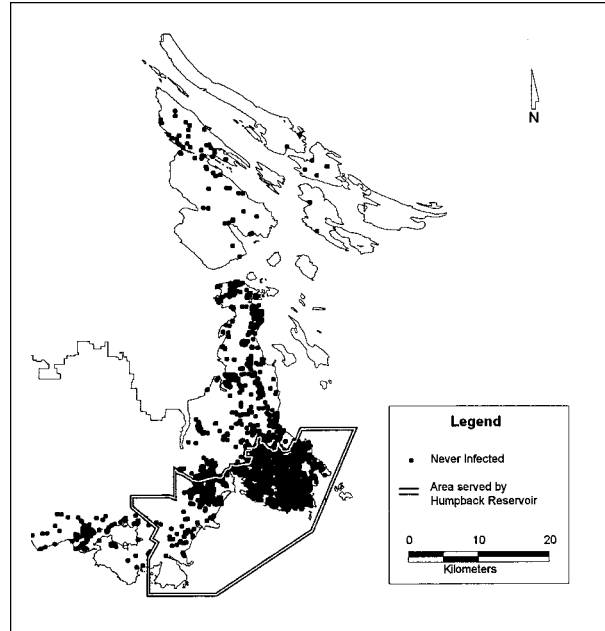
Figure 2. Geographic distribution of the residences of women screened during pregnancy and classified as never infected with toxoplasmosis (n = 3558).
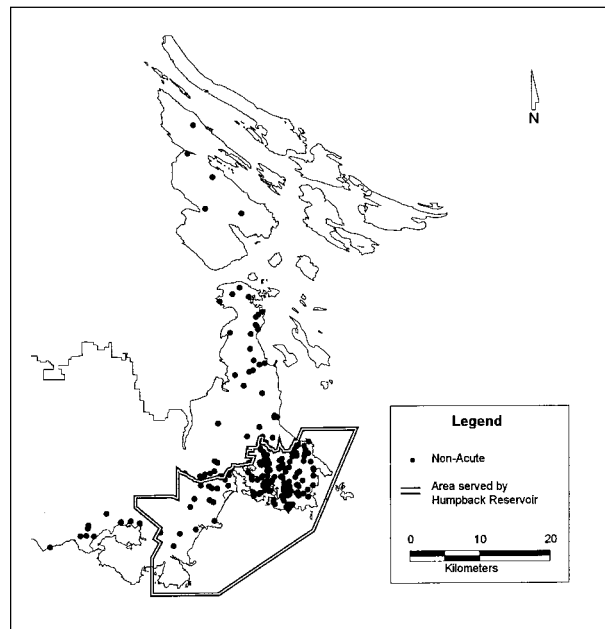
Figure 3. Geographic distribution of the residences of women screened during pregnancy and classified as having nonacute cases of toxoplasmosis (n = 216).
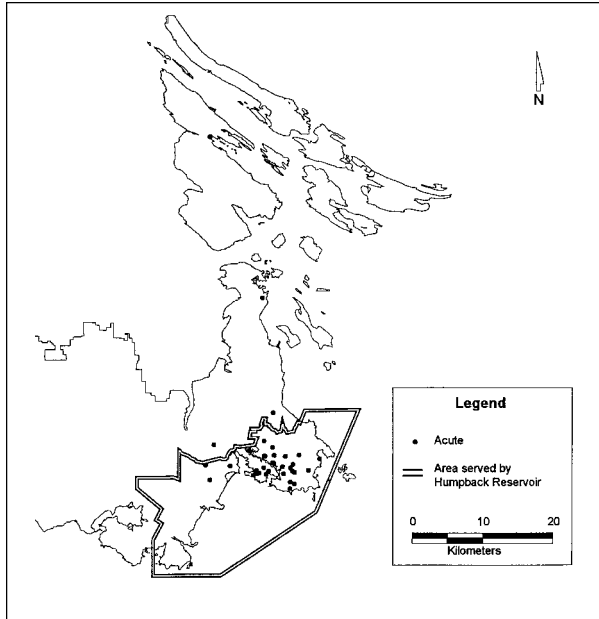
Figure 4. Geographic distribution of the residences of women screened during pregnancy and classified as having acute cases of toxoplasmosis (n = 36).

statistical analysis. These maps are characteristic of an outbreak associated with a one-time event. The geographic distribution of pregnant women who were never infected (Figure 2) indicates that the susceptible population is similar to the distribution of the whole population. The geographic distribution of pregnant women who had nonacute cases (Figure 3) is similar to the population distribution, indicating that before the outbreak, residence was not associated with antibody. Visually comparing the geographic distribution of pregnant women with acute cases (Figure 4) with that of pregnant women who were never infected (Figure 2) suggests that most of the recently infected pregnant women lived in the area served by the Humpback Reservoir. The

difference between the distributions seen in Figures 3 and 4 suggests that this event was new and unusual, rather than an ongoing or intermittent exposure that had not been recognized.

Well-defined geographic areas of the Capital Regional District were classified as receiving high, intermediate, and no exposure to water from the Humpback Reservoir (exposure scores 3, 2, and 1, respectively). An analysis for linear trend in proportions was performed by using StatCalc in Epi Info to demonstrate whether a dose-response relationship was present.

If water from the Humpback Reservoir was the source of infection, the rate of infection would be expected to increase with increased exposure. The Table shows the geographic distribution of women according to the estimated concentration of water from Humpback Reservoir received at their residence. When acutely infected women were compared with seronegative women, the trend in linear proportions across the three exposure scores was significant (chi-square = 6.67; p = 0.01). In contrast, a significant linear trend in proportions was not demonstrated when women with nonacute cases were compared with seronegative women (chi-square = 1.30; p = 0.25).

Conclusions

The conclusions drawn from geographic mapping depend on the accuracy and validity of the datasets and the availability of denominator data to avoid making false associations that are a function of population densities. For the overall population, denominators were available from census data. Firm denominator data were available from the serologic screening study.

With respect to accurate placement on the maps, the addresses and the delineation of the water distribution systems were critical to the evaluation. Although extensive efforts were

Table. Linear trend analysis of acute and nonacute infections with *Toxoplasma gondii* among screened pregnant women, by degree of exposure to Humpback Reservoir water

	Rank of exposure	Exposure score	Infected (cases)	Never infected (controls)	Odds ratio (relative to baseline)	Trend test p value[a]
Acute	No	1	4	983	1.00	
(recent	Intermediate	2	10	1,078	2.28	
infection)	High	3	22	1,497	3.61	0.01
Nonacute	No	1	64	983	1.00	
(past	Intermediate	2	71	1,078	1.01	
infection)	High	3	81	1,497	0.83	0.25

[a]Extended Mantel-Haenszel chi-square, Schlesselmann JJ. Case-control studies, New York, Oxford University Press, 1982.

made to verify addresses, there was some potential for misclassifying exposure. However, any misclassification resulting from the use of the Medical Services Plan database should not have systematically biased our analyses.

The Greater Victoria Water District engineers delineated the boundaries of the Humpback distribution system and ranked the zones in the Capital Regional District according to the proportion of water received from the Humpback Reservoir. As the engineers had no prior knowledge of the distribution of outbreak-related cases of toxoplasmosis, it is unlikely that the determination of these geographic boundaries was biased.

A limitation of the geographic mapping analysis of cases is that rates were not adjusted for age or other covariates. However, the subsequent analyses using data from the population-based screening of pregnant women were not confounded by age. The age distribution of the women in these analyses did not differ when grouped by infection status.

Computer mapping software had the advantages of 1) facilitating the verification and correct placement of addresses, 2) reducing the time required to map the location of large datasets, 3) enabling queries and statistical analysis of the data after mapping, 4) allowing several sets of mapped data to be analysed simultaneously for potential relationships, and 5) generating printouts or overheads for presentations.

Acknowledgments

Mr. Eng is with the Health Protection and Environmental Program for Greater Victoria, British Columbia. His interests focus on environmental epidemiology, manipulation and analysis of computer data, and computer-based mapping.

References

1. Barreto ML. The dot map as an epidemiological tool: a case study of *Schistosoma mansoni* infection in an urban setting. Int J Epidemiol 1993;22:731-41.
2. Glass GE, Schwartz BS, Morgan JM, Johnson DT, Noy PM, Israel E. Environmental risk factors for Lyme disease identified with geographic information system. Am J Public Health 1995;85:944-8.
3. Skorpanich MA, Taylor TH, Anton-Culver H. Mapping disease and risk factors to identify public health concerns. In: URISA 88. Urban & Regional Information Systems Association, Los Angeles, CA 1988;4:310-9.
4. Timmreck TC. An introduction to epidemiology. Boston: Jones and Bartlett Publishers, Inc.; 1994.
5. Rosenberg ML, Haziet KI, Schaefer J, Wells JG, Pruneda RC. Shigellosis from swimming. JAMA 1976;236:1849-52.
6. Merson MH, Goldmann DA, Boyer KM, Peterson NJ, Patton C, Everett LG, et al. An outbreak of *Shigella sonnei* gastroenteritis on Colorado River raft trips. Am J Epidemiol 1974;100:186-95.
7. Bowie WR, King AS, Werker DH, Issac-Renton JL, Bell A, Eng SB, et al. Outbreak of toxoplasmosis associated with municipal drinking water. Lancet 1997;350:173-7.
8. Remington JS, McLeod R, Desmonts G. Toxoplasmosis. In: Remington JS, Klein JO, editors. Infectious diseases of the fetus and newborn. 4th ed. Philadelphia (PA): W.B. Saunders Company; 1995. p. 140-267.
9. Wong S-Y, Remington JS. State of the art clinical article: Toxoplasmosis in pregnancy. Clin Infect Dis 1994;18:853-62.
10. Montoya JG, Remington JS. Studies on the serodiagnosis of toxoplasmic lymphadenitis. Clin Infect Dis 1995;20:781-9.
11. Dannemann BR, Vaughan WC, Thulliez P, Remington JS. Differential agglutination test for diagnosis of recently acquired infection with *T. gondii*. J Clin Microbiol 1990;28:1928-33.
12. Irwin GS, Zanette M, Morris B. Greater Victoria's drinking water system 1994 bacteriological summary. Greater Victoria Water District, Victoria, British Columbia, Canada; 1995.
13. British Columbia Roads and Rivers (TcN data), based on Transportation Centerline Network (1993), Planning Services Branch, Ministry of Transportation and Highways, Province of British Columbia.

HIV Infection as a Risk Factor for Shigellosis[1]

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We investigated cases of shigellosis in San Francisco and Alameda Counties identified during 1996 by active laboratory surveillance to assess the role of HIV infection as a risk factor for shigellosis. Dramatically elevated rates of shigellosis in HIV-infected persons implicate HIV infection as an important risk factor for shigellosis in San Francisco.

Shigella infections are responsible for an estimated 300,000 illnesses and 600 deaths per year in the United States and more than 600,000 deaths per year worldwide (1). *Shigella* species are typically transmitted by direct or indirect fecal-oral contact; as a result, shigellosis has long been associated with outbreaks in day-care centers, nursing homes, and institutionalized populations (2-4). However, several studies have demonstrated an increased frequency of shigellosis cases in young adult men residing in urban settings who have little, if any, exposure to these traditionally recognized risk groups (5-9). These investigations also suggest that *Shigella* infection occurs during the practice of gay sex; however, since most of these studies occurred before the HIV epidemic, the relationship between HIV infection and gay sex and the subsequent risk for shigellosis has yet to be evaluated (5-9).

HIV-infected persons are at increased risk for infection by several common enteric pathogens (10). Previous investigations have demonstrated that HIV-infected patients are at 20 times greater risk for infection with *Salmonella* species and 39 times greater risk for infection with *Campylobacter* species than the general population (11,12). To determine the rate of shigellosis in HIV-infected persons, we investigated all cases of shigellosis in San Francisco, a county with a high prevalence of HIV infection and a high incidence of shigellosis. Alameda, a neighboring county with lower rates of shigellosis and HIV infection, was used as a comparison area for our investigation because of its proximity and differing shigellosis and HIV epidemiology.

During 1996, cases of culture-confirmed shigellosis were identified in San Francisco and Alameda Counties by active surveillance in 28 laboratories for isolates of *Shigella* species cultured from any anatomic site as part of the California Emerging Infections Program. The program comprises one of five sites in the Foodborne Diseases Active Surveillance Network (FoodNet), which is part of the Centers for Disease Control and Prevention's Emerging Infections Program. All available medical records of patients were reviewed by a standardized data collection instrument detailing demographic and medical information. Data concerning sexual activity and orientation and foreign travel were obtained from routine telephone interviews of patients with shigellosis, conducted by the San

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Francisco Department of Public Health. This information was not available for Alameda residents because no telephone interviews were conducted in Alameda County.

Patients were considered HIV-infected if their medical record contained a physician's note or a laboratory report documenting HIV infection. In the absence of such documentation, patients were considered HIV-negative. San Francisco patients were classified as gay if they were male and had identified themselves as gay or bisexual during telephone interviews. Recent sexual contact for San Francisco patients was assessed during the telephone interviews and was defined as having had a sexual encounter within 10 days of the onset of shigellosis symptoms. Foreign travel exposure was defined as travel to an area where shigellosis was endemic 7 days before the onset of symptoms.

Postcensus data for San Francisco and Alameda Counties were obtained for 1996 (13). Estimates of the prevalence of HIV infection in San Francisco by groups at risk were obtained from the 1997 HIV Consensus Report on HIV Prevalence and Incidence in San Francisco (14). This report is based on the findings of a consensus panel that systematically reviewed numerous sources of published and unpublished data. FoodNet incidence rates for culture-confirmed cases of shigellosis were based on aggregated data collected by active laboratory-based surveillance during 1996 in four urban areas in Connecticut, Georgia, Minnesota, and Oregon (15). National incidence rates were based on culture-confirmed cases of shigellosis reported through passive laboratory-based surveillance in 1996 (15).

Data were managed and analyzed by Stata 4.0 (Stata Corporation, College Station, TX) and EpiInfo 6.04b (CDC, Atlanta, GA) software. Univariate analyses of proportions were performed by chi-square test. Incidence rates and incidence rate ratios were compared by using the exact method to calculate confidence intervals and statistical significance. Temporal trends of shigellosis were assessed by conducting chi-square for trend analysis on the number of cases of shigellosis diagnosed by month.

During 1996, 228 and 140 culture-confirmed cases of shigellosis were identified in San Francisco and Alameda, respectively. In San Francisco, 142 (62%) of these cases were caused by *S. sonnei*, 73 (32%) by *S. flexneri*, 7 (3%) by

S. boydi, 2 (1%) by *S. dysenteriae,* and 4 (2%) were not speciated. In Alameda, 93 (66%) of the cases were caused by *S. sonnei*, 28 (20%) by *S. flexneri*, 6 (4%) by *S. boydi*, 2 (2%) by *S. dysenteriae,* and 11 (8%) were not speciated. No difference was observed in the proportion of cases caused by different species in the two counties (p = 0.16).

An analysis of the month of diagnosis for all patients with *S. sonnei* infections demonstrated a distinct trend in both San Francisco (p = .001) and Alameda (p = .03). The number of infections per month was highest in San Francisco between January and May and in Alameda between August and November. No temporal trend was apparent for *S. flexneri* infections in San Francisco (p = .77) or Alameda (p = .36).

San Francisco patients were significantly more likely than Alameda patients to be male, adult, white, and HIV-infected (Table 1). Sixty-six (39%) of 168 shigellosis patients and 30 (54%) of 56 *S. flexneri*-infected patients in San Francisco were HIV-infected; 11 (15%) of 75 HIV-negative patients and 1 (2%) of 56 HIV-infected patients in San Francisco reported recent travel to a shigella-endemic area outside the United States (p = 0.01).

Table 1. Persons with shigellosis, by county of residence, 1996

Characteristic	San Francisco (n = 228)	Alameda (n = 140)	p value
Male	157/228 (69%)	60/140 (43%)	<0.001
Age ≥18	181/227 (80%)	57/140 (41%)	<0.001
Race			
White	117/200 (59%)	15/92 (16%)	<0.001
Black	39/200 (20%)	44/92 (48%)	<0.001
Hispanic	37/200 (19%)	22/92 (24%)	0.285
Hospitalized	22/201 (11%)	12/140 (9%)	0.472
HIV infection	66/168 (39%)	9/125 (7%)	<0.001
Foreign travel	25/185 (14%)	NAª	---
Gay male	96/190 (51%)	NAª	---
Recent sex	70/136 (51%)	NAª	---

ªNA = not available

The annual incidence rates of shigellosis for various population groups in San Francisco, Alameda, other FoodNet sites, and the United States are shown in Table 2. San Francisco had higher overall rates, particularly among men and persons ages 25 to 64 years, than Alameda, other FoodNet sites, and the United States.

In San Francisco, an analysis of the annual incidence rates of shigellosis per 100,000

Table 2. Annual incidence rates of culture-confirmed shigellosis per 100,000 population for selected groups, 1996

Group	San Francisco County	Alameda County	FoodNet sites[a]	United States
Overall rate	30.9	10.5[b]	7.3	5.3
Male	43.2	9.2[b]	7.4	3.3
Female	19.1	11.8[b]	7.1	3.9
Age groups (yrs)				
<5	82.5	47.9[b]	36.7	16.7
5-14	22.1	17.1	12.6	6.8
15-24	19.9	9.3[b]	5.3	2.4
25-39	49.9	7.6[b]	5.6	2.7
40-64	25.9	3.3[b]	2.4	1.0
65+	2.5	4.0	1.5	0.7

[a]Does not include California.
[b]Comparison of rates in San Francisco and Alameda Counties, p value <.05.

population by sexual orientation and HIV status showed rates of 12.4 in heterosexual and HIV-negative persons, 60.1 in gay and HIV-negative persons, 378 in not gay and HIV-infected persons, and 442 in gay and HIV-infected persons. Incidence rate ratios for these groups, relative to the not gay and HIV-negative population, were as follows: gay and HIV-negative 4.9 (95% CI 2.7-8.1); not gay and HIV-infected 30.6 (95% CI 12.8-63.0); and gay and HIV-infected 35.7 (95% CI 25.1-50.4).

Thirty-four (10%) of 341 of patients were hospitalized for shigellosis (median hospital stay 3 days). Furthermore, in San Francisco 13 (22%) of 60 HIV-infected shigellosis patients were hospitalized, while 7 (8%) of 86 HIV-negative persons were hospitalized (p = .02). Twelve (13%) of 93 *S. flexneri* and 19 (9%) of 216 *S. sonnei* patients were hospitalized (p = .27).

These population-based data demonstrate a high overall annual incidence rate of shigellosis in San Francisco compared with neighboring Alameda County, other FoodNet sites, and the United States, and dramatically elevated rates in HIV-infected San Francisco residents. The high proportion of cases in San Francisco in both the gay and the HIV-infected populations suggests that these groups play a major role in the epidemiologic features of endemic shigellosis in San Francisco. Furthermore, the greatly elevated incidence rates of shigellosis in the HIV-infected population suggest that HIV may be a important risk factor for *Shigella* infection.

These data also demonstrate that shigellosis is associated with extensive illness and increased health-care expenditures, particularly in the HIV-infected population, as evidenced by the frequency of hospitalization in these persons.

There are several possible explanations for the high rates of shigellosis observed in HIV-infected patients in this study. The compromised host immunity of HIV-infected persons may increase their risk for clinical infection after exposure. A recent study found that 75% of asymptomatic household contacts of symptomatic shigellosis patients had evidence of *Shigella* infection by polymerase chain reaction, which suggests that host immunity may play an important role in determining which exposed persons progress to clinical infection (16). Increased susceptibility to shigellosis among HIV-infected persons could be mediated through different mechanisms, including compromised cell-mediated immunity or achlorhydria. Alternatively, the high rate of shigellosis in HIV-infected patients may be related to factors other than host immunity, such as sexual or behavioral practices, which were not thoroughly investigated in our study.

Our investigation suggests that HIV infection is an important risk factor for shigellosis. This finding has not been previously described on a population level. This investigation also suggests that HIV infection is an important determinant of the epidemiologic features of shigellosis in San Francisco and that public health prevention strategies in areas with a large HIV-infected and gay male population may need to be revisited. A diagnosis of shigellosis in young adult men who are not part of a recognized outbreak and have not recently traveled to a *Shigella*-endemic area may serve as a marker for HIV infection and may indicate a need for counseling and HIV testing.

Due to the methods of data collection, misclassification of HIV infection status and sexual orientation could have occurred in either shigellosis patients or in the San Francisco Department of Public Health population estimates, thereby altering the incidence rates in an unpredictable manner. Moreover, the propensity of HIV-infected patients with shigellosis to seek medical attention and the likelihood of their health-care providers to obtain cultures may differ from that of HIV-negative patients. This bias, if present, could influence the observed risk

associated with HIV infection. However, it is unlikely that such a bias could account for the entire difference between groups, given the magnitude of the elevation in incidence rates in the HIV-infected population.

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Mr. Baer is a 3rd-year medical student at the University of North Carolina at Chapel Hill. His current research includes examining a possible molecular pathway for the *Chlamydia pneumoniae* and atherosclerosis association.

References

1. Bennett JV, Holmberg SD, Rogers MF, Solomon SL. Infectious and parasitic diseases. Am J Prev Med 1987;3:102-14.
2. Weissman JB, Schmerler A, Weiler P, Filice G, Godbey N, Hansen I. The role of preschool children and day-care centers in the spread of shigellosis in urban communities. J Pediatr 1974;84:797-802.
3. Ryan MJ, Wall PG, Adak GK, Evans HS, Cowden JM. Outbreaks of infectious intestinal disease in residential institutions in England and Wales 1992-1994. J Infect 1997;34:49-54.
4. DuPont HL, Gangarosa EJ, Reller LB, Woodward WE, Armstrong RW, Hammond J,et al. Shigellosis in custodial institutions. Am J Epidemiol 1970;92:172-9.
5. Dritz SK, Back AF. Shigella enteritis venereally transmitted [letter]. N Engl J Med 1974;291:1194.
6. Bader M, Petersen AH, Williams R, Spearman J, Anderson H. Venereal transmission of shigellosis in Seattle-King County. Sex Transm Dis 1977;4:89-91.
7. Dritz SK, Ainsworth TE, Garrard WF, Back A, Palmer RD, Boucher LA, et al. Patterns of sexually transmitted enteric diseases in a city. Lancet 1977;2:3-4.
8. Tauxe RV, McDonald RC, Hargrett-Bean N, Blake P. The persistence of *Shigella flexneri* in the United States: Increasing role in adult males. Am J Public Health 1988;78:1432-5.
9. Quinn TC, Stamm WE, Goodell SE, Mkrtichian E, Benedetti J, Corey L, et al. The polymicrobial origin of intestinal infections in homosexual men. N Engl J Med 1983;309:576-82.
10. Angulo FJ, Swerdlow DL. Bacterial enteric infections in persons infected with human immunodeficiency virus. Clin Infect Dis 1995;21 Suppl 1:S84-93.
11. Sorvillo FJ, Lieb LE, Waterman SH. Incidence of campylobacteriosis among patients with AIDS in Los Angeles County. J Acquir Immune Defic Syndr Hum Retrovirol 1991;4:598-602.
12. Celum CL, Chaisson RE, Rutherford GW, Barnhart JL, Echenberg DF. Incidence of salmonellosis in patients with AIDS. J Infect Dis 1987;156:998-1002.
13. U.S. Census Bureau. Annual time series of county population estimates by age, sex, race, and Hispanic origin; 1996; [accessed 1998 Aug 1]. Available from: URL http://www.census.gov/population/estimates/countypop.html.
14. Shafer KP, McFarland W, Katz MH. 1997 consensus report on HIV prevalence and incidence in San Francisco. San Francisco Department of Public Health HIV Seroepidemiology Unit 1997.
15. Centers for Disease Control and Prevention. Revised FoodNet 1996 final report. 1998 Sep.
16. Gaudio PA, Sethabutr O, Echeverria P, Hoge CW. Utility of a polymerase chain reaction diagnostic system in a study of the epidemiology of shigellosis among dysentery patients, family contacts, and well controls living in a shigellosis-endemic area. J Infect Dis 1997;176:1013-8.

Dengue Seroconversion among Israeli Travelers to Tropical Countries

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We tested for dengue seroconversion among 104 Israeli young adults who traveled to tropical countries for at least 3 months. Seven (6.7%) seroconverted during travel; four (3.8%) had immunoglobulin (Ig) M antibodies; one was symptomatic with borderline IgM and a rise in IgG; two others (1.9%) had a rise in IgG titers, without detectable IgM. All four IgM-positive patients had traveled to Southeast Asia.

Dengue fever is a rapidly spreading mosquito-borne viral disease. Some 40% of the world's population lives in disease-endemic areas, and dengue outbreaks occur in more than 100 countries (1). Infected persons usually have high fever, chills, frontal headache, rash, severe myalgia, and malaise. Sometimes, the disease goes unrecognized (2).

According to surveillance data, nearly 200,000 cases of dengue fever occur in 31 countries in Central and South America (3); the attack rate among disease-endemic populations may be as high as 6,400 per 100,000 persons exposed. In contrast to data regarding the rate of dengue among populations in disease-endemic areas, data on the attack rate among travelers are scarce.

The rate of dengue fever has been examined in selected Japanese, Spanish, Swiss, and German travelers and in U.S. troops deployed in Somalia (4-8). The rate of dengue in these febrile patients was 6.9% to 65%. To the best of our knowledge, the rate of dengue fever has never been examined prospectively in a cohort of healthy, long-term travelers to disease-endemic areas.

The Study

The study was approved by the Helsinki Committee of the Bnai Zion Medical Center, which provides service to approximately 1,500 travelers per year. Each traveler is requested to fill out a questionnaire including demographic,

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itinerary, and vaccination data, which are stored in a computerized database. The purpose of the study was explained to the travelers, and informed consent was obtained upon enrollment. Serum was drawn from random volunteers before starting the recommended vaccinations. Eligibility for inclusion was based on a minimum length of travel of at least 3 months and donation of a serum sample before and after travel. One hundred and four travelers fulfilled the inclusion criteria. The second serum sample was taken 1 to 4 months after returning home. Travelers who had positive dengue immunoglobulin (Ig) G serologic results were sent a questionnaire. In addition to demographics, the patients were asked to indicate their destination, season and length of stay, mosquito bites, use of repellents, fever, chills, nausea or vomiting, muscle aches, headache, cough, rash, or arthralgia. A case of dengue fever was defined according to Centers for Disease Control and Prevention (CDC) criteria (9). An asymptomatic dengue infection was one that met the laboratory criteria for diagnosis without clinical signs. Confidence intervals (CIs) were calculated with Statmate (GraphPad Software, San Diego, CA).

After thawing, 104 posttravel serum samples were tested for antibodies to dengue by an IgG enzyme-linked immunosorbent assay (ELISA) (Pan-Bio Pty, Ltd., Queensland, Australia). All positive sera were then tested in parallel with pretravel sera to confirm seroconversion. Seroconverting pairs were sent to the CDC laboratories in San Juan, Puerto Rico, for

confirmation and dengue IgM and IgG antibody determination.

IgM antibody was measured as a qualitative ELISA. An optical density of 0.2 or greater compared with a negative control was considered positive. The test may show some cross-reactivity to other flaviviruses (10). IgG antibody was measured as a quantitative ELISA using mixed dengue antigens. This test may also show cross-reactivity with other flaviviruses (11).

The mean age of the 104 study participants was 22.4 ± 2.2 years. The average length of stay abroad was 6.1 months (3 to 16 months), and the total time abroad for all 104 travelers was 53 person-years. The average time between serum samples was 11.1 months (4 to 22 months). The destinations were Southeast Asia (70%), South America (24%), Africa (4%), and both Southeast Asia and South America (2%).

Seven travelers (6.7%; 95% CI = 2.7-13.3%) had either an IgM antibody or a fourfold (or greater) rise in IgG titers after the trip. The median age of this group was 22 years, and four travelers were women. All seven stayed at their destinations (Table) during the summer months (some also spent the spring or autumn there). Their mean length of stay abroad was 5.3 months. The rates of conversion per month of exposure by continent were as follows: Southeast Asia, 5 (1.1%) of 451 (95% CI = 0.36-2.6%); South America, 1 (0.6%) of 159 (95% CI = 0.02-3.5%); and Africa, 1 (4%) of 25 (95% CI = 0.1-20.3%).

Four patients (3.8%) tested positive for dengue IgM after travel. All four had negative IgM dengue serologic test results before travel, and all but one had negative IgG titers. All four (two of them male) visited Southeast Asia, with Thailand being the only common destination. Two of these patients, who had traveled separately, indicated that they became ill on the island of Ko-Pangan. All four were engaged in extensive outdoor activities and consequently had mosquito exposure. All four used mosquito repellents (containing 20% to 25% DEET), but only three recalled being bitten by mosquitoes. Three travelers had fever, which in two (both female) was also accompanied by chills, headache, and protracted fatigue. One patient had an apparently asymptomatic infection.

Only two of the four IgM-positive patients had received Japanese B encephalitis vaccine after the initial blood collection, which could have interfered with after-travel testing. Of the three travelers who had a fourfold IgG rise, two were asymptomatic, and one had a clinical picture compatible with dengue fever 1 month after arriving in Thailand. This traveler also had a marginal IgM test. Among these three travelers, two had received yellow fever vaccine and one Japanese B encephalitis vaccine, which could have interfered with after-travel testing.

Conclusions

Researchers have found that the younger the age, the higher the rate of illness among travelers, but dengue fever has not been thoroughly investigated (12,13). Dengue fever has not been reported in Israel over the last 50

Table. Dengue serologic results in seroconverting Israeli travelers to tropical countries

Serum number	Age/sex	Destination	Length of stay (mos.)	Season of travel	Disease status	Results IgM	IgG
4	52/F	Africa	3	Summer	Asymptomatic	Negative	40
4a[a]						Negative	640
10	22/F	Southeast Asia	6	Spring-summer	Symptomatic	Negative	Negative
10a						+/- Pos.[b]	160
11	21/F	Southeast Asia	4	Summer-autumn	Symptomatic	Negative	40
11a						Positive	2,560
12	25/F	Southeast Asia	3	Summer-autumn	Symptomatic	Negative	Negative
12a						Positive	160
13	26/M	Southeast Asia	3	Summer-autumn	Symptomatic	Negative	Negative
13a						Positive	640
14	22/M	South America	6	Summer-autumn	Asymptomatic	Negative	160
14a						Negative	640
18	21/M	Southeast Asia	12	Spring-summer	Asymptomatic	Negative	Negative
18a						Positive	160

[a]a = after-travel sample.
[b]This test was marginally positive.

years, which made our group of young travelers particularly suitable for this study. In addition, there has been a dramatic increase in the number of Israeli travelers to tropical areas during the past decade. Approximately 40,000 Israelis travel to the tropics each year, more than 25,000 of whom are backpackers who travel for 3 to 12 months off the beaten track (14) and are exposed to the same diseases as travelers from other countries. Dengue fever has not been mentioned as a real hazard to Israeli travelers, despite an incidence in our study that may be as high as the rate of malaria without prophylaxis, and higher than the rates of hepatitis A, giardiasis, or typhoid fever (15). Nevertheless, it would be premature to extrapolate from our group of youngsters traveling on prolonged journeys, to groups with other travel characteristics. Older and perhaps short-term travelers may, for example, choose other tracks or adhere more closely to recommendations regarding insect repellents (16).

Four travelers (3.8%) of our group had IgM antibodies, indicating acute infection. Another traveler had symptoms of dengue fever with borderline IgM and a rising titer of IgG, which most probably reflected a recent infection. This traveler could have been infected earlier during her 6-month trip, and by the time the serum was taken, the IgM level might have dropped. Two additional travelers had a rise in IgG titers without detectable IgM.

Our results are tentative, as the serologic tests for dengue are not devoid of cross-reactivity (11). Both IgM and IgG may cross-react with other flaviviruses, such as Japanese B encephalitis, West Nile encephalitis, or yellow fever. The rate of IgG cross-reactivity between dengue infection and Japanese B encephalitis or yellow fever vaccine may be 17% to 40%; however, IgM cross-reactivity was not found after vaccination (E. Schwartz, pers. comm.). As none of our travelers had signs of encephalitis and yellow fever does not exist in Southeast Asia, the five travelers with IgM antibodies (including the one with a borderline case) contracted dengue fever. Indeed, four of them had clinical symptoms compatible with dengue fever. The diagnosis in the two travelers who had a fourfold rise in IgG titers was uncertain, since both were asymptomatic and had received yellow fever vaccines, which may have caused a cross-reaction in IgG assays. Dengue infection may be asymptomatic.

Only three of the four IgM-positive patients were febrile; two of these also had chills. Asymptomatic dengue, which was found in three of our patients, has been described in populations of disease-endemic areas but never in travelers (2,17).

Two additional points in our study deserve comment. First, all four IgM-positive cases and the borderline IgM case occurred in travelers to Southeast Asia. A higher density of the vector and virus in Southeast Asia or visits by many of our travelers to Thai destinations—known for their high rate of dengue (18-20)—may have played a major role in this trend (our data are insufficient to permit conclusions regarding travel to Africa and South America). Secondly, all seven cases occurred during the summer, a season known for its high rate of mosquito activity and dengue transmission. The rate of dengue fever among travelers has been studied by four groups of researchers (4-7); an additional group described dengue in U.S. troops deployed in Somalia (8). The rate of dengue in their cohorts was 6.9% to 65%. However, all of these researchers focused on febrile patients with either fever of unknown origin, suspected dengue, or malaria. Hence, it is impossible to extrapolate from their data the actual risk of acquiring dengue during travel to the tropics.

Other travel clinics in Israel also have indicated that (after malaria) dengue is the second most frequent cause of hospitalization of returning travelers (20). Based on a minimum figure of the four IgM-positive patients, the calculated risk for dengue during a 1-month trip is thus 630 out of 100,000 travelers, which puts dengue high on the list of diseases contracted in the tropics. This statement holds true at least for Israeli travelers to the Far East. As patients may be evacuated because of dengue fever or may become sick after returning home, physicians need to be all the more vigilant in the face of this diagnostic possibility.

We conclude that dengue fever is perhaps the most common mosquito-borne disease of long-term young travelers, particularly those visiting Southeast Asia. The present results should serve as a further impetus toward the development of a vaccine for dengue fever.

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References

1. World Health Organization/CTD. Dengue and DHF prevention and control. Geneva: The Organization; 1998.
2. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg 1988;38:172-80.
3. Lifson AR. Mosquitoes, models, and dengue [commentary]. Lancet 1996;347:1201-2.
4. Yabe S, Nakayama M, Yamada K, Kitano T, Arai Y, Horimoto T, et al. Laboratory virological diagnosis of imported dengue cases. Kansenshogaku Zasshi 1996;70:1160-9 (In Japanese).
5. Lopez-Velez R, Perez-Casa C, Vorndam A, Rigau J. Dengue in Spanish travelers returning from the tropics. Eur J Clin Microbiol Infect Dis 1996;15:823-6.
6. Settah SG, Vernazza PL, Morant R, Schultze D. Imported dengue fever in Switzerland—serological evidence for a hitherto unexpectedly high prevalence. Schweiz Med Wochenschr 1995;125:1673-8 (In German).
7. Jelinek T, Dobler G, Holscher M, Loscher T, Nothdurft H-D. Prevalence of infection with dengue virus among international travelers. Arch Intern Med 1997;157:2367-70.
8. Sharp TW, Wallace MR, Hayes CG, Sanchez JL, DeFraites RF, Arthur RR, et al. Dengue fever in U.S. troops during Operation Restore Hope, Somalia, 1992-1993. Am J Trop Med Hyg 1995;53:89-94.
9. Centers for Disease Control. Dengue fever. MMWR Morb Mortal Wkly Rep 1990;39:10-1.
10. Burke DS, Nisalak A, Ussery MA. Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. J Clin Microbiol 1982;16:1034-42.
11. Makino Y, Tadano M, Saito M, Maneekam N, Sittisombut N, Sirisanthana V, et al. Studies on serological cross-reaction in sequential flavivirus infections. Microbiol Immunol 1994;38:951-5.
12. Steffen R, Rickenbach M, Wilhelm U, Helminger A, Schar M. Health problems after travel to developing countries. J Infect Dis 1987;156:84-91.
13. Reid D, Grist NR, Najera R. Illness associated with "package tours": a combined Spanish-Scottish study. Bull World Health Organ 1978;56:117-22.
14. Berger SA, Giladi M, Shapira I. Health concerns of Israelis traveling to third world countries—experience of a travel advisory clinic. Harefuah 1994;126:410-2 (In Hebrew).
15. Reid D, Keystone J. Health risks abroad: general considerations. In: DuPont H, Steffen R, editors. Textbook of travel medicine and health. Vol 1. Hamilton, Canada: BC Decker; 1997. p. 3-9.
16. Fradin MS. Mosquitoes and mosquito repellents: a clinician's guide. Ann Intern Med 1998;128:931-40.
17. da Cunha RV, Dias M, Nogueira RM, Chagas N, Miagostovich MP, Schatzmayr HG. Secondary dengue infection in schoolchildren in a dengue endemic area in the state of Rio de Janeiro, Brazil. Rev Inst Med Trop Sao Paulo 1995;37:517-21.
18. Pick N, Potasman I. Dengue fever. Harefuah 1995;129:30-2 (In Hebrew).
19. Thavara U, Tawatsin A, Phan-Urai P, Ngamsuk W, Chansang C, Liu M, et al. Dengue vector mosquitos at a tourist attraction, Ko Samui, in 1995. Southeast Asian J Trop Med Public Health 1996;27:160-3.
20. Schwartz E, Mendelson E, Sidi Y. Dengue fever among travelers. Am J Med 1996;101:516-20.

Effectiveness of Pneumococcal Polysaccharide Vaccine for Preschool-Age Children with Chronic Disease

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To estimate the effectiveness of pneumococcal polysaccharide vaccine, we serotyped isolates submitted to the Pneumococcal Sentinel Surveillance System from 1984 to 1996 from 48 vaccinated and 125 unvaccinated children 2 to 5 years of age. Effectiveness against invasive disease caused by serotypes included in the vaccine was 63%. Effectiveness against serotypes in the polysaccharide vaccine but not in a proposed seven-valent protein conjugate vaccine was 94%.

Streptococcus pneumoniae is a leading cause of pneumonia, meningitis, bacteremia, and death in young children. A polysaccharide vaccine has been recommended for use in chronically ill children and adults 2 to 64 years of age, as well as all adults $\geq$65 (1). While many studies have assessed the immunogenicity of the polysaccharide vaccine, scant data exist on its effectiveness in younger children.

More than 90 serotypes of *S. pneumoniae* have been described (2); however, most invasive infections in the United States are caused by $\leq$10 serotypes (3). Pneumococcal vaccines available since 1978 consist of a mixture of capsular polysaccharides from the most common serotypes causing invasive disease. This vaccine is recommended for children $\geq$2 years of age with underlying diseases or immunosuppressive medical treatments that are risk factors for invasive pneumococcal disease (1,3).

Clinical trials of pneumococcal polysaccharide vaccine effectiveness in children have shown conflicting results (4-7). Vaccine failure in immunized children has been reported (8), and one study comparing immunization with antibiotic prophylaxis in children with sickle cell disease concluded that the vaccine was inferior

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to penicillin prophylaxis (9). Uncertainty regarding the effectiveness of vaccination may contribute to low vaccination rates among persons at risk for pneumococcal disease (1).

In indirect cohort analysis (10), the distribution of pneumococcal serotypes causing invasive disease among vaccinated and unvaccinated groups is compared. If the vaccine is effective, vaccinated persons have fewer infections with serotypes represented in the vaccine than unvaccinated persons. This method has been used to calculate an overall effectiveness of 57% in persons >5 years of age, based on serotypes of invasive isolates obtained through a national, voluntary sentinel surveillance system (11). Using data from national surveillance, we examined vaccine effectiveness for children 2 through 5 years of age.

The Study

Since 1978, a national, hospital laboratory-based surveillance system has collected data on invasive pneumococcal disease (12). Participating institutions are requested to report all pneumococcal isolates obtained from normally sterile body sites, along with information on the patient's age, sex, symptoms, underlying diseases, and vaccination history. The specifics of how demographic and vaccination information is collected are the responsibility of participating institutions. Isolates are serotyped at the

Centers for Disease Control and Prevention on the basis of capsular swelling with serotype-specific antisera (Quellung reaction).

Children included in the analysis were 24 to 59 months of age with one or more chronic illnesses, had vaccination status and date indicated on the surveillance form, received vaccine between January 1984 and April 1996, and had onset of invasive pneumococcal disease between January 1984 and April 1996. Only isolates from cerebrospinal fluid (CSF) or blood were considered in the analysis. Information on antibiotic prophylaxis was not collected. Chronic illness was defined as an underlying illness considered a risk factor for invasive pneumococcal disease and an indication for vaccination (3).

Vaccine effectiveness was defined as the percentage of reduction in the risk for infection from serotypes included in the vaccine (vaccine-type serotypes) among vaccinated persons compared with unvaccinated persons. Infections with vaccine-related serotypes (6A, 9A, 9L, 18B, 18F, 23A) not specifically included in the vaccine were categorized as infections with nonvaccine serotypes, except where noted. Effectiveness was expressed as 1 minus the odds ratio x 100%; the 95% confidence intervals (also x 100%) were calculated by the methods of Cornfield when cell sizes were all greater than five subjects and by exact methods otherwise. Calculations were performed with Epi-Info version 6.02 (CDC/World Health Organization, Atlanta, GA) with the EXACT supplemental program (David O. Martin).

We performed a preliminary analysis of all pneumococcal isolates from children in the database to determine the proportion of vaccine-type organisms in unvaccinated persons by sex, underlying illness, or state of residence. Proportions of vaccine-type serogroups did not differ by underlying illness or by sex. Because the proportion of vaccine-type isolates from children from Alaska was 92.3%, compared with the 85.4% of isolates from children from other states (chi-square = 6.3; p <0.02), children from Alaska were excluded from the analysis.

The analysis included 173 children, 52% male, median age 3 years; 48 children (28%) had received vaccine before acquiring invasive pneumococcal disease. Isolates were obtained from blood only from 156 children (90%), from CSF only from 10 children (6%), and from both sites from 7 children (4%). The median time between date of vaccination and date of specimen collection was 338.5 days (33 days to 1,341 days), and no child had been vaccinated within 30 days of invasive pneumococcal infection. Of serotypes from the 173 invasive infections, serotypes 4, 6A, 6B, 14, 23F, 19F, 9V, and 18C accounted for 81% of the isolates (Figure 1).

Forty-six (27%) of children in the study had sickle cell disease (Figure 2). The "other" category included children with congenital anomalies such as congenital heart or lung defects, children with anatomic asplenia, and children on immunosuppressive medication regimens. Thirty-three (69%) of 48 vaccinated children had sickle cell disease.
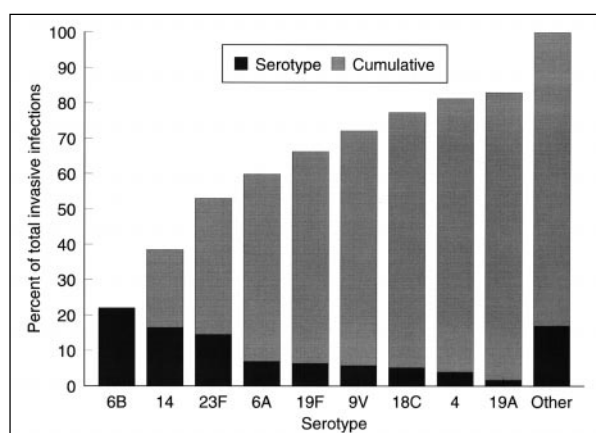
Figure 1. Invasive pneumococcal infections among 173 children ages 2 through 5 years (24-59 months), by serotype. Bottom bar represents proportion of total invasive infections in the cohort caused by each serotype. Top bar depicts cumulative proportion of invasive infections caused by serotypes represented by the bars to the left. Serotypes in the "other" category included 19 serotypes with three or fewer isolates. Two isolates could not be serotyped.
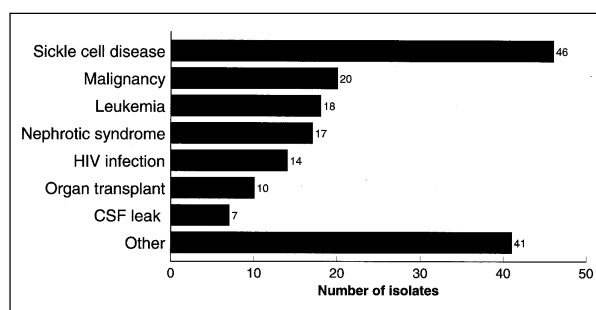
Figure 2. Frequency of various underlying chronic illnesses among 173 children with invasive pneumococcal disease. The category "malignancy" excluded hematopoetic malignancies, which are included in the leukemia category. Organ transplant includes both solid organ and bone marrow transplants. CSF is cerebrospinal fluid.

The Table presents vaccine effectiveness estimates for the overall cohort and for children with and without sickle cell disease. For children with the disease, the lower bound of the 95% confidence interval included 0%. The estimate of vaccine effectiveness for children without sickle cell disease was higher than the estimate for children with the disease. Point estimates of effectiveness for children with nephrotic syndrome or HIV infection were 80%; however, the 95% confidence intervals included 0% (data not shown). Other chronic diseases reported in this cohort included leukemia, nonhematopoetic malignancy, and organ transplant; however, none of the children with these underlying diseases were vaccinated, and effectiveness could not be calculated.

Protein conjugate vaccines offer the advantage of being effective in the first 2 years of life, when response to polysaccharide vaccines is poor. However, the number of serotypes that can be represented in these vaccines is limited. To evaluate polysaccharide effectiveness for serotypes not represented in a protein conjugate vaccine under evaluation for license (13), we excluded children infected with serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. Polysaccharide vaccine was highly effective in preventing invasive disease due to serotypes included in the polysaccharide vaccine but not in the conjugate vaccine (Table). If the 14 children with serotypes 6A, 9A, 9L, 18B, 18F, and 23A are also excluded (because of potential protection conferred by the proposed conjugate vaccine for these vaccine-related serotypes), the vaccine effectiveness estimate is 92% (exact 95% confidence intervals 17% to 100%).

Conclusions

Case-control studies have demonstrated that pneumococcal capsular polysaccharide vaccines are effective (14-16) and cost-effective (17,18) in the prevention of invasive pneumococcal disease among elderly and chronically ill adults. We used data from a national sentinel surveillance system for invasive pneumococcal disease to determine whether children ages 2 to 5 years were also protected. An overall vaccine effectiveness of 63% was demonstrated by indirect cohort analysis (15). The indirect cohort analysis presented here strengthens the case for the use of pneumococcal polysaccharide vaccine for children with underlying conditions. For children with sickle cell disease, penicillin prophylaxis remains the most effective preventive measure for reducing pneumococcal disease.

Accuracy of vaccine history is critical to this analysis and may vary between surveillance sites. To minimize inaccuracies, patients with no indication of vaccine history were excluded. For those with a reported vaccine history, misclassification due to inaccurate history should be as likely among patients with vaccine-type as among nonvaccine-type infections because the serotype of patient isolates was not known when vaccine status was determined (serotyping was done at CDC). Bias due to this nondifferential misclassification will be towards the null hypothesis (no effect of vaccination) (19).

Newly developed pneumococcal protein conjugate vaccines are safe and immunogenic for infants and young children (13,20,21). Preliminary results from a large, Phase-III trial of a heptavalent conjugate vaccine among healthy children indicate substantial efficacy in preventing invasive disease (13). However, the expense and technical difficulty of creating conjugates for each serotype will likely limit the number of serotypes represented in a polyvalent conjugate vaccine to fewer than 12. Available data suggest that polysaccharide vaccine, when administered after primary immunization with a conjugate vaccine, elicits a significant booster effect in

Table. Estimates of pneumococcal polysaccharide vaccine effectiveness among 173 children 2 through 5 years of age, using the indirect cohort method

| Group | Vaccine serotype/total(%) | | Effectiveness (95% CI)[b] |
	Vaccinated children[a]	Unvaccinated children[a]	
All children	35/48 (73)	110/125 (88)	63% (8% to 85%)
Children with SCD	27/33 (82)	12/13 (92)	62% (-294% to 98%)
Children without SCD	8/15 (53)	98/112 (88)	84% (40% to 96%)
Nonconjugate vaccine serotype[c]	1/14 (7)	18/33 (55)	93% (45% to 100%)

[a]23-valent pneumococcal polysaccharide vaccine.
[b]Effectiveness (95% confidence interval) estimated as (1- odds ratio or 95% confidence bound) x 100%.
[c]Children infected with a serotype not in proposed conjugate vaccine (15) (excludes children infected with serotypes 4, 6B, 9V, 14, 18C, 19F, 23F).
SCD, sickle-cell disease.

healthy infants (22) equivalent to the booster response engendered by a second conjugate vaccine series (23). These results and the level of effectiveness seen with pneumococcal polysaccharide vaccine in our study suggest that the polysaccharide vaccine will still be a useful adjunct to conjugate vaccine, by providing additional protection to children ≥2 years of age for whom polysaccharide vaccine is currently indicated.

Acknowledgments

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References

1. Centers for Disease Control and Prevention. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep 1997;46(RR-8).
2. Henrichsen J. Six newly recognized types of *Streptococcus pneumoniae*. J Clin Microbiol 1995;33:2759-62.
3. American Academy of Pediatrics. Pneumococcal infections. In: Peter G, editor. 1997 Red book: report of the Committee on Infectious Diseases. 24th ed. Elk Grove Village (IL): American Academy of Pediatrics; 1997. p. 410-9.
4. Riley ID, Everingham FA, Smith DE, Douglas RM. Immunisation with a polyvalent pneumococcal vaccine: Effect on respiratory mortality in children living in the New Guinea highlands. Arch Dis Child 1981;56:354-7.
5. Rosén C, Christensen P, Hovelius B, Prellner K. Effect of pneumococcal vaccination on upper respiratory tract infections in children: Design of a follow-up study. Scand J Infect Dis 1983;Suppl 39:39-44.
6. Douglas RM, Miles HB. Vaccination against *Streptococcus pneumoniae* in childhood: lack of demonstrable benefit in young Australian children. J Infect Dis 1984;149:861-9.
7. Ammann AJ, Addiego J, Wara DW, Lubin B, Smith WB, Mentzer WC. Polyvalent pneumococcal-polysaccharide immunization of patients with sickle-cell anemia and patients with splenectomy. N Engl J Med 1977;297:897-900.
8. Ahonkhai VI, Landesman SH, Fikrig SM, Smalzer EA, Brown AK, Cherubin CE, et al. Failure of pneumococcal vaccine in children with sickle-cell disease. N Engl J Med 1979;301:26-7.
9. John AB, Ramlal A, Jackson H, Maude GH, Waight-Sharma, A, Serjeant GR. Prevention of pneumococcal infection in children with homozygous sickle cell disease. BMJ 1984;288:1567-70.
10. Broome CV, Facklam RR, Fraser DW. Pneumococcal disease after pneumococcal vaccination: an alternative method to estimate the efficacy of pneumococcal vaccine. N Engl J Med 1980;549-52.
11. Butler JC, Breiman RF, Campbell JF, Lipman HB, Broome CV, Facklam RR. Pneumococcal vaccine efficacy: an evaluation of current recommendations. JAMA 1993;270:1826-31.
12. Broome CV. Efficacy of pneumococcal polysaccharide vaccines. Rev Infect Dis 1981;Suppl 3:S82-8.
13. Black SB, Shinefield H, Ray P, Lewis E, Fireman P, et al. Efficacy of heptavalent conjugate pneumococcal vaccine (Wyeth Lederle) in 37,000 infants and children: results of the Northern California Kaiser Permanente Efficacy Trial. In: Programs and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998; San Diego, California. Washington: American Society for Microbiology, 1998.
14. Shapiro ED, Berg AT, Austrian R, Schroeder D, Parcells V, Margolis A, et al. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. N Engl J Med 1991;325:1453-60.
15. Sims RV, Steinmann WC, McConville JH, King LR, Zwick WC, Schwartz JS. The clinical effectiveness of pneumococcal vaccine in the elderly. Ann Intern Med 1988;108:653-7.
16. Farr BM, Johnston BL, Cobb JK, Fisch MJ, Germanson TP, Adal KA, et al. Preventing pneumococcal bacteremia in patients at risk: Results of a matched case-control study. Arch Intern Med 1995;155:2336-40.
17. Gable CB, Holzer SS, Engelhart L, Friedman RB, Smeltz F, Schroeder D, et al. Pneumococcal vaccine: efficacy and associated cost savings. JAMA 1990;264:2910-5.
18. Sisk JE, Moskowitz AJ, Whang W, Lin JD, Fedson DS, McBean AM, et al. Cost-effectiveness of vaccination against pneumococcal bacteremia among elderly people. JAMA 1997;278:1333-9.
19. Copeland KT, Checkoway H, Holbrook RH, McMichael AJ. Bias due to misclassification in the estimate of relative risk. Am J Epidemiol 1977;105:488-95.
20. Käyhty H, Åhman H, Rönnberg P-R, Tillikainen R, Eskola J. Pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine is immunogenic in infants and children. J Infect Dis 1995;172:1273-8.
21. Rennels MB, Edwards KM, Keyserling HL, Reisinger KS, Hogerman DA, Madore DV, et al. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM_{197} in United States infants. Paediatrics 1998;101:604-11.
22. Åhman H, Käyhty H, Lehtonen H, Leroy O, Froeschle J, Eskola J. *Streptococcus pneumoniae* capsular polysaccharide-diphtheria toxoid conjugate vaccine is immunogenic in early infancy and able to produce immunologic memory. Pediatr Infect Dis J 1998;17:211-6.
23. Obaro SK, Huo Z, Banya WAS, Henderson DC, Monteil MA, Leach A, et al. A glycoprotein conjugate vaccine primes for antibody responses to a pneumococcal polysaccharide vaccine in Gambian children. Pediatr Infect Dis J 1997;16:1135-40.

Commentary

Stimulating the Development of Orphan (and Other) Vaccines

Drs. Lang and Wood (this issue, pp. 749-756) highlight factors that affect vaccine development decisions at large pharmaceutical companies and suggest measures to make development of orphan vaccines more attractive. Because of the importance of economic assessment in corporate decision-making, development of vaccines for rare diseases is usually problematic. Exceptions may include vaccines for potential bioterrorism agents (the government may support development and production) and therapeutic vaccines for chronic or deadly diseases (the price of a vaccine could be high, commensurate with the cost of therapy). Of seven vaccines defined by the Institute of Medicine as being "most favorable" for development, three were therapeutic vaccines (for diabetes mellitus, rheumatoid arthritis, and multiple sclerosis) (1).

In the developing world, price has been a major impediment to the introduction of new vaccines. Whether this reflects limitations in ability or willingness to pay, the end result is that a company could not expect sales in developing countries to provide the desired return on investment (2). Clearly, novel solutions are needed if vaccines that could save millions of children's lives are to be used effectively. Support for vaccination from the Bill and Melinda Gates Foundation and the promotion of vaccines as an acceptable component of bilateral loans by the World Bank may begin shifting the balance between market imperatives and public health needs.

Drs. Lang and Wood propose a package of incentives that may help promote development of orphan vaccines by major manufacturers. But will these measures be enough to alter vaccine development priorities? Lowering the risks or costs of vaccine development may be much less important than increasing the potential for profit. The vaccine development pipeline is full of products that will never come to market, not because they cost more to develop but because the company projects insufficient profit from their eventual use. Promoting a greater appreciation of the benefits of prevention in both developing and industrialized countries and enhancing the size of the market and the willingness to pay will likely have a greater impact on investment decisions than an incremental decrease in vaccine development costs.

If large manufacturers shift vaccine development priorities on the basis of incentives and other measures so that the total number of products brought to market is not increased but one set of priorities is substituted for another, the overall impact on disease prevention may be not change. The greatest increase in disease prevention and in the development of orphan vaccines would occur by increasing the total number of vaccines produced. The therapeutics industry differs from the vaccine industry in that it includes a substantially greater number of players that can bring a new product to market. In the United States currently, 194 drugs and biologics have been brought to market as orphan products, but none are vaccines. Thus, incentives that draw new companies to invest in vaccine development may be extremely useful for the development of orphan vaccines.

Vaccines prevent more than 3.2 million deaths per year (3). Developments in biotechnology have created the promise of prevention for many more infectious and chronic diseases (4). Realizing this promise will require bringing to licensure more of the vaccines now in development. Finally, our credibility in designating disease areas as priorities for vaccine development rests on our ability to use the new vaccines already in hand.

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References

1. Institute of Medicine. Vaccines for the 21st century: a tool for decision-making. Washington: National Academy Press, 1999.
2. Hausdorf WP. Prospects for the use of new vaccines in developing countries: cost is not the only impediment. Vaccine 1996;14:1179-86.
3. World Health Organization, United Nations Children's Fund. State of the world's vaccines and immunization. Geneva, Switzerland: The Organization, 1996.
4. Jordan Report. Accelerated development of vaccines 1998. Washington: National Institute of Allergy and Infectious Diseases, 1998.

Letters

Swine as a Potential Reservoir of Shiga Toxin-Producing *Escherichia coli* O157:H7 in Japan

To the Editor: Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 has become a major meat safety issue worldwide. Cattle, an important reservoir of human infection (1), may not be the only source of this organism (2,3). In a survey of pigs in England (4), non-STEC O157 was isolated from four (0.4%) fecal samples collected (after slaughter) from 1,000 pigs. We found that, although an unlikely source of infection for humans, pigs are a potential reservoir of STEC O157:H7 in Japan.

In 1997, there were 14,400 pig farms and 9,823,000 pigs (average 682 per farm) in Japan. Thirty-five (0.24%) of these farms were randomly selected for study, and rectal swabs were taken from 221 healthy pigs during May and June 1997. The average number of animals examined on each farm was 6.3.

Fecal samples were dipped into test tubes containing Cary-Blair transport medium (Nissui, Japan) and kept refrigerated until processing (usually within 48 hours). Swabs were then incubated overnight at 42°C in 10 ml of mEC broth (Kyokuto, Japan) containing 20 µg/ml of novobiocin (Sigma, USA), after which one loop of the broth was spread onto MacConkey sorbitol agar medium (Difco, USA). After overnight incubation at 37°C, sorbitol-negative colonies from the agar plates were tested by slide agglutination with *E. coli* O157-latex test (Oxoid, UK). Strains that agglutinated were confirmed as *E. coli* by using the API 20E system (BioMerieux, France). Strains confirmed as *E. coli* O157 were subcultured in a motility medium for 3 to 4 days to enhance development of flagella, then they were tested by tube agglutination with *E. coli* H7 antiserum (Denka-seiken, Japan). The swine *E. coli* O157:H7 isolates were examined by polymerase chain reaction for the presence of Shiga-toxin genes *stx1* and *stx2* and to elucidate intimin (*eaeA*) DNA sequences (5), for a plasmid of 92 kb (pO157) by agarose gel electrophoresis (6), and for phage type by the previously described method (7).

Although the numbers sampled were too small to allow comparisons between farms, samples from three (1.4%) apparently healthy pigs (ages: 2, 6, and 9 months) from three farms (8.6%) were positive for STEC O157:H7. The three strains from the pigs were biochemically typical of STEC O157:H7 that did not ferment sorbitol and lacked ß-glucuronidase; agglutinated with *E. coli* O157-latex and with H7 antiserum; possessed *stx1*, *stx2*, and *eaeA* genes; and harbored pO157 plasmid characteristic of STEC O157:H7. The strains belonged to phage type 21, 37, or 43.

The 1.4% carriage rate of STEC O157:H7 in pigs in this investigation is almost the same as that in cattle in Japan (8), which suggests that STEC O157:H7 strains are probably widespread in Japanese pig populations. The STEC O157-positive pigs were each housed in a concrete-floored pen and kept separate from cattle. Whether these pig isolates are the same as cattle or human isolates needs to be clarified; however, they had the same biochemical and genetic markers as STEC O157:H7 isolated from cattle and humans (6,9). The phage type 21 that we found among pig isolates was also observed in bovine and human STEC O157:H7 isolates in Japan (7). These results suggest that common vehicles for dissemination of the organism may exist.

So far, pork has not been identified as a source of human STEC O157:H7 illness in industrialized countries, but our results indicate that eating pork, contact with pigs, and contamination with pig feces should be considered potential sources of this pathogen. This is the first isolation of naturally occurring STEC O157:H7 in pigs in Japan.

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References

1. Wells JG, Shipman LD, Greene KD, Sowers EG, Green JH, Cameron DN, et al. Isolation of *Escherichia coli* serotype O157:H7 and other shiga-like-toxin-producing *E. coli* from dairy cattle. J Clin Microbiol 1991;29:985-9.
2. Chapman PA, Siddons CA, Harkin MA. Sheep as a potential source of verocytotoxin-producing *Escherichia coli* O157. Vet Rec 1996;138:23-4.

3. Chalmers RM, Salmon RL, Willshaw GA, Cheasty T, Looker N, Davies I, et al. Vero-cytotoxin-producing *Escherichia coli* O157 in a farmer handling horses. Lancet 1997;349:1816.

4. Chapman PA, Siddons CA, Cerdan Malo AT, Harkin MA. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiol Infect 1997;119:245-50.

5. Sueyoshi M, Fukui H, Tanaka S, Nakazawa M, Ito K. A new adherent form of an attaching and effacing *Escherichia coli* (*eaeA+,bfp-*) to the intestinal epithelial cells of chicks. J Vet Med Sci 1996;58:1145-7.

6. Chapman PA, Siddons CA, Wright DJ, Norman P, Fox J, Crick E. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. Epidemiol Infect 1993;111:439-47.

7. Akiba M, Masuda T, Sameshima T, Katsuda K, Nakazawa M. Molecular typing of *Escherichia coli* O157:H7(H-) isolates from cattle in Japan. Epidemiol Infect 1999;122:337-41.

8. Sekiya J. *Escherichia coli* O157:H7 in livestock in Japan. Revue Scientifique et Technique Office International des Épizooties 1997;16:391-4.

9. Ratnam S. March SB, Ahmed R, Bezanson GS, Kasatiya S. Characterization of *Escherichia coli* serotype O157:H7. J Clin Microbiol 1988;26:2006-12.

Hospitalizations for Rotavirus Gastroenteritis in Gipuzkoa (Basque Country), Spain

To the Editor: Rotavirus is the main cause of severe acute gastroenteritis among children both in developing and in industrialized countries. The incidence of rotavirus gastroenteritis in northern Europe is similar to or greater than the estimated incidence of the disease in the United States (1-3); however, little is known about the impact of rotavirus infection on health in southern Europe.

We examined the incidence of hospitalization for rotavirus gastroenteritis during 3 years (July 1993-June 1996) in Gipuzkoa (population 400,480, of whom 58,896 are <15 years of age). The presence of rotavirus antigen was prospectively investigated by enzyme immunoanalysis (IDEIA Rotavirus, Dako Diagnostics, UK) in stool samples from all patients <15 years of age in the study area for whom a microbiologic analysis was requested for acute gastroenteritis. Children hospitalized for rotavirus gastroenteritis were sought retrospectively through searching both the computerized records of microbiology laboratory and hospital medical records for the diagnoses 558.9 (other gastroenteritis and presumably noninfectious colitis) and 008.6-009.3 (enteritis due to specific viruses and presumably infectious intestinal disorders) (4). All children in this study lived in the study area, had been hospitalized for gastroenteritis, and had one stool sample positive for rotavirus in the first 5 days of hospitalization without another gastroenteritis agent detected in the stool.

One hundred fifty-two (82 male and 70 female) of 1,004 children <15 years of age with rotavirus gastroenteritis had been hospitalized for rotavirus infection. No deaths were recorded. Cases usually occurred in epidemic waves, with the highest incidence in January-March. An additional 133 children with rotavirus in stools had been hospitalized but were not included in this study because they had hospital-acquired infections (67 cases), were coinfected by another microorganism (11 cases), came from outside the geographic study area (19 cases), or had a main reason for hospitalization other than gastroenteritis (36 cases). The mean annual incidence of hospitalization was 0.86 per 1,000 children (1 month to 14 years old) and 3.11 per 1,000 children (1 month to 5 years old). The maximum incidence occurred in 6- to 11-month-old children (11.81 per 1,000 children). Children were hospitalized for a mean of 4.8 ± 2.2 days. Rotavirus gastroenteritis was responsible for 152 (2%) of 7,403 pediatric admissions. For the 1- to 35-month age group, community-acquired rotavirus gastroenteritis was responsible for 140 (4.6%) of 3,026 admissions.

Although the incidence is based solely on confirmed cases, the findings closely reflect disease incidence in our region. The National System of Health covers 100% of the reference population, and hospitalization of children in private institutions is rare. Stool cultures were taken for most children for gastroenteritis (94.5%), and the presence of rotavirus was investigated in every case.

The hospitalization rate observed in this study was similar to that reported in other studies from Sweden (2), Denmark (5), and the United States (6) and lower than that found in England and Wales (3). In Spain, reporting of rotavirus infection is not required, is not included in mortality registers, and is not the object of specific vigilance by sentinel surveillance systems. Therefore, information about the incidence and impact of rotavirus infection in Spain is scarce. However, two reports from Spain must be highlighted: one is based on a theoretical prediction using a statistical model (7) and the

other is a small clinical and epidemiologic study of hospitalized children <2 years of age in Santiago de Compostela (8). Data from both studies are consistent with our results. Rotavirus gastroenteritis is a common cause of hospitalization and produces a heavy load on the health-care system in our region. After years of research, vaccines that effectively prevent rotavirus infections in humans have been developed (9,10). These data should be considered in evaluating the potential benefits of introducing rotavirus vaccine in our region and monitoring its effectiveness.

Acknowledgments

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References

1. Glass RI, Bresee JS, Parashar UD, Holman RC, Gentsch JR. First rotavirus vaccine licensed: Is there really a need? Acta Paediatr 1999;88 Suppl 426:S2-8.
2. Johansen K, Bennet R, Bondesson K, Eriksson M, Hedlund K-O, De Verdier Klingenberg, et al. Incidence and estimates of the disease burden of rotavirus in Sweden. Acta Paediatr 1999;88 Suppl 426:S20-3.
3. Ryan MJ, Ramsay M, Brown D, Gay NJ, Farrington CP, Wall PG. Hospital admissions attributable to rotavirus infection in England and Wales. J Infect Dis 1996;174 Suppl 1:S12-8.
4. Ministerio de Sanidad y Consumo. Clasificación internacional de enfermedades. Novena revisión. Modificación clínica, 1994.
5. Hjelt K, Krasilnikoff PA, Grauballe PC. Incidence of hospitalization and outpatient clinical visits caused by rotavirus and non-rotavirus acute gastroenteritis. A study of children living in the southern district of Copenhagen County. Dan Med Bull 1984;31:249-51.
6. Matson DO, Estes MK. Impact of rotavirus infection at a large pediatric hospital. J Infect Dis 1990;162:598-604.
7. Visser LE, Cano Portero R, Gay NJ, Martinez Navarro JF. Impact of rotavirus disease in Spain: an estimate of hospital admissions due to rotavirus. Acta Paediatr 1999;88 Suppl 426:S72-6.
8. Rodríguez-Cervilla J, Peñalver MD, Curros MC, Pavón P, Alonso C, Fraga JM. Rotavirus: Estudio clínico y epidemiológico en niños hospitalizados menores de dos años. An Esp Pediatr 1996;45:499-504.
9. Parashar UD, Bresee JS, Gentsch JR, Glass RI. Rotavirus. Emerg Infect Dis 1998;4:561-70.
10. Bernstein DI, Sack DA, Rothstein E, Reisinger K, Smith VE, O'Sullivan D, et al. Efficacy of live, attenuated, human rotavirus vaccine 89-12 in infants: a randomized placebo-controlled trial. Lancet 1999;354:287-90.

Israeli Spotted Fever Rickettsia (*Rickettsia conorii* Complex) Associated with Human Disease in Portugal

To the Editor: Mediterranean spotted fever is endemic in Portugal, where it is a reportable disease with approximately 1,000 new cases per year (1). *Rickettsia conorii* has been thought to be the only pathogenic rickettsia of the spotted fever group in Portugal (2), as well as in the Western Mediterranean area. Another rickettsia in this group, the Israeli spotted fever rickettsia, which belongs to the *R. conorii* complex (3-5), was isolated in 1974 from ticks and humans; however, its distribution appeared to be restricted to Israel (6). We report three cases of rickettsiosis in Portugal caused by Israeli spotted fever rickettsia.

Case 1. A 71-year-old woman was hospitalized with a history of fever (39°C) for 6 days, headache, and icterus. The influenzalike syndrome was treated with an antipyretic. In the next 4 days, the patient had myalgias, malaise, and mental confusion. Ten hours after being transferred to an intensive care unit, she died with septic shock and multiorgan failure, despite intravenous administration of doxycycline and other antibiotics.

Case 2. A 79-year-old woman, who was previously healthy except for high blood pressure, was hospitalized with a 4-day history of gastrointestinal disorders, nausea, and vomiting, which were attributed to food poisoning; high fever (40°C) developed, and 3 days later a cutaneous rash, which spread to the palms and soles. The diagnosis of Mediterranean spotted fever was made by indirect immunofluorescent assay against *R. conorii* (immunoglobulin [Ig] M 1:40; IgG 1:512). The patient was treated with doxycycline and was discharged from the hospital 20 days after admission.

Case 3. A 65-year-old woman was hospitalized with a 6-day history of fever (39°C), headache, vomiting, and epigastric pain, which had been treated with penicillin. Rash and

icterus developed, and the patient died of shock and multiorgan failure 9 hours after hospitalization, despite treatment with a mixture of antibiotics, which contained doxycycline.

Rickettsiae of the spotted fever group were isolated by the shell vial technique from the blood of the three patients. Sequences of polymerase chain reaction-amplified fragments of 16SrRNA (1440 bp), citrate synthase (382 bp), and rompA (590 bp) genes of the isolates show 100% similarity with the homologous sequence of Israeli spotted fever rickettsia (4,7,8).

All three patients lived in semirural areas, along the River Tejo (Setubal District). None had left Portugal during the previous year. Although none had a tache noire, contact with ticks cannot be excluded. The absence of tache noire is typical in Israeli spotted fever (6). These findings indicate that the geographic distribution of Israeli spotted fever is wider than had been thought and includes the Iberian Peninsula. Because initial signs and symptoms of the disease are particularly uncharacteristic and appropriate treatment may be delayed, this rickettsia can cause life-threatening disease.

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References

1. Tavares L, Botas J, Antunes F, Araújo FC. A febre escaronodular em Portugal. O Médico 1985;113:838-46.
2. Bacellar F, Regnery RL, Nuncio S, Filipe AR. Genotypic evaluation of Rickettsial isolates recovered from various species of ticks in Portugal. Epidemiol Infect 1995;114:169-78.
3. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of *Rickettsiae* and estimation of interspecies sequence divergence for portions of two rickettsial genes. J Bacteriol 1991;173:1576-89.
4. Fournier PE, Roux V, Raoult D. Phylogenetic analysis of spotted fever group *Rickettsiae* by study of the outer surface protein rOmpA. Int J Sys Bacteriol 1998;48:839-49.
5. Roux V, Raoult D. Phylogenetic analysis and taxonomic relationships among the genus *Rickettsia*. In: *Rickettsiae* and rickettsial diseases at the turn of the third millennium. Raoult D, Brouqui P, editors. Paris: Elsevier; 1999. p. 52-66.
6. Goldwasser RA, Steiman Y, Klingberg W, Swartz TA. The isolation of strains of *Rickettsiae* of the spotted fever group in Israel and their differentiation from other members of the group by immunofluorescence methods. Scand J Infect Dis 1974;6:53-62.
7. Roux V, Raoult D. Phylogenetic analysis of the genus *Rickettsia* by 16S rDNA sequencing. Res Microbiol 1995;146:385-96.
8. Roux V, Rydkina E, Eremeeva M, Raoult D. Citrate syntase gene comparison, a new tool for phylogenetic analysis and its application for *Rickettsiae*. Int J Sys Bacteriol 1997;47:252-61.

Avoiding Misdiagnosis of Malaria: A Novel Automated Method Allows Specific Diagnosis, even in the Absence of Clinical Suspicion

To the Editor: We report three cases of malaria to illustrate a novel method that allows diagnosing the disease, even if clinicians do not suspect it or request malaria smears. Lack of clinical suspicion is a well-known factor for malaria misdiagnosis and may be responsible for almost 40% of deaths from *Plasmodium falciparum* infections in industrialized countries (1-3). A recent study from Canada showed that in 59% of cases malaria was initially misdiagnosed, and in 16% three or more physician contacts occurred before malaria smears were ordered (4).

Early diagnosis of malaria relies crucially on clinical suspicion. A clinician suspecting the disease has to explicitly request malaria smears. This problem has not been solved with the advent of several methods alternative to microscopy, including recently introduced rapid dipstick tests (5). Performing any of these tests blindly without a specific request is impractical. On the other hand, routinely performed laboratory tests in the work-up of febrile patients, e.g., automated full blood counts, have so far detected only nonspecific changes, such as anemia or thrombocytopenia, which are associated with many other conditions (6). These changes on their own are therefore not specific enough to trigger malaria smears without an explicit request.

New automated full blood counts-analyzers incorporate flow-cytometric principles. The Cell-Dyn 3500 (Abbott, Santa Clara, CA) uses scattered laser-light of leukocytes at four different angles to generate a white-blood-cell differential (7). Monocytes and neutrophils may

ingest birefringent depolarizing malaria pigment that can be detected by the instrument. The appearance of monocytes (purple dots) above the separation line, in the eosinophil area (green dots), is a highly specific sign of the presence of ingested malaria pigment and consequently malaria.

A study from South Africa investigating 224 directed samples for malaria diagnosis found a sensitivity of 72% and specificity of 96% (8). In Portugal, we observed 45 positives in 120 directed samples. So far, all cases identified by microscopy showed the typical changes in the full-blood-count plots, suggesting a near 100% sensitivity in imported malaria cases. Several thousand full-blood-count plots from patients with a wide range of underlying pathologic features did not show such changes, making them highly specific for malaria diagnosis. However, the changes may persist for some time despite clinical and parasitologic cure, as pigment-containing monocytes may remain in the circulation for 2 to 3 weeks (9). Consequently, the observed changes may not necessarily indicate acute disease but may persist during convalescence.

We report three cases in which clinical suspicion did not lead to the request of a malaria diagnostic test. The final diagnosis of malaria was made only because of the changes observed in the color monitor of the Cell-Dyn 3500. As part of a preliminary investigation of this new method, we reviewed all full-blood-count plots at 24-hour intervals. During a 2-week period, three full-blood-count granularity/lobularity plots compatible with malaria were identified and the full-blood-count results and clinical notes were reviewed. The principal symptoms in the three cases were fever and aches in bones and muscles, case 1; complications of assault, case 2; and feeling generally unwell (from drug abuse), case 3. In all cases, the full-blood-count results were within normal ranges, except for a thrombocyte count of 23,000 in case 2. In cases 1 and 3, the patients were discharged with a clinical diagnosis of flulike syndrome and drug abuse-related problems, respectively, while in case 2, the patient was to be admitted with a diagnosis of assault-related injuries. As attending clinicians had not requested malaria smears, we performed blood films on the recovered specimens that confirmed a diagnosis of malaria. (Case 1: *P. ovale,* 10,000 µl; case 2: *P. falciparum,*

9,000 µl; case 3: *P. falciparum,* 1,500 µl). In case 2, our findings permitted appropriate treatment in the emergency room; in the other two cases, it allowed patients to be contacted at home. All three patients (two male, one female) were of Black African origin but lived in Portugal. They had returned to Portugal after visiting Africa (Angola and Guinea). None of them had taken malaria prophylaxis during their journey.

Anisotropic malaria pigment has been the basis for several microscopy methods for malaria diagnosis (10). However, sensitivities are similar to that of conventional microscopy, and these methods have to be ordered specifically. In contrast, automated full-blood-count is regarded as routine for febrile patients, and the new automated method has the potential to detect additional, unsuspected cases, in which clinical suspicion did not lead to requests for malaria testing. If further studies validate this technique, the instrument could be modified to specifically flag such results, which would alert laboratory staff to perform blood films on these samples, even in the absence of a clinician's request. Finally, if software algorithms are adjusted to enumerate pigment-containing leukocytes, the usefulness of this indicator as a prognostic marker (11) could be further evaluated. The instrument may greatly facilitate quantification of pigment-containing leukocytes, which have been determined by time-honored but cumbersome microscopy.

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References

1. World Health Organization. World malaria situation in 1994. Wkly Epidemiol Rec 1997;36:269-274.
2. Day KP. Malaria: a global threat. In: Krause RM, editor. Emerging Infections. New York: Academic Press; 1998. p. 463-97.
3. Greenberg AE, Lobel HO. Mortality from *Plasmodium falciparum* malaria in travelers from the United States (1959-1987). Ann Intern Med 1990;113:326-7.
4. Kain KC, Harrington MA, Tennyson S, Keystone JS. Imported malaria: prospective analysis of problems in diagnosis and management. Clin Infect Dis 1998;27:142-9.
5. Hänscheid T. Diagnosis of malaria: a review of alternatives to conventional microscopy. Clin Lab Haematol 1999;21:235-45.

6. Giacomini T, Lusina D, Foubard S, Baledent F, Guibert F, Le Pennec MP. Dangers of hematological automated analysis for malaria diagnosis. Bull Soc Pathol Exot 1991;84:330-3.

7. de Grooth BG, Terstappen LW, Puppels GJ, Greve J. Light-scattering polarization measurements as a new parameter in flow cytometry. Cytometry 1987;8:539-44.

8. Mendelow BV, Lyons C, Nhlangothi P, Tana M, Munster M, Wypkema E, et al. Automated malaria detection by depolarization of laser light. Br J Haematol 1999;104:499-503.

9. Day NPJ, Thi Diep P, Thi Ly P, Xuan Singh D, Phu Loc P, Van Chuong L, et al. Clearance kinetics of parasite and pigment-containing leukocytes in severe malaria. Blood 1996;88:4694-700.

10. Lawrence C. Laveran remembered: malaria haemazoin in leucocytes. Lancet 1999;353:1852.

11. Phu NH, Day N, Thi Diep P, Ferguson DJP, White NJ. Intraleucocytic malaria pigment and prognosis in severe malaria. Trans R Soc Trop Med Hyg 1995;89:200-4.

The First Reported Case of *Aerococcus* Bacteremia in a Patient with HIV Infection

To the Editor: We report the first case of *Aerococcus viridans* bacteremia in a patient with HIV infection. Two species in the genus *Aerococcus* have been implicated as rare pathogens in humans. *A. urinae* causes urinary tract infections; the other species, *A. viridans*, a gram-positive coccus considered a contaminant in cultures, has been associated with human infections that included bacteremia (1,2), septic arthritis (3), and infectious endocarditis (4,5). Widely distributed in the environment, the organism has been recovered from dust, vegetables, and crustaceans (6) and was isolated from different areas in a hospital (recovery room, intensive care unit, delivery room, treatment room, premature nursery) and from numerous objects (7).

We describe the first case of *A. viridans* bacteremia in a patient with HIV. A 34-year-old man without notable medical history sought medical attention after several weeks of epigastric midabdominal pain associated with a 15-lb weight loss; the pain did not respond to antacid medications. The patient said that he did not have fever, chills, night sweats, or history of transfusions and did not use alcohol, tobacco, or drugs. He had engaged in homosexual activity 2 to 3 years earlier.

Physical examination showed moderate cachexia and low-grade fever (38.8ºC) associated with tachycardia, but the heart and lung examination was otherwise normal. The abdomen was soft, flat, and tender to palpation in the midabdominal epigastric area, without hepatosplenomegaly, guarding, or rebound tenderness. No other abnormalities were identified. The patient was admitted to the hospital, and the initial set of routine blood cultures (Bactec 9240 instrument, Becton Dickinson, Sparks, MD) showed no growth. On hospital day 2, he began to have severe rigors, along with persistent fever. A second set of blood cultures drawn at that time grew paired gram-positive cocci in less than 24 hours. The patient was empirically started on penicillin G, and cefotaxime was added shortly thereafter because of the possibility of intermediately resistant pneumococcus. The rigors responded to antibiotic treatment, and a third set of blood cultures showed no growth. The negative blood cultures before and after appropriate antimicrobial therapy and the short time to detection (which suggests a large initial inoculum) led us to believe that the organism in this case was a true pathogen and not a contaminant.

The patient's work-up included a normal abdominal computer tomography; abdominal ultrasound showed nonobstructing cholelithiasis. Laboratory tests demonstrated anemia of chronic disease diagnosed by a hematocrit of 25% associated with a low reticulocyte production index, high serum ferritin, and an elevated erythrocyte sedimentation rate (91 mm/hr), with polyclonal hypergammaglobulinemia and hypoalbuminemia on serum protein electrophoresis. Stool samples were negative for occult blood, and serologic tests showed no *Helicobacter pylori* antibodies. The patient's total lymphocyte count was 300 cells/µl, HIV serologic testing by enzyme-linked immunosorbent assay and Western blot was positive, and flow cytometry revealed an absolute CD4+ T-lymphocyte count of 19 cells/µl, with an HIV-1 retroviral titer of 280,000 by polymerase chain reaction. Gallium scanning was negative for *Pneumocystis carinii* pneumonia and gastrointestinal lymphoma. A follow-up endoscopy showed esophageal ulcers, with disruption of the mucosal barrier. Blood cultures were negative for cytomegalovirus or mycobacteria, but the aerobic isolate initially reported as paired gram-positive cocci was later identified as *A. viridans.*

The identification of *A. viridans* was made on

the basis of the following characteristics: catalase negativity, α-hemolytic gram-positive cocci forming pairs and tetrads (not chains) in broth culture; growth in the presence of 40% bile and 6.5% NaCl and ability to hydrolyze esculin; pyrrolidony l-aminopeptidase positivity, leucine-aminopeptidase negativity; and production of acid from trehalose, sucrose, maltose, and lactose but not from sorbitol.

Susceptibility testing by the agar dilution method showed that the isolate was susceptible to penicillin-G (MIC = 0.12 µg/ml) and vancomycin (MIC = 0.25 µg/ml). On the basis of this case and previous reports (1,2), we believe that *A. viridans* is a potential pathogen that can cause serious infections in immunocompromised patients. The presumed route of infection in this patient was esophageal ulcers. Clinical microbiologists should pay close attention to α-hemolytic, catalase-negative streptococci recovered from sterile body sites that form tetrads rather than chains on Gram stain.

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References

1. Swanson H, Cutts E, Lepow M. Penicillin-resistant *Aerococcus viridans* bacteremia in a child receiving prophylaxis for sickle-cell disease. Clin Infect Dis 1996;22:387-8.
2. Kern W, Vanek E. *Aerococcus* bacteremia associated with granulocytopenia. European Journal of Clinical Microbiology 1987;6:670-3.
3. Taylor PW, Trueblood MC. Septic arthritis due to *Aerococcus viridans*. J Rheumatol 1985;5:1004-5.
4. Untereker WJ, Hanna BA. Endocarditis and osteomyelitis caused by *Aerococcus viridans*. Mt Sinai J Med 1976;43:248-52.
5. Janosek J, Eckert J, Hudac A. *Aerococcus viridans* as a causative agent of infectious endocardititis. Journal of Hygiene, Epidemiology, Microbiology and Immunology 1980;1:92-6.
6. Pien FD, Wilson WR, Kunz K, Washington JA II. *Aerococcus viridans* endocarditis. Mayo Clin Proc 1984;59:47-8.
7. Kerbaugh MA, Evans JB. *Aerococcus viridans* in the hospital environment. Applied Microbiology 1968;16:519-23.

Proficiency in Detecting Vancomycin Resistance in Enterococci among Clinical Laboratories in Santiago, Chile

To the Editor: Vancomycin-resistant enterococci (VRE) can be difficult to detect because of limitations in the susceptibility testing methods commonly used in clinical laboratories. Although VRE have not been reported in Chile, clinical isolates have been reported in Argentina (1) and Brazil (2). It is important to detect vancomycin resistance as early as possible, so infection control preventive measures can be instituted when they have their greatest impact. The microbiology laboratory is the first line of defense against VRE, as it plays a critical role in its recognition. In Chile, most laboratories follow the National Committee for Clinical Laboratory Standards recommendations for antimicrobial susceptibility testing and use disk-diffusion methods (3); however, these methods have limitations in detecting low levels of resistance to vancomycin in enterococci.

We evaluated the ability of referral microbiology laboratories in Chile to detect vancomycin resistance in five *Enteroccocus* spp. isolates with different susceptibility patterns for vancomycin, penicillin, and ampicillin. Of six referral laboratories that agreed to participate, four used the disk-diffusion method to evaluate antimicrobial susceptibility. Two used an agar dilution minimum inhibitory concentration (MIC) method, one as the only susceptibility testing method and the other in addition to disk diffusion. The participants correctly evaluated vancomycin susceptibility in 17 (57%) of 30 isolates.

The accuracy of detecting vancomycin resistance varied according to the level of resistance. Isolate 1, which had a high level of resistance (Van A phenotype, MIC 256 µg/ml), was evaluated correctly in 5 (83%) of 6 laboratories. Isolate 2, with a lower level of resistance (Van B, MIC 64 µg/ml), was evaluated correctly in 4 (67%) of 6 laboratories. Isolates 3 and 4, both with intermediate resistance (Van B, MIC 16-32 µg/ml, and Van C, MIC 8 µg/ml, respectively), were evaluated correctly by one laboratory each. Isolate 5 (vancomycin susceptible) was evaluated correctly by all laboratories. Susceptibility to penicillin and ampicillin was correctly identified in 53 (96.4%) of 55 isolates. Although laboratories

correctly identified *E. faecium* and *E. faecalis* to the species level, most (4 of 5) did not correctly identify *E. gallinarum* (three misidentified it as *E. casseliflavus* and one as *E. faecalis*).

The results of this study are consistent with those of previous studies in the United States (4,5), South America (6), Spain (7), and Mexico (8). Although in countries like Chile, disk diffusion is practical and reliable for most susceptibility testing, detecting low-level vancomycin resistance in enterocci is difficult without supplementary testing. In Chile, as in other countries, strategies should be implemented to improve detection of these strains, including improvement of phenotypical and genotypical methods for VRE detection and species identification. Documentation of proficiency in detecting VRE is important for improving laboratory performance, detecting clinical isolates, and accurate and reliable reporting to local, national, and international surveillance systems.

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References

1. Marin ME, Mera JR, Arduino RC, Correa AP, Coque TM, Stambulian D, et al. First report of vancomycin-resistant *Enterococcus faecium* isolated in Argentina. Clin Infect Dis 1998;26:235-6.
2. Cereda RF, Medeiros EA, Vinagre A, Rego ST, Hashimoto A, Febre N, et al. Epidemiologic analysis for acquisition of vancomycin-resistant enterococcus (VRE) in an intensive care unit in Brazil. In: Proceedings of the Eighth Annual Meeting of the Society for Healthcare Epidemiology of America; 1998; São Paulo, Brazil.
3. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial disk susceptibility tests: approved standard M2-A6, 6th ed. Villanova (PA): The Committee.
4. Tenover FC, Tokars J, Swenson J, Paul S, Spitalny K, Jarvis WR. Ability of clinical laboratories to detect antimicrobial agent-resistant enterococci. J Clin Microbiol 1993;31:1695-9.
5. Rosenberg J, Tenover FC, Wong J, Jarvis W, Vugia DJ. Are clinical laboratories in California accurately reporting vancomycin-resistant enterococci? J Clin Microbiol 1997;35:2526-30.
6. Cookson ST, Lopardo H, Marin M, Arduino R, Rial MJ, Altschuler M, et al. Study to determine the ability of clinical laboratories to detect antimicrobial-resistant *Enterococcus* spp. in Buenos Aires, Argentina. Diagn Microbiol Infect Dis 1997;29:107-9.
7. Alonso-Echanove J, Robles B, Jarvis WR. Proficiency of clinical laboratories in Spain in detecting vancomycin-resistant *Enterococcus spp*. The Spanish VRE Study Group. J Clin Microbiol 1999;37:2148-52.
8. McDonald LC, Garza LR, Jarvis WR. Proficiency of clinical laboratories in and near Monterrey, Mexico, to detect vancomycin-resistant enterococci. Emerg Infect Dis 1999;5:143-6.

Food-Related Illness and Death in the United States

To the Editor: Dr. Mead and colleagues should be commended for attempting to estimate the prevalence of foodborne disease in the United States (1). Their study provides more complete estimates than previous studies in terms of the number of foodborne pathogens included; for example, it includes the first realistic estimate of the number of cases of disease due to Norwalk-like caliciviruses. However, the publication of these estimates raises some important issues.

Even though "accurate estimates of disease burden are the foundation of sound public health policy" (2), most of these estimates (in particular, the assumption that unknown agents are transmitted by food in the same proportion as known agents) were derived from assumptions rather than data. Known foodborne agents clearly cannot account for most gastrointestinal illnesses (1). However, illnesses from unknown agents may be as likely to have the transmission characteristics of rotavirus (1% foodborne) or *Cryptosporidium* (10% foodborne) as those of the Norwalk-like viruses (40% foodborne). Furthermore, it was assumed that detecting outbreaks or cases of toxin-mediated illnesses (e.g., due to *Bacillus cereus*, *Staphylococcus aureus*, or *Clostridium perfringens)* follows the model of *Salmonella*. In the authors' entire list of known foodborne agents, data are presented for cases identified both from outbreaks and active surveillance for only three agents: *Salmonella, Shigella*, and *Campylobacter*. *Salmonella* is clearly the most highly characterized, hence the most attractive as a model. However, the ratios of the numbers of cases detected through active surveillance to the numbers of cases detected through outbreaks range from 10 for *Salmonella* to more than 400 for *Campylobacter*. What if the ratios for toxin-mediated illnesses were more

similar to *Campylobacter* than to *Salmonella* ratios? The total estimated cases of these illnesses would increase by a factor of 40. The inadequacy of simply applying a *Salmonella*-based multiplier to the number of cases reported from outbreaks can be demonstrated by applying that multiplier to the total number of cases reported in all foodborne disease outbreaks, typically 15,000 to 20,000 per year (3,4). On the basis of these estimates, the number of foodborne illnesses would range from 5.7 million to 7.6 million, including illnesses caused by unknown agents.

The authors make similar assumptions for hospitalizations and deaths: unknown agents are estimated to account for 81% of hospitalizations and 65% of deaths due to foodborne illnesses. In a retrospective review of death certificate data similar to that used by Mead and colleagues, Perkins et al. projected the number of unexplained deaths possibly due to infectious diseases they expected to find in the Emerging Infections Program sites (5). Prospectively, a much smaller number of unexplained deaths was actually found, because known causes were identified through a detailed review of the death certificates and cases (6). A prospective examination of death certificates for foodborne diseases might also result in a smaller than expected yield.

The need to rely on assumptions to generate estimates highlights the gaps in our understanding of foodborne diseases. A dozen different studies could address these data gaps. However, once the 76 million figure is agreed upon, the perceived need for these studies will decrease.

Finally, if these estimates are accepted as reasonable, do current food safety efforts represent sound public policy? If 82% of foodborne illnesses, 81% of hospitalizations, and 65% of deaths are caused by agents we have not yet identified, where is the commitment of resources needed to identify them? If eradicating *Campylobacter, Salmonella, Escherichia coli* O157:H7, and *Listeria* would reduce the number of foodborne illnesses by only 5%, hospitalizations by 10%, and deaths by 25%, why are these agents the primary focus of our national foodborne disease control efforts? Overestimating the occurrence of foodborne diseases caused by unknown agents may lead us to undervalue the public health importance of these and other well-known agents.

Estimating the occurrence of foodborne diseases is daunting. The numerous efforts, including this one by Mead et al., to provide estimates have serious shortcomings. The real challenge is to identify the gaps in our knowledge so that they can be systematically addressed and updated estimates of foodborne illness can be provided to guide prevention efforts and assess the effectiveness of current food safety measures (2).

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References
1. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis 1999;5:607-25.
2. Centers for Disease Control and Prevention. CDC data provides the most complete estimate on foodborne disease in the United States. Press release available at URL: http://www.cdc.gov/od/oc/media/pressrel/r990917.htm
3. Foodborne disease outbreaks, 5-year summary, 1983-1987. MMWR Morb Mortal Wkly Rep 1992;39(SS-1):1-15.
4. Surveillance for foodborne disease outbreaks. United States, 1988-92. MMWR Morb Mortal Wkly Rep 1996;45(SS-5):2-55.
5. Perkins BA, Flood JM, Danila R, Holman RC, Reingold AL, Klug LA, et al. Unexplained deaths due to possibly infectious causes in the United States: defining the problem and designing surveillance and laboratory approaches. Emerg Infect Dis 1996;2:47-53.
6. Minnesota Department of Health. Annual summary of communicable diseases reported to the Minnesota Department of Health, 1998. Disease Control Newsletter 1999;27:29-30.

Food-Related Illness and Death in the United States–Reply to Dr. Hedberg

To the Editor: Like all scientific undertakings, our estimates require assumptions. Because the actual frequency of foodborne transmission of unknown agents cannot be measured directly, it must be assumed. If unknown agents had transmission characteristics similar to those of rotavirus (1% foodborne transmission) or cryptosporidium (10% foodborne transmission), as Dr. Hedberg suggests, the number of cases of foodborne illness caused by unknown agents would be substantially lower than we estimated. However, unknown agents could just as easily have the transmission characteristics of *Escherichia coli* O157:H7 or *Campylobacter* (80% foodborne transmission), which just 30 years ago

were "unknown agents." For the sake of objectivity, we based our assumption on the aggregate of information for known pathogens rather than on "expert opinion." Interestingly, however, the Council of Science and Technology's "expert opinion" of the percentage of diarrheal illness due to foodborne transmission was 35% (1), nearly identical to the figure we developed.

As noted in our article, pathogen-specific multipliers for underreporting are needed for many diseases. For lack of a better model, we assumed that the underreporting of toxin-mediated diseases follows the model of *Salmonella*. The alternative Dr. Hedberg suggests, *Campylobacter*, is also a nontoxin-mediated bacterial infection like *Salmonella*, but one for which the degree of underreporting is less well documented. Extrapolating from outbreak data to the number of sporadic cases does indeed have limitations, which is the reason we used it for only the few diseases for which other surveillance data were not available.

Regarding deaths attributed to unknown agents, prospective studies may show that some of these deaths are in fact caused by known agents. However, this would not necessarily lessen the overall impact of foodborne illness: it would merely shift the number of deaths from the unknown category to the known category. The possibility that some deaths attributed to unknown agents are in fact caused by *Salmonella* and other known pathogens supports our use of data on known pathogens to estimate the frequency of foodborne transmission for unknown agents.

Improved estimates will require expanded research into the etiologic spectrum of undiagnosed illness. In the meantime, documenting the substantial impact of foodborne illness neither devalues current surveillance and prevention efforts nor undermines future efforts to determine the causes and impact of foodborne diseases. Our estimates help define gaps in existing knowledge and provide a more rational basis for public health policy than reliance on decades-old data.

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References

1. Foodborne pathogens: risks and consequences. Ames, (IA): Council of Agricultural Science and Technology; 1994.

Specimen Collection for Electron Microscopy

To the Editor: We read with interest the excellent article "Smallpox: an attack scenario," by Tara O'Toole (1). At a critical point in the scenario, the author states, "The infectious disease specialist takes a swab specimen from the … skin lesions… and requests that it be examined by an experienced technician…. electron microscopy shows an orthopoxvirus consistent with variola." In fact, swab specimens of skin lesions for the detection by electron microscopy of viruses such as pox and herpes viruses are far from ideal; the chances of viral detection would be greatly enhanced if a skin scraping were provided to the electron microscopist.

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References
1. O'Toole T. Smallpox: an attack scenario. Emerg Infect Dis 1999;5:540-6.

News and Notes

Upcoming Keystone Symposia

"Pathogen Discovery: From Molecular Biology to Diseases"
Organizers: Georg Hess and Helen H. Lee

"Genetics, Pathogenesis and Ecology of Emerging Viral Diseases"
Organizers: Michael J. Buchmeier and Clarence J. Peters

The two symposia will be held concurrently at the Civic Center, Taos, New Mexico, January 24-30, 2000. For information on registration and abstract submission, see the Keystone Symposia Web Site at: http://www.symposia.com.

Summary of Symposia Contents

There is still a long list of serious diseases whose causes remain undetermined. Recently, new technologies have allowed the discovery of a number of new and emerging pathogens. Elucidation of pathogenesis and disease associated with these agents has been challenging and controversial.

The first of these concurrent symposia, "Pathogen Discovery: From Molecular Biology to Diseases," seeks to foster discussion of important subjects related to pathogen discovery, including the assessment of evidence implicating candidate infectious agents in disease, the value of animal models, technologies used to search for candidate pathogen sequences, and approaches to the study of disease pathogenesis. Interactions between the host and the infectious agent will be addressed, with reference to susceptibility, viral persistence, and disease expression.

The second symposium will explore these issues in detail as they apply to emerging viruses. Emerging viruses have attracted attention in the scientific and popular press, and their recognition as serious human disease threats has resulted in a flood of reports describing their appearance and spread. Examination of this body of literature during the second symposium will allow the identification of several themes central to the understanding and control of emerging viruses. These themes include interpreting the role of genetic variation in animal and human disease, understanding the ecologic relationships of the virus to its natural host and vectors, and delineating how these relationships affect humans.

The mechanisms of viral pathogenesis must be understood so that therapeutic and vaccination strategies can be designed. The combination of a rapidly expanding world population and the ease of travel on a global scale provide opportunities for transport of viral vectors or infected persons from disease-endemic areas to other regions, heightening the urgent need for detection and prevention strategies. Key investigators in each of these areas will present recent findings in a setting that facilitates comparisons with other presentations highlighting the social and economic problems posed by emerging viral diseases.

Applied Epidemiology Course
Wellington, New Zealand, February 14-18, 2000

This course, offered by the Wellington School of Medicine as part of its Third International Summer School, provides participants with core skills in applied epidemiology: the ability to analyze data from an epidemiologic perspective, explore data using geographic information systems (GIS), set up surveillance systems, and investigate disease outbreaks and clusters. The course also teaches analytical skills, including practical use of EpiInfo and GIS software.

Highly interactive teaching and case studies provide the knowledge and skills needed for work in public health. The course is specifically designed for medical and nonmedical staff who undertake field investigations, for infection control staff, and for policy analysts who interpret data.

The course is convened by Michael Baker, a public health physician at New Zealand's national communicable disease surveillance center (ESR) `and senior lecturer at the Department of Public Health, Wellington School of Medicine. Other contributors, from ESR, the Wellington School of Medicine and the Wellington public health service, include epidemiologists with training and experience in New Zealand, Australia, and the United States.

Cost for the 5-day course is NZ$1,150. For further information or application, contact the Department of Public Health, Wellington School of Medicine, P.O. Box 7343, Wellington South; telephone: 64-4-385-5999, ext. 6052; fax: 64-4-389-5139; e-mail: comhtw@wnmeds.ac.nz; homepage: http://www.wnmeds.ac.nz/academic/dph.

News and Notes

4th Decennial International Conference on Nosocomial and Health Care-Associated Infections
Atlanta, Georgia, USA, March 5–9, 2000

On-Line Abstract Submission Deadline—November 15, 1999

Every 10 years, the Centers for Disease Control and Prevention sponsors the International Conference on Nosocomial and Health Care-Associated Infections in Atlanta, Georgia. The 1970, 1980, and 1990 conferences set the agenda for research and infection control practices for the respective decades; the Fourth Decennial Conference will set the agenda for the new millennium.

Call for abstracts
The deadline for on-line submission of abstracts is November 15, 1999. The deadline for abstracts submitted by mail is November 1, 1999. Selected late-breaker abstracts must be submitted by Monday, January 17, 2000. Abstracts can be submitted on-line starting in June at http://www.decennial.org/. Abstract submission forms can be downloaded from the Internet site or mailed upon request. Abstract submission forms will be automatically distributed to all Association for Professionals in Infection Control and Epidemiology (APIC), Infectious Disease Society of America (IDSA), and Society for Health Care Epidemiology of America (SHEA) members, as well as attendees at the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC).

Program Registration
Program and registration information will be distributed to all members of APIC, IDSA, and SHEA, as well as to ICAAC attendees. If you are not a member of one of these organizations, please contact the 4th Decennial Conference (see below) and ask to be placed on the mailing list. The conference will be held in conjunction with the 10th annual meeting of SHEA; separate registration is required for SHEA events.

For more information contact the 4th Decennial Conference, 6220 Montrose Road, Rockville, MD 20852, USA; telephone: 301-984-9450; fax: 301-984-9441; e-mail: info@decennial.org; Internet: www.decennial.org.

Towards a Healthy Europe for the Year 2010
V European Conference on Health Promotion and Health Education
Santander, Spain, May 10-13, 2000

The theme of cultural diversity has been chosen for this conference, which will deal with professional practices, communication in social settings, and health policies that should be developed to achieve a healthy Europe for the next millennium.

We believe that the coexistence of different cultures is sometimes the cause of health problems but also increasingly the source of solutions and opportunities for health promotion and for the achievement of a healthy Europe.

The subjects related to this central theme will be addressed in plenary sessions, open debates, oral presentations, and poster sessions. There will also be workshops which will serve as a forum for professionals with common interests in specific areas.

For additional information, please contact Asociación de Educación para la Salud (ADEPS), Servicio de Medicina Preventiva, 4ª Norte, Hospital Clínico San Carlos, c/o Profesor Martín Lagos, 28040 Madrid, Spain; telephone: 34-91-330-3422; fax: 34-91-543-7504; e-mail: msainz@hcsc.es.

Erratum Vol. 5 No. 3

In the article "Use of Antimicrobial Growth Promoters in Food Animals and *Enterococcus faecium* Resistance to Therapeutic Antimicrobial Drugs in Europe," by H.C. Wegener et al., reference 3 is incorrect. The correct reference is

3. Centers for Disease Control and Prevention. Summary of Notifiable Diseases, United States, 1997. MMWR Morb Mortal Wkly Rep 1998;46:1-87.

We regret any confusion this error may have caused.

ICEID 2000

Call for Abstracts

Abstract submission to ICEID 2000 is now possible through the Conference Web site, and we strongly encourage you to make use of this electronic method. Not only will you be able to submit your abstract in a finished form (prepared using your own word processing program such as Word or WordPerfect, on PC or Macintosh platforms), but you can check back to learn acceptance status, presentation location, and time information. In addition, it will save you both the costs of postage and the worry of a lost submission.

ICEID 2000 is using the American Society for Microbiology's Web-based Abstract Submission System. If you have used the system to submit to either the 1999 or 2000 ASM General Meetings or the 1999 ICAAC Meeting, you will be able to reenter the system as a returning user.

For more information on ICEID abstract submission, see the ASM Web site: www.asmusa.org. Follow the links to Meetings and ICEID 2000.

News and Notes

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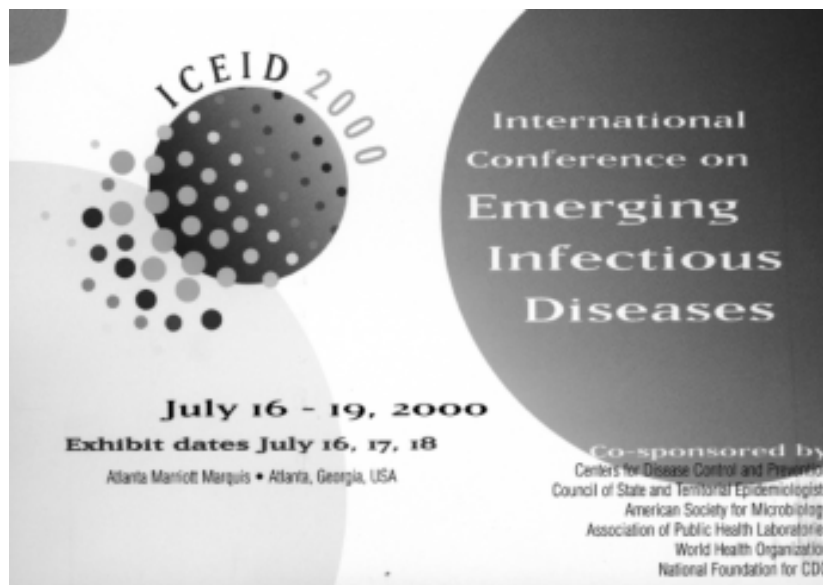
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Current Issue

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Press Releases

Frog Die-Offs Caused by Two New Infections
November 15, 1999
Mass deaths of frogs worldwide, due to two new infections, could predict far-reaching effects on the ecosystem.

ATLANTA—Two newly discovered infections—one fungal, the other viral—may be causing mass die-offs of frogs, sometimes to the brink of extinction, according to an article in the current issue of Emerging Infectious Diseases, CDC's peer-reviewed journal, which tracks new and reemerging infectious diseases worldwide. Go

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Editorial Policy and Call for Articles

Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal has an international scope and is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, demography, sociology, and other disciplines. Inquiries about the suitability of proposed articles may be directed to the Editor at 404-371-5329 (tel), 404-371-5449 (fax), or eideditor@cdc.gov (e-mail).

Emerging Infectious Diseases is published in English and features the following types of articles: Perspectives, Synopses, Research Studies, Policy Reviews, and Dispatches. The purpose and requirements of each type of article are described in detail below. To expedite publication of information, we post journal articles on the Internet as soon as they are cleared and edited.

Spanish and French translations of some articles can be accessed through the journal's homepage at www.cdc.gov/eid. Articles by authors from non-English-speaking countries can be made simultaneously available in English and in the author's native language (electronic version of the journal only).

Instructions to Authors

Manuscript Preparation

Follow "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Ann Int Med 1997:126[1]36-47) (http://www.acponline.org/journals/resource/unifreqr.htm).

Begin each of the following sections on a new page and in this order: title page, abstract, text, acknowledgments, references, tables, figure legends, and figures.

Title page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Also provide address for correspondence (include fax number and e-mail address).

Abstract and key words. Avoid citing references in the abstract. Include up to 10 key words; use terms listed in the Medical Subject Headings from Index Medicus (http://www.nlm.nih.gov/mesh/meshhome.html).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Type only on one side of the paper and number all pages, beginning with the title page. Indent paragraphs 5 spaces; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use Courier font size 10 and ragged right margins. Italicize (rather than underline) scientific names when needed.

Electronic formats. For word processing, use WordPerfect or MS Word. Send graphics in either (TIFF), or .EPS (Encapsulated Postscript) formats. The preferred font for graphics files is Helvetica. Convert Macintosh files into one of the suggested formats. Submit slides or photographs in glossy, camera-ready photographic prints.

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

Tables and figures. Create tables within the word processing program's table feature (not columns and tabs within the word processing program). For figures, use color as needed; send files, slides, photographs, or prints. Figures, symbols, lettering, and numbering should be clear and large enough to remain legible when reduced. Place figure keys within the figure.

Manuscript Submission

Include a cover letter verifying that the final manuscript has been seen and approved by all authors.

Submit three copies of the original manuscript with three sets of original figures and an electronic copy (on diskette or by e-mail) to the Editor, Emerging Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd., MS D-61, Atlanta, GA 30333, USA; e-mail eideditor@cdc.gov.

Types of Articles

Perspectives, Synopses, Research Studies, and Policy Reviews: Articles should be approximately 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch.

Perspectives: Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases or related issues. Perspectives may also address factors known to influence the emergence of diseases, including microbial adaptation and change; human demographics and behavior; technology and industry; economic development and land use; international travel and commerce; and the breakdown of public health measures. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Synopses: This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. Use of subheadings in the main body of the text is recommended. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text. Photographs and illustrations are encouraged.

Research Studies: These articles report laboratory and epidemiologic results within a public health perspective. Although these reports may be written in the style of traditional research articles, they should explain the value of the research in public health terms and place the findings in a larger perspective (e.g., "Here is what we found, and here is what the findings mean").

Policy Reviews: Articles in this section report public health policies that are based on research and analysis of emerging disease issues.

Dispatches: These brief articles are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome. Dispatches (1,000 to 1,500 words) need not be divided into sections. Provide a short abstract (50 words); references, not to exceed 10; figures or illustrations, not to exceed two; and a brief biographical sketch.

Book Reviews: Short reviews (250 to 500 words) of recently published books on emerging disease issues are welcome.

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

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