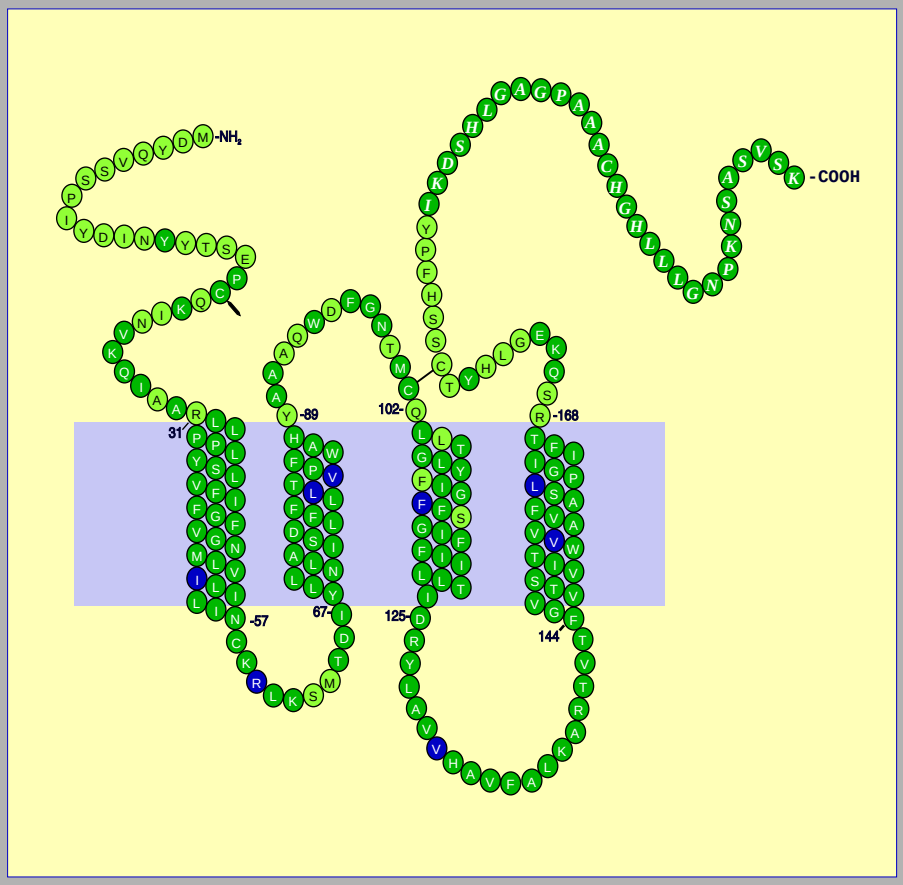
EMERGING INFECTIOUS DISEASES

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

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

Vol. 3, No. 3, July-Sept 1997



Recombination in HIV

Host Genes and HIV

Vancomycin Resistance

Flea-borne Rickettsioses

Lyme Disease

Epidemic Typhus

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

Contents

EMERGING INFECTIOUS DISEASES

Volume 3 • Number 3 *July–September 1997*

Cover photo: Predicted structure and amino acid sequence of the mutant form of human CCR5. Modified and reprinted with permission from Nature (Vol. 382:723), Copyright (1996), Macmillan Magazines Ltd and the authors.

Emerging Infectious Diseases is indexed in Index Medicus/Medline, Current Contents, and several other electronic databases.

Electronic Access

If you have Internet access, you can retrieve the journal electronically on the World-Wide Web (WWW), through file transfer protocol (FTP), or by electronic mail (e-mail). WWW and FTP: Access the journal at http://www.cdc.gov/ncidod/EID/eid.htm or from the CDC home page (http://www.cdc.gov). You can also download it through anonymous FTP at ftp.cdc.gov. The files can be found in the directory pub/publications/eid. LISTSERVer (e-mail lists): To have the table of contents automatically sent to your e-mail box,subscribe to the EID-TOC mailing list. You can then obtain individual articles on WWW or through FTP, or you can request copies by e-mail. To receive the entire journal, subscribe to the EID-ASCII mailing list To subscribe to a list, send an e-mail to listserv@cdc.gov with the following in the body of your message: subscribe listname (e.g., subscribe EID-TOC).

Emerging Infectious Diseases

Emerging Infectious Diseases is published four times a year by the National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, Mailstop C-12, Atllanta, GA 30333, USA. Telephone 404-639-3967, fax 404-639-3075, e-mail eideditor@cdc.gov.

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

Recombination in HIV: An Important Viral Evolutionary Strategy

Donald S. Burke
Walter Reed Army Institute of Research, Rockville, Maryland, USA

Human immunodeficiency virus (HIV) is a diploid virus: each virion carries two complete RNA genomic strands. Homologous recombination can occur when a cell is coinfected with two different but related strains. Naturally occurring recombinant HIV strains have been found in infected patients in regions of the world where multiple genotypic variants cocirculate. One recombinant HIV strain has spread rapidly to millions of persons in Southeast Asia. Recombination is a mechanism whereby high level and multidrug-resistant strains may be generated in individual treated patients. Recombination also poses theoretical problems for the development of a safe HIV vaccine. Certain features of HIV replication, such as syncytium formation and transactivation, may be best understood as components of a sexual reproductive cycle. Recombination may be an important HIV evolutionary strategy.

Human immunodeficiency virus (HIV)-1, like all retroviruses, is "diploid." Each viral particle contains two RNA strands of positive polarity, each full length and potentially able to replicate (1). No other virus families, RNA or DNA, are diploid. Typically both RNA strands in a retroviral particle derive from the same parent provirus. However, if an infected cell simultaneously harbors two different proviruses, one RNA transcript from each provirus can be encapsidated into a single "heterozygous" virion. When this virion subsequently infects a new cell, the reverse transcriptase may jump back and forth between the two RNA templates so that the newly synthesized retroviral DNA sequence is recombinant between that of the two parents (2). All subsequent progeny virions will be of this recombinant genotype. HIV-1 strains with chimeric genomes thought to have arisen through homologous recombination have recently been discovered in nature (3).

Temin observed that the replication strategy of HIV-1 suggests a form of primitive sexual reproduction (4), which is apparently genderless but sexual in that 1) two parental gametes must fuse into a single progeny, 2) the genetic information of the parental strains is recombined, and 3) subsequent offspring carry genetic information from both parents.

Address for correspondence: Col. Donald S. Burke, Walter Reed Army Institute of Research, 1600 East Gude Drive, Rockville, MD 20850 USA; fax 301-294-1898; e-mail: dburke@pasteur.hjf.org.

Theoretical Advantages and Disadvantages of Recombination

The replication error rate for HIV is such that each newly synthesized HIV genome carries on average approximately one mutation (5). This high mutation rate, common to most RNA viruses, permits rapid exploration of nucleotide sequence space (the universe of all possible RNA sequences) (6). Only certain regions of sequence space encode replication-competent viruses; these regions can be conceptualized as "peaks" on a "fitness landscape" of sequence space. Although a high mutation rate can lead to rapid evolution, too high a mutation rate carries the danger that the encoded information may degenerate into gibberish. For an organism with a very high mutation rate, an efficient recombination mechanism provides at least two significant theoretical advantages.

Escape from Muller's Ratchet (Within a Fitness Peak)

For any organism with a genome sequence exactly on a peak on the fitness landscape, every new mutation is by definition not beneficial. Furthermore, unfavorable mutations accumulate more rapidly than restorative back-mutations. Muller showed that in the absence of recombination, the net effect is an inexorable stepwise

"ratcheting down" in fitness of the entire population, where each step in the "ratchet" represents loss of the previously most highly fit genomic sequence. Genetic recombination can readily bring about regeneration of perfectly fit organisms from less than perfectly fit parents (7).

Evolutionary Broad Jumping (Between Fitness Peaks)

On rugged fitness landscapes—regions of sequence space where as few as one or two mutations can be lethal—an organism may be trapped on a fitness peak because locations in sequence space near the peak may all be nonviable. In such circumstances, step-by-step mutation is not an option for exploration of sequence space. Recombination between two such highly niched organisms can generate progeny that may fortuitously "land" on unexplored fitness peaks at positions in sequence space between those of the parents. Kaufman has dubbed this process "evolutionary broad jumping" (8).

For conventional plus-stranded RNA viruses, replication occurs in the cytoplasm. A plus RNA strand growing on a negative strand template can transiently hybridize with other template (negative) strands to form a replication complex, thereby confusing the polymerase into a "copy choice" that can lead to template switching (9). In contrast, transcription of a retroviral genomic plus strand is restricted to a fixed location in the nucleus where the negative strand template is integrated into the host chromosomal DNA. Unable to utilize the conventional "replication complex" mechanism for recombination during forward transcription, retroviruses may have evolved an alternative mechanism to bring two strands together within a single virion to permit recombination during reverse transcription.

As for all organisms that reproduce sexually, the cost is high: half the genetic information in each generation is wasted. Furthermore, the efficiency of retroviral recombination is unclear. Many "matings" occur between sibling strands derived from the same provirus, which may be identical or differ at only one or two nucleotides. At the other extreme, coinfection of a single cell by two very different lentiviruses may not give rise to any heterozygous virions, and even if copackaging does occur, the degree of sequence identity may be insufficient to permit homologous recombination (10).

Evidence of HIV Recombination in Nature

In the research laboratory, recombination is widely considered a dominant feature of retroviral genetics. When cell cultures are coinfected with retroviruses that contain genetic markers at specific sites on their genomes, recombinant progeny arise frequently, and markers as close as 1,000 nucleotides segregate "as if unlinked" (1).

The possibility that HIV-1 strains might recombine in nature was proposed early in the epidemic (11). However, the first compelling evidence for lentivirus recombination in nature was the discovery that isolates from sabeus monkeys in western Africa were chimeras between the simian immunodeficiency virus (SIV) of African green monkeys and the SIV of sooty mangabeys (12). The sabeus monkey SIV was shown to be a recombinant resulting from at least two interstrand crossovers between genomes of the green monkey and mangabey viruses.

Most HIV-1 strains from around the world can be placed into one of nine nucleotide sequence-defined clades; these clades have been given the letter designations A through I. However, more than a dozen HIV-1 strains isolated from patients have now been shown to have chimeric genomes in that their gag and env genomic regions cluster with different clades (13). Interclade recombination is relatively easy to demonstrate because strains from different clades typically differ substantially in their nucleotide sequence identities. For example, the env gene sequences of HIV-1 strains of different clades may differ by 20% or more (14). As might be expected, interclade HIV-1 recombinants have most often been detected in geographic regions where two or more clades are prevalent (14). For example, A clade and D clade viruses cocirculate in East Africa, and several A/D recombinant viruses have been detected in this region. In western equatorial Africa, multiple HIV-1 clades (A, C, D, E, F, G, and H) as well as the outlier "group O" HIV-1 strains are known to cocirculate, and preliminary studies suggest that recombinant forms are quite common in this region. Most are recombinants between the A clade, which is predominant, and another clade. The rapidly spreading HIV-1 strain in Southeast Asia is one such recombinant, of A with E (15). This strain consists of A clade gag and pol genes, but the env gene is chimeric: the surface gp120 envelope protein and external domain of transmembrane

gp41 envelope protein are contributed by the E clade, while the cytoplasmic domain of gp41 is again A genotype. In effect, this epidemic strain is a pseudotyped A virus that carries an E envelope. A wide variety of genetically similar recombinant E/A strains have been found in equatorial Africa, so it is likely that the recombinant event occurred there and a subclone was introduced into Southeast Asia by an infected traveler. B/F recombinants have been found in Brazil, where both parental clades are found (16).

Intraclade recombinants are much more difficult to detect and demonstrate convincingly because of the genetic similarity of the parental strains. Clones of B clade viruses from the blood of a patient with acute retroviral syndrome who had had multiple sex partners were found to belong to three distinct clade B env variants (17). Some of the clones appeared to be probable recombinants. Strains from an infant who had been transfused with blood from two HIV-infected donors in 1984 were found to include probable B intraclade recombinants (18).

Studies of specimens from an A/C-infected spousal pair in Zambia have shown that a variety of recombinants can be present in one small epidemiologic cluster at different times, suggesting that recombination may be continuous and ongoing in vivo in patients who are coinfected with two or more distinct strains (19).

Mechanisms of Retroviral Recombination

The exact mechanism by which two retroviral RNA genome strands are copackaged into a single virion—"mating"—is only partially understood. A key step is thought to be the dimerization of the two strands near their 5' genome termini (20), which in turn permits interaction of the RNA packaging signals with gag proteins.

Two genomes per virion is a necessary but not sufficient condition for retroviral recombination: the reverse transcriptase must also readily switch strands (21). Low processivity (loose adherence to the template RNA) is an inherent property of retroviral reverse transcriptases. In the normal retroviral replication cycle, the reverse transcriptase and approximately 1,000 bp of the nascent DNA minus strand jump from the plus RNA strand 5' repeat region to the identical repeat region at the 3' end of the genome. Presumably the low processivity required to permit this jump from one end of the genome to the other also permits ready interstrand switching (4).

Preferred sites for HIV recombination, if any, remain uncertain. If recombination "hot spots" are found, they may be dictated by RNA secondary structures that retard polymerase movement, as with other viruses. Alternatively, physical sites of recombination may be essentially randomly distributed along the genome, and apparent recombination hot spots might simply reflect selection for viability.

HIV as a Primitive Sexual Organism

Once the replication of HIV is viewed as that of a primitive sexual organism (diploid with mating), it is instructive to reexamine the biology of HIV for other features that might facilitate a sexual life style.

Syncytium Induction

Some HIV strains induce formation of multinucleated syncytia in cell cultures in vitro; this property has been associated with clinical virulence (22). Syncytia formation might facilitate multiple infection of a single cell by fusing two or more infected cells into one. Syncytium-inducing strains may be more virulent not because syncytium induction per se leads directly to immunopathogenesis, but because this property permits more efficient generation of rapidly growing variants through recombination and selection. Syncytia induction might, therefore, represent a mechanism to optimize the spatial interactions between strains: a mating ground.

tat/tar Transactivation

Integrated HIV provirus remains transcriptionally inactive unless the LTR promoter region is activated by cellular activation factors. HIV transcription can also be autocatalytically increased by binding of the HIV tat protein to the tar region of the LTR. If two different proviruses are present in the same cell, transactivation through tat produced by either provirus could lead to synchronization of replication of both proviruses. There is also evidence that tat can be released from infected cells and be taken up into and transactivate tar sequences in other infected nearby cells (23). The tat/tar interaction might be thought of as a pheromone, or a specific mate recognition system that optimizes the temporal interactions between strains.

The Lentivirus Gene Pool and Origins of Contemporary HIV Clades

At least 17 HIV clades have now been reported in humans: nine HIV-1 clades in the major grouping (A through I), three HIV-1 group O group "outlier" clades, and five HIV-2 clades. An additional three lentiviruses are known in nonhuman primate species (African green monkeys, mandrils, and Syke's monkeys). Thus the potential gene pool for primate lentivirus recombination is on the order of 20, e.g., 20 gag genes and 20 pol genes. The current HIV-1 clades may have arisen in part through past recombination between some of these genes.

Rates of transspecies infections with lentiviruses have not been measured. SIV has infected persons handling SIV-infected monkeys or virus cultures in the United States (24). Furthermore, phylogenetic data suggest that the human HIV-2 virus is almost certainly derived from SIV of sooty mangabeys in West Africa. Nucleotide sequence data suggest multiple sooty mangabey-to-human transspecies transmissions (25).

Viable recombinants between SIV and HIV ("SHIV" strains) have been genetically engineered in research laboratories for use in animal modeling experiments (26). No naturally occurring HIV-1/HIV-2 recombinants have been detected in human populations, but efforts to detect such strains have been very limited. Although SIV strains can productively infect humans and, therefore, might recombine with HIV-1, the lentiviruses of cats, horses, cows, and sheep have not productively infected humans and are unlikely to contribute to the pool of human lentivirus genetic elements.

Barriers to HIV Recombination

Not all the theoretically possible combinations between HIV-1, HIV-2, and SIVs may give rise to recombinants in nature because of epidemiologic and biologic barriers.

Segregation by Host Species

The frequency of transmission of viruses between primate species is not known. Intense surveillance for pox virus infections in equatorial Africa during the final decade of the smallpox eradication effort detected only 400 human monkeypox cases (27). Worldwide surveillance for herpes B virus infection, a common infection in nonhuman primates that is uniformly fatal in humans, has identified only 40 cases.

Segregation by Geography

Although there are now perhaps 20 million HIV-infected persons worldwide, few (except in equatorial Africa) are likely to encounter partners infected with another clade. This is because, except in equatorial Africa, the HIV epidemic is genetically relatively homogeneous: B clade viruses predominate in Europe and North and South America, C clade viruses predominate in southern Africa and India, and E clade viruses predominate in Southeast Asia (28,29).

The current geographic distribution of clades and recombinants may not remain static. B clade and F clade mixing has begun in South America, and B/F recombinants have been detected. B clade and C clade mixing is occurring in South Africa, and E/A clade and C clade mixing is occurring in Asia, but new interclade recombinants have not yet been detected in these regions.

Requirement for Multiple Infections in a Single Human

HIV-1 can superinfect persons who are chronically infected with HIV-2, but there is substantial heterotypic protection (30). Human infections with two or more HIV-1 clades have been recognized only rarely (31,32). While HIV-1-infected chimpanzees can be superinfected with a closely related strain under experimental conditions (33), it is still unclear if chronically HIV-1-infected humans are susceptible to superinfection by another HIV-1 strain through natural transmission. It may be that multiple infections with HIV-1 can occur in humans only when exposure to both viruses is near simultaneous.

Requirement for Dual Infection of a Single Cell

Different variant HIV-1 strains can concurrently infect single cells in cell cultures in vitro, as can HIV-1 and HIV-2 (34). However, HIV downregulates its CD4 cell surface receptor, and dual infection of a single cell in vivo may require simultaneous attachment and penetration.

Viral Structural Incompatibilities

The replication and synthesis of HIV virions is a complex process with molecular interactions at several levels: overlapping reading frames, RNA/RNA secondary structures, protein/RNA interactions, and intra- and intermolecular protein interactions. Recombination between two highly replication competent parent viruses might give rise to nothing but nonviable recombinant

progeny because of incompatibilities in these molecular interactions.

Consequences of Recombination for Prevention and Treatment of HIV Infections

The propensity of HIV strains to recombine has serious implications for epidemic control efforts.

Epidemic Forecasting

If new strains—with new epidemiologic properties—can arise through HIV recombination as readily as new strains arise through reassortment in influenza A, then the long-term epidemiology of HIV may similarly be characterized by epidemic shifts and drifts. The emergence of the E/A recombinant clade in Southeast Asia may have simply been a chance event. However the fact that the E/A clade has spread much more rapidly than the B clade in this region raises concerns that some recombinants may emerge through natural selection based on their transmission efficiency (35).

Antiviral Drug Resistance

Most HIV strains that are highly resistant to zidovudine (AZT) or to other antiviral drugs have multiple mutations, which act synergistically to confer the resistant phenotype to that drug. In vitro experiments in which single-mutant strains are grown in the presence of AZT show strong selection for recombinants bearing two or more resistance mutations (36). Multidrug resistant HIV-1 strains are likely to arise in patients treated with multiple drugs through recombination of variants that are resistant to single drugs (37). For example, crossover recombinants between strains singly resistant to a nucleoside analog and a protease inhibitor would be generated frequently in any cell coinfected with variants resistant to only one of these antiviral drugs.

Paradoxically, mutations in the reverse transcriptase that confer drug resistance might also serve to limit recombination. Mutations that confer AZT resistance increase the processivity of HIV-1 reverse transcription in vitro, but recombination rates of viruses bearing these mutations have not yet been studied (38).

Vaccines

If new HIV strains are continually generated in nature through recombination, matching vaccines with prevalent genotypes in a particular geographic region may prove difficult. This may be less of a problem if small subunit vaccines are effective but may be more serious if complex vaccines constructed from antigens corresponding to two or more HIV gene products are needed. The need for a new influenza vaccine with each shift in the dominant epidemic influenza strain may be an instructive model. Another concern is that HIV genetic information from a vaccine might recombine with wild-type virus in vivo in an HIV-infected patient who was vaccinated, giving rise to new variants. Recombinant vaccinia strains containing HIV genes have already been tested in humans, and live attenuated and naked DNA HIV vaccines are being considered (39). Although in most instances it is unlikely that the new genetic information from the vaccine could give rise to more transmissible or more virulent strains, it is nonetheless possible. A relevant example is a recent epidemic in China, where gene sequences from the live attenuated oral polio vaccine were found to be stably recombined into the dominant virulent wild-type virus (40).

Conclusion

Recombination may be an important fitness search strategy in the ongoing evolution of HIV. Many of the strains around the world appear to have arisen through recombination, and it is likely that recombination may be an important mechanism by which HIV evades drug or immune pressures. Future epidemiologic and clinical trials should examine the role of recombination in HIV evolution and adaptation, and computer models that simulate HIV mutation and recombination should be developed. The conceptual approach to HIV replication as a primitive sexual reproductive cycle might lead to new classes of interventions that block HIV evolution and adaptation.

Acknowledgments

The author acknowledges valuable discussions with Drs. Francine McCutchan, Jean Carr, and Dan St. Louis; library search assistance from Ms. Pam Bell; and manuscript preparation assistance from Ms. Mary Hall.

Dr. Burke is interested in the molecular epidemiology of viral diseases. He conducted research on tropical infectious diseases, especially arthropod-borne viruses, hepatitis viruses, and HIV/AIDS. Dr. Burke is now professor of international health and director of the Center for Immunization Research at the Johns Hopkins University School of Hygiene and Public Health.

References

1. Coffin JM. Genetic diversity and evolution of retroviruses. Curr Top Microbiol Immunol 1992; 176:143-64.
2. Hu W-S, Temin HM. Genetic consequences of packaging two RNA genomes in one retroviral particle: pseudodiploidy and high rate of genetic recombination. Proc Natl Acad Sci USA 1990;87:1556-60.
3. Robertson DL, Hahn BH, Sharp PM. Recombination in AIDS viruses. J Mol Evol 1995;40:249-59.
4. Temin HM. Sex and recombination in retroviruses. Trends Genet 1991;7:71-4.
5. Wain-Hobson S. The fastest genome evolution ever described: HIV variation in situ. Curr Opin Genet Dev 1993;3:878-83.
6. Holland J, Spindler K, Horodyski F, Grabau E, Nichol S, VandePol S. Rapid evolution of RNA genomes. Science 1982;215:1577-85.
7. Felsenstein J, Yokoyama S. The evolutionary advantage of recombination. II. Individual selection for recombination. Genetics 1976;83:845-59.
8. Kauffman SA. The origins of order. New York: Oxford University Press; 1993. p. 114-7.
9. Kirkegaard K, Baltimore D. The mechanism of RNA recombination in poliovirus. Cell 1986;47:433-43.
10. Zhang J, Temin HM. Retrovirus recombination depends on the length of sequence identity and is not error prone. J Virol 1994;68:2409-14.
11. Li WH, Tanimura M, Sharp PM. Rates and dates of divergence between AIDS virus nucleotide sequences. Mol Biol Evol 1988;5:313-30.
12. Jin MJ, Hui H, Robertson DL, Muller MC, Barre-Sinoussi F, Hirsch VM, et al. Mosaic genome structure of simian immunodeficiency virus from West African green monkeys. EMBO J 1994;13:2935-47.
13. Robertson DL, Sharp PM, McCutchan FE, Hahn BH. Recombination in HIV-1 [letter]. Nature 1995;9:374:124-6.
14. Louwagie J, Janssens W, Mascola J, Heyndrickx L, Hegerich P, van der Groen G, et al. Genetic diversity of the envelope glycoprotein from human immunodeficiency virus type 1 isolates of African origin. J Virol 1995;69:263-71.
15. McCutchan FE, Artenstein AW, Sanders-Buell E, Salminen MO, Carr JK, Mascola JR, et al. Diversity of the envelope glycoprotein among human immunodeficiency virus type 1 isolates of clade E from Asia and Africa. J Virol 1996;70:3331-8.
16. Sabino EC, Shpaer EG, Morgado MG, Korber BT, Diaz RS, Bongertz V, et al. Identification of human immunodeficiency virus type 1 envelope genes recombinant between subtypes B and F in two epidemiologically linked individuals from Brazil. J Virol 1994;68:6340-6.
17. Zhu T, Wang N, Carr A, Wolinsky S, Ho DD. Evidence of coinfection by multiple strains of human immunodeficiency virus type 1 subtype B in an acute seroconvertor. J Virol 1995;69:1324-7.
18. Diaz RS, Sabino EC, Mayer A, Mosley JW, Busch MP, the Transfusion Safety Study Group. Dual human immunodeficiency virus type 1 infection and recombination in a dually exposed transfusion recipient. J Virol 1995;69:3273-81.
19. Salminen MO, Carr JK, Robertson DL, Hegerich P, Gotte D, Koch C, et al. Evolution and probable transmission of inter-subtype recombinant HIV-1 in a Zambian couple. J Virol 1997;71:2647-55.
20. Paillart J-C, Skripkiin E, Ehresmann B, Ehresmann C, Marquet R. A loop-loop "kissing" complex is the essential part of the dimer linkage of genomic HIV-1 RNA. Proc Natl Acad Sci USA 1996;93:5572-7.
21. Huber HE, McCoy JM, Seehra JS, Richardson CC. Human immunodeficiency virus 1 reverse transcriptase: template binding, processivity, strand displacement synthesis, and template switching. J Biol Chem 1989;264:4669-78.
22. Fouchier RA, Meyaard L, Brouwer M, Hovenkamp E, Schuitemaker H. Broader tropism and higher cytopathicity for CD4+ T cells of a syncytium-inducing compared to a non-syncytium-inducing HIV-1 isolate as a mechanism for accelerated CD4+ T cell decline in vivo. Virology 1996;219:87-95.
23. Thomas CA, Dobkin J, Weinberger OK. Tat-mediated transcellular activation of HIV-1 long terminal repeat directed gene expression by HIV-1-infected peripheral blood mononuclear cells. J Immunol 1994;153:3831-9.
24. Khabbaz RF, Heneine W, George JR, Parekh B, Rowe T, Woods T, et al. Brief report: infection of a laboratory worker with simian immunodeficiency virus. N Engl J Med 1994;330:172-7.
25. Chen Z, Telfer P, Cettie A, Reed A, Zhang L, Ho D, et al. Genetic characterization of new West African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop. J Virol 1996;70:3617-27.
26. Luciw PA, Pratt-Lowe E, Shaw KE, Levy JA, Cheng-Mayer C. Persistent infection of rhesus macaques with T-cell-line-tropic and macrophage-tropic clones of simian/human immunodeficiency virus (SHIV). Proc Natl Acad Sci USA 1995;92:7490-4.
27. Jezek Z, Fenner F. Human monkeypox. In: Virology monographs. Basel (Switzerland): Karger; 1988. p. 17.
28. Hu DJ, Dondero TJ, Rayfield MA, George JR, Schochetman G, Jaffe HW, et al. The emerging genetic diversity of HIV. JAMA 1996;275:210-6.
29. Burke DS, McCutchan FE. Global distribution of HIV-1 clades. In: Devita VT, Hellman S, Rosenberg S, editors. AIDS: biology, diagnosis, treatment, and prevention. 4th ed. New York: Lippincott-Raven Publishers; 1997.
30. Marlink R, Kanki P, Thior I, Travers K, Eisen G, Siby T, et al. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science 1994;265:1587-90.
31. Artenstein AW, VanCott TC, Mascola JR, Carr JK, Hegerich PA, Gaywee J, et al. Dual infection with human immunodeficiency virus type 1 of distinct envelope subtypes in humans. J Infect Dis 1995;171:805-10.
32. Heyndrickx L, Alary M, Janssens W, Davo N, van der Groen G. HIV-1 group O and group M dual infection in Benin [letter]. Lancet 1996;347:902-3.
33. Fultz PN, Srinivasan A, Greene CR, Butler D, Swenson RB, McClure Hm. Superinfection of a chimpanzee with a second strain of human immunodeficiency virus. J Virol 1987;61:4026-9.

34. Kim JH, McLinden RJ, Mosca JD, Burke DS, Boswell RN, Birx DL, et al. Transcriptional effects of super-infection in HIV chronically infected T cells: studies in dually infected clones. J Acquir Immune Defic Syndr Hum Retrovirol 1996;12:329-42.
35. Soto-Ramirez LE, Renjifo B, McLane MF, Marlink R, O'Hara C, Sutthent R, et al. HIV-1 Langerhans' cell tropism associated with heterosexual transmission of HIV. Science 1996;271:1291-3.
36. Kellam P, Larder BA. Retroviral recombination can lead to linkage of reverse transcriptase mutations that confer increased zidovudine resistance. J Virol 1995;69:669-74.
37. Gu Z, Gao Q, Faust EA, Wainberg MA. Possible involvement of cell fusion and viral recombination in generation of human immunodeficiency virus variants that display dual resistance to AZT and 3TC. J Gen Virol 1995;76:2601-5.
38. Caliendo AM, Savara A, An D, DeVore K, Kaplan JC, D'Aquila RT. Effects of zidovudine-selected human immunodeficiency virus type 1 reverse transcriptase amino acid substitutions on processive DNA synthesis and viral replication. J Virol 1996;70:2146-53.
39. Burke DS. Review: human trials of experimental HIV vaccines. AIDS 1995;9:S171-S80.
40. Zheng DP, Zhang LB, Fang ZY, Yang CF, Mulders M, Pallansch MA, et al. Distribution of wild type 1 poliovirus genotypes in China. J Infect Dis 1993;168:1361-7.

Host Genes and HIV: The Role of the Chemokine Receptor Gene CCR5 and Its Allele (Δ32 CCR5)

Janet M. McNicholl, Dawn K. Smith, Shoukat H. Qari, and Thomas Hodge
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Since the late 1970s, 8.4 million people worldwide, including 1.7 million children, have died of AIDS, and an estimated 22 million people are infected with human immunodeficiency virus (HIV)(1). During 1995 and 1996, major clinical and laboratory discoveries regarding HIV pathogenesis provided new hope for the prevention and treatment of HIV infection. One major discovery was that members of the chemokine receptor family serve as cofactors for HIV entry into cells. We describe the role of allelic polymorphism in the gene coding for the CCR5 chemokine receptor with regard to susceptibility to and disease course of HIV infection. We also examine the effect of this discovery on medical and public health practices.

The HIV Epidemic: a Global Problem

The World Health Organization has estimated that 22.6 million people are infected with human immunodeficiency virus (HIV) as of mid-1996 (1). The highest prevalence of HIV occurs in parts of Asia and sub-Saharan Africa. In 1995, HIV-associated illnesses caused the deaths of 1.3 million people, including 300,000 children under 5 years of age. No protective vaccine or cure is available, and while prevention methods are reducing incidence in some countries, HIV disease is expected to increase. Earlier HIV infection diagnosis, inhibition of ongoing HIV replication with antiretroviral therapy (in industrialized countries), and prevention and treatment of opportunistic infections and cancers delay the onset of AIDS and increase the life expectancy of HIV-infected persons.

An Overview of Host Genes and HIV Infection

Since the early years of the HIV epidemic, significant differences in the rate of disease progression have been observed in longitudinally followed HIV-infected persons. The role of genes of the human leukocyte antigen (HLA) system in determining the course of disease has been examined by using such measures as the CD4+ cell count or the length of time between HIV infection and AIDS (2-6). Several HLA genes or haplotypes appear to influence disease progression, although the effects are complex and may depend on interactions with other host genes.

Studies of the effect of host genes on susceptibility to HIV infection were facilitated by the identification of persons who were persistently exposed to HIV but remained uninfected (7-12). Before the discovery of the role of chemokine receptor gene polymorphism in HIV infection, only genes of the HLA system were thought to protect against HIV infection. For example, certain distributions of HLA class I alleles were observed in uninfected female commercial sex workers in Africa (13,14) and Thailand (11), who had been highly exposed to HIV; additional class I and II alleles that may be associated with remaining uninfected have been identified (6). Several mechanisms, some related to cytotoxic T-cell function, have been suggested to explain these findings (7,10,11,15).

Non-HLA genetic factors also influence susceptibility to HIV infection and the course of HIV disease. In 1996 and 1997, studies confirmed the protective role of homozygosity for a 32 base pair (bp) deletion in the chemokine receptor gene, CCR5 (Δ32 CCR5), against HIV infection (9,12,16-19). Until recent reports of HIV infection in three persons homozygous for the 32 bp CCR5

Address for correspondence: Janet McNicholl, Centers for Disease Control and Prevention, MS A-25, 1600 Clifton Rd. N.E., Atlanta, GA 30333 USA; fax: 404-639-2149; e-mail: jkm7@cdc.gov.

deletion (20-22), no cases of HIV infection had been reported in studies of more than 60 persons homozygous for the CCR5 32 bp deletion. Presence of one copy of the deleted CCR5 gene also influences the course of disease as the onset of AIDS occurs later for some heterozygous persons than for those homozygous for the wild type CCR5 (12,17-19,23). The discovery of the role of CCR5 alleles has prompted studies of the possible role of many other host genes in HIV infection (24-26).

What Are Chemokine Receptors?

Chemokine receptors are cell surface proteins that bind small peptides called chemokines (27). Chemokines can be classified into three groups based on the number and location of conserved cysteines: C, CC, and CXC. Chemokine receptors are grouped into families on the basis of the chemokine ligands they bind: CC, CXC, or both (27; Table 1). Some receptors are promiscuous, while others are selective in terms of ligand binding. The receptors are widely distributed on hematopoietic and other cells, but the Duffy antigen of erythrocytes (DARC) is the only member expressed on cells of erythroid lineage. Several human chemokine receptors have been classified as such on the basis of similarity of gene sequences and predicted protein structures, but their ligands have not been identified (orphan receptors). Among these is the recently identified *TER1* gene (28).

The characteristic feature of all chemokine receptors is a serpentine 7 transmembrane-spanning domain structure, which is shared with other receptors; e.g., the rhodopsin and the thyrotrophin receptors (Figure 1). Extracellular portions are involved in chemokine binding, while intracellular portions are involved in cell signaling. The effect of receptor-ligand interactions is usually mediated through G-protein coupled interactions; results in alterations in cell

Table 1. Human chemokine receptors

Receptors (Old names)[a]	Ligands		Predominant expression/ Tissue distribution	Pathogens[b]	Chromosome location	GenBank Acc. #
	C-C chemokines	CXC chemokines				
CC Receptors						
CCR1 (CC CKR1)	MIP-1α, ß, RANTES, MCP-3		monocytes, T cells		3p21	L10918
CCR2A (MCP-1Ra)	MCP-1		T cells, basophils monocytes,		3p21	U03882
CCR2B (MCP-1Rb)	MCP-1, 3, 4		HIV-1 (NSI)			U03905
CCR3 (CKR3)	Eotaxin, RANTES, MCP-2,3,4		eosinophil, basophils, microglial cells, and possibly monocytes; little expression in peripheral blood T-lymphocytes or dendritic cells	HIV-1 (NSI)	3p21	U28694
CCR4	TARC		basophils, T cells		3p24	X85740
CCR5[c] (CC CKR5)	RANTES, MIP-1α, ß		monocytes, dendritic cells, microglial cells, T cells	HIV-1, (NSI) HIV2	3p21	U57840
CXC Receptors						
CXCR1 (IL-8 RA)		IL-8	neutrophils, NK cells		2q35	M68932
CXCR2 (IL-8 RB)		IL-8, MGSA, *gro*-α, NAP-2, IP-10, ENA-78, Mig				M73969
CXCR3		IP-10, Mig	activated T cells			X95876
CXCR4 (Fusin, LESTR, HUMSTR)		SDF-1	wide: $CD4^+$ and $CD4^-$ cells, monocytes, macrophages, dendritic cells, B cells; other tissues, e.g., brain, lung, spleen	HIV-1 (SI) HIV-2	2q21	M99293

function such as activation, motion, or migration, usually along a chemokine concentration gradient; and varies depending on the chemokine bound and the cell type (27).

Some chemokine receptors have a role in infectious disease susceptibility or pathogenesis. Several of the CC or CXC receptors are used by HIV-1 or HIV-2 as entry cofactors (Table 1). DARC serves as a cofactor for entry of *Plasmodium vivax* into erythrocytes (29); and as is the case with the 32bp-deleted CCR5 and HIV, resistance to *P. vivax* malaria is associated with lack of DARC expression (29), probably due to polymorphism of the DARC gene promoter (30). Several viruses (Epstein-Barr virus, cytomegalovirus, and *Herpes virus samiri*) contain functional homologues of human chemokine receptors, which suggests that the viruses may use these receptors to subvert the effects of host chemokines (27). They may also serve as HIV entry cofactors for human cells, as observed for HCMV-US28 (31).

Chemokine Receptors and HIV Infection

The first clue that chemokine-related events were important in HIV pathogenesis came from work in R. Gallo's laboratory, which showed that high levels of chemokines could inhibit HIV replication in vitro (32). Then a cohort of highly exposed, HIV-negative men had high circulating levels of several chemokines, such as RANTES, MIP-1α, and MIP-1ß (8). These data led to the hypothesis that chemokines might prevent HIV infection by binding to the elusive HIV entry cofactor. Since the discovery of CD4 in the 1980s as an HIV receptor, it had become apparent that other factors were required for HIV to enter cells. For example, mouse cells expressing CD4 could not be infected with HIV (33). Using a novel cloning strategy, E. Berger and colleagues first demonstrated in May 1996 that CXCR4, a chemokine receptor for which no ligand had yet been determined, was an entry cofactor for T-cell tropic or syncytium-inducing (SI) viruses (34).

Table 1. Human chemokine receptors (continued from p. 2)

Receptors (Old names)[a]	Ligands		Predominant expression/ Tissue distribution	Pathogens[b]	Chromosome location	GenBank Acc. #
	C-C chemokines	CXC chemokines				
CC/CXC Receptor						
DARC (Duffy antigen)	RANTES, MCP-1, TARC etc.	IL-8, MGSA, *gro*-α *etc.*	endothelial cells, erythrocytes	*Plasmodium vivax*	1	U01839
Others [d]						
STRL33	ND	ND	lymphoid tissues and activated T cells	HIV-1	3	U73529
HCMV-US28	MIP-1α, ß, RANTES		fibroblasts infected with CMV	HIV-1	N/A	X17403
ChemR1	ND	ND	T lymphocytes, polymorphonuclear cells		3p21-24	Y08456
CMKBRL1	ND	ND	neutrophils, monocytes, brain, liver, lung, skeletal muscles		3p21	U28934
*TER*1	ND	ND	thymus, spleen		3p21	U62556
V28	ND	ND	Neural and lymphoid tissue		3p21	U20350
D2S201E	ND	ND	wide, including cells of hemopoietic origin		2q21	M99293
BLR1	ND	ND	B lymphocytes			X68149
EBI1	ND	ND	B lymphocytes			L08176
GPR1,2,5	ND	ND		ND		L36149

[a] New nomenclature for CC and CXC chemokine receptors was adopted at the Gordon Research Conference on Chemotactic Cytokines, June 23–28, 1996. [b]Pathogens using this receptor for infection. [c] The 32bp deleted allele of CCR5 has been referred to as CCR5-2 (19). [d]Chemokine receptor-like genes whose predicted proteins have 7 transmembrane domains.

Abbreviations: BLR1, Burkitt's lymphoma receptor-1; CMKBRL1, Chemokine ß receptor like-1; DARC, duffy antigen/receptor for chemokines; EBI1, Epstein-Barr virus-induced receptor; ENA78, epithelial-derived neutrophil-activating peptide-78; GPR, G protein coupled receptor; *gro*, growth related gene product; HCMV, human cytomegalovirus; HUMSTR, human serum transmembrane segment receptor; IL, interleukin; IP-10, interferon-gamma inducible 10kD protein; LESTR, leukocyte-expressed seven-transmembrane-domain receptor; MCP, monocyte chemotactic protein; Mig, monokine induced by interferon gamma; MIP, macrophage inflammatory protein; NSI, non-syncytium inducing; N/A, not applicable; NAP-2, neutrophil-activating protein-2; ND, not determined; RANTES, regulated on activation, normal T cell expressed and secreted; SDF-1, stromal cell-derived factor-1; STRL33, seven transmembrane-domain receptor from lymphocyte clone 33;TARC, thymus and activation regulated chemokine.

Figure 1. Predicted structure and amino acid sequence of CCR5. The typical serpentine structure is depicted with three extracellular (top) and three intracellular (bottom) loops and seven transmembrane (TM) domains. The shaded horizontal band represents the cell membrane. Amino acids are listed with a single letter code. Residues that are identical to those of CCR2b are indicated by dark shading, and highly conservative substitutions are indicated by light shading. Extracellular cysteine residues are indicated by bars, and the single N-linked glycosylation consensus site is indicated by an asterisk. Reprinted and modified with permission from the authors and Cell (39). Copyright (1996) Cell Press.

Shortly before this, Samson et al. had cloned CCR5 (then known as CC-CKR5) and shown that its ligands included RANTES, MIP-1α, and MIP-1ß (35). These data encouraged several groups to examine the role of CCR5 in HIV entry, and within weeks of the CXCR4 publication, five additional publications reported CCR5 and CCR3 as HIV entry cofactors for macrophage-tropic or non-syncytium-inducing (NSI) viruses (33,36-39) and CCR3 and CCR2b as entry factors for dual tropic HIVs (38,39). Recent studies of brain-derived microglial cells have shown that CCR5 and CCR3 permit entry of HIV into these cells (40).

The tropism of HIV strains appears to be determined in part by the way they use chemokine receptors (Table 1, Figure 2). In peripheral blood, although both T cells and monocytes express CXCR4 and CCR5 (41), HIV strains that use CXCR4 tend to infect predominantly T cells, while strains that use CCR5 infect T cells and monocytes. This tropism correlates with the ability of the virus to induce syncytia in T-cell lines. SI viruses (often present late in the course of HIV infection), tend to be CXCR4 tropic, while NSI viruses (which appear to be the viruses transmitted in vivo) tend to be CCR5 tropic (33,34,36-39). However, some primary SI viruses can use either CXCR4 or CCR5 (42), and some may use both coreceptors, as well as CCR2b or CCR3 (39). When classified based on chemokine receptor use, HIV types are independent of their genetic relatedness (43).

The exact mechanism by which HIV interacts with CXCR4 or CCR5 and by which this interaction, together with CD4 binding, leads to virus entry has not been clearly defined (44). It appears to involve the interactions of the V3 loop and other parts of the outer envelope protein gp120 (45-47) with extracellular domains of CCR5 or CCR2b (48,49) and may involve multistep

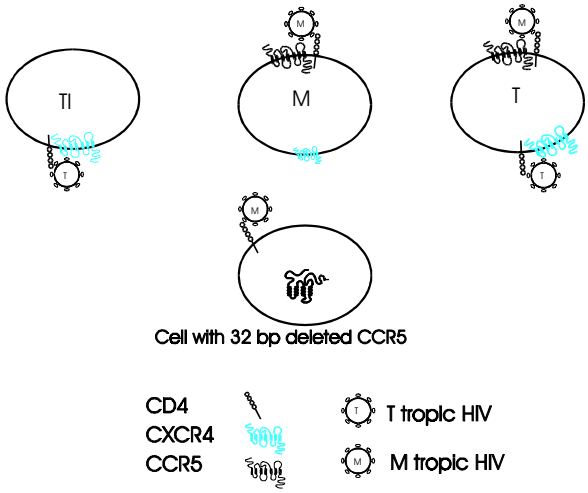


Figure 2. Chemokine receptors and cell tropism of HIV. Three cell types are illustrated, an in vitro passaged T-cell line (Tl), a monocyte/macrophage (M), and a circulating T-cell (T). T-cell lines express CXCR4 but not CCR5; macrophages and circulating peripheral blood T-cells express both receptors, although the amounts of CXCR4 are lower on macrophages (as indicated by the small CXCR4 symbol). M-tropic HIV, because of certain envelope amino acid sequences, binds to CCR5 and can enter both macrophages and circulating T cells. T-tropic HIV preferentially binds CXCR4 and enters T cells or T-cell lines. After binding to the chemokine receptor and to CD4, the viruses enter by fusion with the cell membrane. Cells with the 32bp deleted form of CCR5 do not express cell surface CCR5, and, although M-tropic HIV can bind CD4, it cannot enter the cell. If the cells express CXCR4, they can still be infected with T-tropic viruses (not shown). Note that this figure does not depict the actual size relationships of the proteins, cells, or viruses.

interactions with CD4, the chemokine receptor, and other cell surface components (50). Chemokines may inhibit HIV entry through receptor blockade, desensitization, sequestration, or internalization; through alterations in receptor affinity; or by inhibiting postbinding steps (51), such as phosphorylation, through G-coupled mechanisms.

Chemokine Receptor Gene Polymorphism and Susceptibility to HIV Infection

In 1995 and 1996, researchers at the Aaron Diamond AIDS Research Center began studies of highly HIV-exposed seronegative men whose cells produced high levels of chemokines in vitro (8,52). Cells from several of these men were not infectable with primary or NSI type viruses, but were infectable with SI viruses (52). These data suggested some protective mechanism related to the CCR5 rather than to the CXCR4 receptor because of the chemokine ligand profile of the CCR5 receptor (Table 1) and stimulated studies that led to the cloning and restriction digestion of CCR5 in these persons (52). The novel deletion of 32bp in CCR5 reported by Liu et al. (9) in August 1996, to be present in three of the 15 HIV-exposed but seronegative men was also independently reported in September by Samson et al. (16) and Dean et al. (17; Table 2). In these studies, the distribution of homozygosity and heterozygosity of the deleted allele, in small and large cross-sectional or prospective studies of HIV-unexposed, -exposed, or -infected persons (mainly European or North American Caucasians) strongly supported the protective effect of the deletion. Since these initial observations, several studies of other populations have reported the same finding: an enrichment of the homozygous Δ32/Δ32 CCR5 genotype in highly HIV-exposed persons who remain uninfected (12,18,19; Table 2). In persons with well-documented HIV exposure, the prevalence of homozygosity for the Δ32/Δ32 CCR5 genotype increases with increasing HIV exposure (12), the wild type and Δ32 CCR5 alleles (referred to by some as CCR5-1 and CCR5-2 [19]) can be easily detected by polymerase chain reaction (PCR) techniques, with or without enzymatic digestion (9,12,16,17), or more recently, by heteroduplex studies (19). An example of the results of a PCR followed by restriction digestion is given in Figure 3. Persons who are homozygous for the wild type CCR5 (W) or the allele with the 32 bp deletion (Δ32) or heterozygous for both alleles can be easily distinguished because the deleted fragment reduces the size of the amplified product (Figure 3).

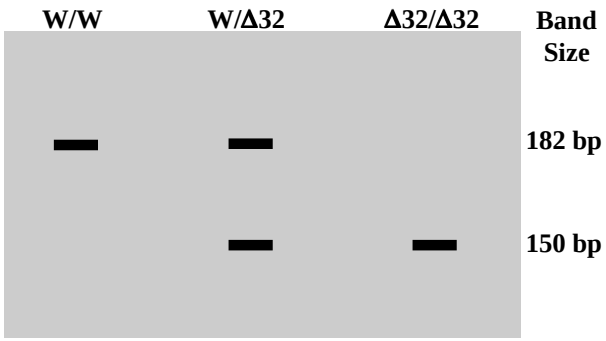


Figure 3. Differentiation of CCR5 genotypes by gel electrophoresis. Band patterns of persons with homozygous wild type (W/W), homozygous 32 bp deletion (Δ32/Δ32) or heterozygous W/Δ32 CCR5 genotypes are shown. PCR amplification of the C-terminal of the CCR5 gene, subsequent digestion with the EcoRI restriction enzyme, and agarose gel electrophoresis of the digested DNA yield a 182 bp band for the wild type CCR5 gene, a 150 bp band for the 32 allele, and both bands in the case of a heterozygous person.

The 32 bp deletion occurs at a site of a repeat motif in the CCR5 gene (16; Figure 4) and results in a frame shift in the coding sequence that produces a defective CCR5, which is not expressed on the surface of cells (Figures 2 and 5). The deletion results in the loss of three of the seven transmembrane domains, two of the three outer loops, and the intracellular signaling domain (Figure 5).

Does the CCR5 32 Bp Deletion Provide Absolute Protection Against HIV Infection?

The genetic link to remaining HIV-negative in spite of continued HIV exposure was the subject of much discussion during the summer and fall of 1996. Was the protection absolute? Who should be tested for the gene? Would persons told they were homozygous for the deletion engage in high-risk behavior more frequently? Although at that point no one homozygous for the deletion had been found HIV-positive, the fact that CXCR4 gene and several other chemokine receptors could mediate HIV entry suggested caution in assuming that persons with the CCR5 Δ32/Δ32 genotype would be absolutely resistant to HIV infection. Since then, three HIV-infected persons with this genotype have been reported (20-22; Table 2); for one, the mode of

Table 2. Population studies of CCR5 genotypes

Author	Population	Ethnicity	HIV Status	No.	[a]CCR5 Genotypes % (No.)			Δ32 allele freq. (%)
					W/W	W/ Δ32	Δ32/Δ 32	
Liu et al. (9)	High-risk, USA	Caucasian	Neg	15	80.0 (12)	0 (0)	20.0 (3)	0.200
	General, USA	Caucasian	Neg	122	80.3 (98)	19.7 (24)	0 (0)	0.098
	General, S. Amer.	Venezuelan	Neg	46	100 (46)	0 (0)	0 (0)	0
Samson et al. (16)	General, Europe	Caucasian	Neg	704	82.7 (582)	16.2 (114)	1.1 (8)	0.092
	General, Asia	Japanese	Neg	248	100 (248)	0 (0)	0 (0)	0
	General, Africa	Ctrl/W. African	Neg	124	100 (124)	0 (0)	0 (0)	0
	AIDS clinics, Paris	Caucasian	Pos	723	89.2 (645)	10.8 (78)	0 (0)	0.054
Dean et al.[b](17)	High-risk, USA	Mixed	Neg	883	81.9 (724)	15.6 (138)	2.4 (21)	0.10
	High-risk, USA	Mixed	Pos	1883	85.9 (1618)	14.0 (264)	0.0005 (1)	0.071
	High-risk, USA	African-Amer.	Mixed	620	96.6 (599)	3.4 (21)	0 (0)	.017
	High-risk, USA	Caucasian	Mixed	1250				0.115
	Low-risk, USA	Caucasian	Mixed	143				0.080
Huang et al.(12)	High-risk, USA	Caucasian	Pos	461	79.8 (368)	20.2 (93)	0 (0)	0.101
	High-risk, USA	Caucasian	Neg	446	78.0 (348)	16.7 (82)	3.6	0.128
	Blood donors, USA	Caucasian, 95%	Neg	637	85.2	13.3	1.4	0.08
	General, Africa/Haiti	Black	Mixed	137	100	0 (0)	0 (0)	0
	General, Asia	China/Thai/ other	Mixed	191	100	0 (0)	0 (0)	0
Michael et al.(18)	High-risk, USA	Mixed	Pos	406	87.5 (348)	14.3 (58)	0 (0)	0.071
	High-risk, USA	Mixed	Neg	21	71.4 (15)	9.5 (2)	19.1 (4)	0.238
	Intermed.-risk, USA	Mixed	Neg	240	78.3 (188)	20.4 (49)	1.3 (3)	0.115
Biti et al. (20)	High-risk, Australia	Caucasian	Pos	265	N/A[c]	N/A	0.004 (1)	N/A
Theodoru et al. (22)	High-risk, Europe		Pos	412	N/A	N/A	0.002 (1)	N/A
Eugenolsen et al. (23)	High-risk, Denmark	Caucasian	Neg	35	74 (26)	20 (7)	6 (2)	0.157
	High-risk, Denmark	Caucasian	Pos	99	78 (77)	22 (22)	0	0.111
	Blood donors, Denmark	Caucasian	Neg	37	73 (27)	24 (9)	3 (1)	0.149
Zimmerman et al. (19)	Blood donors, N. America	Caucasian	Neg	387	77.5 (300)	21.7 (84)	0.8 (3)	0.116
		African-Am.	Neg	294	94.2 (277)	5.8 (17)	0 (0)	0.29
		Hispanic	Neg	290	92.8 (269)	6.9 (20)	0.3 (1)	0.38
		Asian	Neg	164	99.4 (163)	0.6 (1)	0 (0)	0.003
		Native Amer.	Neg	87	83.9 (73)	12.6 (11)	3.4 (3)	0.098
	Blood donors, India	Tamil	Neg	46	100 (46)	0 (0)	0 (0)	0
	Blood donors, W. Africa	Black	Neg	40	100 (40)	0 (0)	0 (0)	0
	High risk, USA	Caucasian	Pos	614	77.4 (475)	22.6 (139)	0 (0)	0.113
	High risk, USA	African-Amer.	Pos	86	97.7 (84)	2.3 (2)	0 (0)	0.012
	High risk, USA	Hispanic	Pos	45	93.3 (42)	6.7 (3)	0 (0)	0.033
	High risk, USA	Caucasian	Neg	111	73.9 (82)	21.6 (24)	4.5 (5)	0.153
	High risk, USA	African-Amer.	Neg	2	50.0 (1)	0 (0)	0.250	
	High risk, USA	Hispanic	Neg	12	91.7 (11)	8.3 (1)	0 (0)	0.042

[a] CCR5 genotypes: W/W, homozygous wild type; W/ Δ32, heterozygous wild type/32bp deletion; Δ32/Δ 32, homozygous for the 32 bp deletion.

[b] Numbers updated to include additional Multicenter Hemophilia Cohort Study patients studied by O'Brien et al. (21) and Dean et al. (pers .comm.).

[c]Not available.

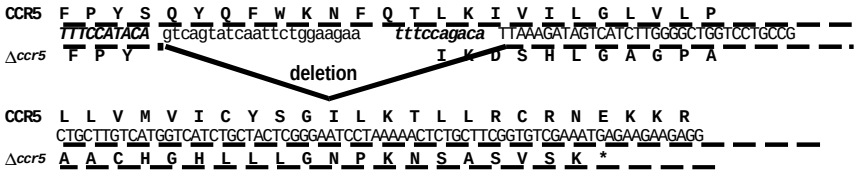


Figure 4. Partial CCR5 gene and amino acid sequence with 32 bp deletion. Nucleotide sequence of the CCR5 gene surrounding the deleted region, and translation into the normal receptor (top lines) or the truncated mutant (Δ 32 CCR5, bottom lines). The 10-bp direct repeat is represented in bold italics and the deleted nucleotides are represented in noncapitalized font.

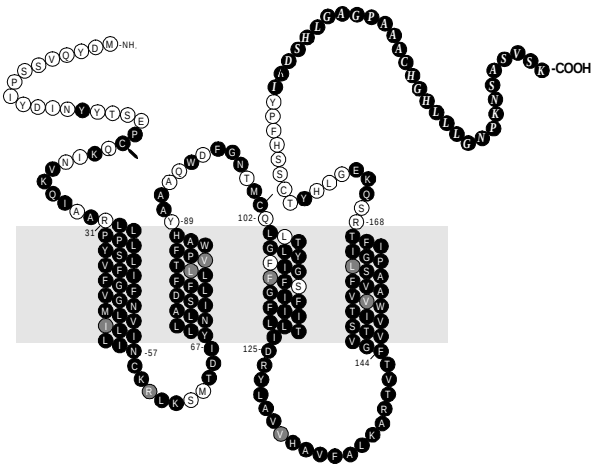


Figure 5. Predicted structure and amino acid sequence of the mutant form of human CCR5. The mutant protein lacks the last three transmembrane segments of CCR5, as well as the regions involved in G-protein coupling. The transmembrane organization is given by analogy with the predicted transmembrane structure of the wild-type CCR5, although the correct maturation of the mutant protein up to the plasma membrane has not been demonstrated. The shaded horizontal band represents membrane (s) of intracellular organelles. Amino acids represented in italic and dark shading correspond to unnatural residues resulting from the frame shift caused by the deletion. Figures 4 and 5 reprinted and modified with permission from the authors and Nature (16) Copyright (1996) Macmillan Magazines Ltd.

transmission was most likely blood products (21), while the only reported exposure risk for the other two was homosexual behavior (20,22). Sequences isolated from these persons suggested the SI virus phenotype, i.e., viruses that most likely used CXCR4 as entry cofactors (21; R. Ffrench, pers. comm.). As several other non-CCR5 receptors (CCR3 and CCR2b) are also HIV entry cofactors, even non-SI viruses might be transmissible in vivo in persons homozygous for the CCR5 32 bp deletion. Thus, HIV entry by non-CCR5 dependent mechanisms can occur in vivo, and persons who have the CCR5 Δ32/Δ32 genotype are not absolutely protected.

Does CCR5 Gene Polymorphism Determine All Resistance to HIV?

The highest reported prevalence of homozygosity for the Δ32 CCR5 allele deletion in persons highly exposed to HIV but uninfected is 33% (9,12). However, most (96% according to one estimate [19]) highly exposed HIV-seronegative persons are not homozygous for the Δ32 CCR5 allele. Moreover, among exposed but persis-tently HIV-seronegative per-sons in Africa and Thailand, no one positive for the Δ32 CCR5 allele has been found (CDC unpub. data; S. Rowland-Jones, F. Plummer, pers. comm.). These data suggest that many mechanisms may contribute to remaining seronegative despite high HIV exposure: chemokine-related mechanisms, such as altered CCR5 levels on the cell surface (53) or the level of circulating chemokines; other immune system genes, such as those of the HLA system (10,11,14); and immune responses, such as those of cytotoxic T cells (7,15). The relative importance of these mechanisms in populations with differing modes of exposure or genetic backgrounds needs to be elucidated.

CCR5 Polymorphism and Its Interaction with Other Factors in the Course of HIV Disease

Several large studies of the effect of CCR5 genotypes on the course of HIV disease have been published (Table 2). Dean et al. examined the course of HIV (time to AIDS) in several U. S. populations with different exposure to HIV (homosexuals, intravenous drug users, persons with hemophilia) and found that persons with one copy of the deleted CCR5 gene had a delayed (approximately 2 years longer) progression to AIDS when compared with those with the homozygous wild type genotype (17). Similar findings have been reported (19,23) in Caucasian HIV-positive homosexual men. Another study of HIV-positive homosexuals did not find such a striking effect of heterozygosity, although progression to AIDS was again slowed (12). One clue

to the varying effects of the heterozygous CCR5 genotype on progression to AIDS in different groups comes from another U. S. study of male homosexuals, in which men with one copy of the Δ32 CCR5 genotype had delayed progression to AIDS, particularly if their circulating viruses were of the NSI phenotype (18). Paradoxically, once AIDS is diagnosed, persons with one copy of Δ32 CCR5 may have a more rapid disease course.[1] Thus, the effect of the CCR5 genotype on disease progression may depend on the phenotype and chemokine receptor use of circulating viruses. The finding that microglial cells can be infected with HIV through CCR3- or CCR5-dependent mechanisms (40) also suggests that the clinical spectrum of HIV disease may also depend on the CCR5 genotype.

Thus, complex interactions between virus and host chemokine receptor genotype and virus and cell chemokine receptor phenotype exist. Studies to elucidate the interaction of these genotypes with each other, with virus phenotypes, and with other factors that influence the course of HIV disease are ongoing (24,25). Preliminary data from the Multi Center AIDS Cohort Study suggest that the effect of CCR5 and HLA genotype on survival or disease course are independent and that the effect of HLA on disease outcomes may be greater than the effect of CCR5 genotype (54).

Population Studies of CCR5 Genotypes

The distribution of the 32bp deletion in different populations is summarized in Table 2. Among Caucasians in North America or Europe, the prevalence of homozygosity for the 32 bp deletion is approximately 1%, and 10% to 20% are heterozygous. In smaller numbers of non-Caucasians, heterozygosity is found in approximately 6% of African-Americans, 7% of Hispanics, 13% of Native Americans, and fewer than 1% of Asians (17,19; CDC, unpub. data). Several studies of non-Caucasian populations including persons from parts of Africa (Zaire, Burkina Faso, Cameroon, Senegal, Benin, Uganda, Rwanda, Kenya, Malawi, Tanzania, Sierre Leone) (12,16,19), Haiti (12), parts of Asia (Thailand, India, China, Korea, Japan, the Philippines) (12,16,19; CDC, unpub. data), and Venezuela (9) have not found the 32 bp deletion among the HIV-infected or -uninfected persons tested (Table 2). One additional study (of more than 3,000 persons) found similar global distributions of CCR5 genotypes (and noted a decreasing frequency from north to south in Europe and Asia).[2]

Other Chemokine Receptor Polymorphisms

Several other point mutations in CCR5 have been observed (17), but their population prevalence and their role in HIV infection or disease progression have not been reported. No polymorphisms in CXCR4 have been reported to date. CCR3 has two alleles (S or T at position 276) (55-57), and similarly, the population prevalence of these alleles and their role in HIV infection have not yet been determined.

Implications for Management and Understanding of the Global HIV Epidemic

The discovery of the role of chemokine receptors as coreceptors, along with CD4, for HIV entry has led to a burgeoning of public and private research in HIV pathogenesis, new therapies, and new approaches to vaccines (52,58). For example, in addition to the classification of HIV types on the basis of genetic or neutralization phenotype relatedness, a new virus classification made on the basis of their receptor use has emerged. New therapies based on receptor mimics, receptor ligands, chemokines, or chemokine analogs are being developed and tested in vitro. Genetic engineering with chemokine receptors is being used to develop animal models for HIV transmission and disease progression. And, in the arena of vaccines, a new concept has developed: the induction of high levels of HIV-blocking chemokines such as RANTES, MIP-1α, and MIP-1ß as desirable properties of an HIV vaccine.

New treatments and prevention measures for HIV raise ethical, social, and legal issues that also relate to a number of other infectious and chronic diseases (e.g., malaria, breast cancer, and diabetes) as new genes influencing the risk for these diseases are discovered. Should all

[1]Garred P, Eugen-Olsen J, Iversen AKN, Benfield TL, Svejgaard A, Hofmann, B, the Copenhagen AIDS Study Group. Dual effect of CCR5 Δ32 gene deletion in HIV-1-infected patients. Lancet 1997; 349:1884.

[2]Martinson JJ, Chapman NH, Rees DC, Lui Y-T, Clegg JB. Global distribution of the CCR5 gene 32-basepair deletion [letter]. Nature Genetics 1997;16:100-103.

persons at risk for HIV infection be tested for CCR5 gene polymorphism? Should persons homozygous for the Δ32 CCR5 genotype receive specific counseling regarding their risk for HIV and other sexually transmitted infections? If a person is heterozygous and infected with HIV, should HIV treatment be altered? Could knowledge of one's genotype favorably or unfavorably influence health insurance, life insurance, or employment opportunities? As a result of these questions, educational materials have become available for the public and public health professionals.[3]

Another finding that provoked discussion was the uneven distribution of the genotype across geographic and racial groups. One hypothesis was that some previous epidemic, restricted to Europe, had led to a survival advantage among persons homozygous or heterozygous for the CCR5 Δ32 genotype and had resulted in a concentration of the CCR5 Δ32 genotype in persons of European ancestry. The "black death" of Northern Europe (1347-1350) and other large epidemics have been used as examples (59,60). Thus, certain populations appear to have an increased survival advantage in the new HIV epidemic. Conversely, populations with lower prevalence of the Δ32 CCR5 genotype might be expected to have a higher prevalence of HIV infection or a more rapid course of the epidemic. In the United States, a greater risk for HIV infection has been found among African-Americans than among Caucasians, when known risk factors, including social class, were controlled (61). The prevalence of the Δ32 CCR5 allele is lower in African-Americans than in Caucasians. While increasing HIV prevalence in parts of Asia and Africa may be attributed to social and demographic factors, as well as differences in the phenotype of circulating viruses (62), the racial distribution of HIV risk raises the possibility that differences in the distribution of the Δ32 CCR5 allele or other heritable host factors might influence the rate of transmission or the speed of the epidemic in different racial groups.

Conclusions

These findings have defined a new role for a gene that normally controls cell migration; have identified new alleles of the gene that determines whether someone becomes infected with HIV and how the disease progresses once infection has occurred; and have identified several other new HIV-receptors that mediate viral entry into different cell types. The findings have precipitated new classifications of HIV phenotypes by cell tropism and receptor use and opened new areas for drug and vaccine development. These findings have also enhanced other research: studies of host factors affecting the acquisition and clinical course of infectious diseases, the differential distribution of genetic characteristics in populations and their potential effects on health status, the translation of genetics research into public health measures, and the social implications of genetic differences affecting illness and death caused by infectious and other diseases.

Acknowledgments

We thank J.S. McDougal and Renu Lal for critical discussion, Clair Kiernan for assistance with graphics, and Wendy Paris for secretarial help.

Dr. McNicholl is a scientist in the Immunology Branch, Division of AIDS, STD, TB, and Laboratory Research, NCID, CDC, and an Assistant Professor of Medicine (Rheumatology) and Pathology at Emory University. Her research includes immunogenetics and T cell antigen recognition in infectious and autoimmune diseases, and their application to vaccines. In collaboration with other scientists at CDC and in Thailand, she is investigating immunologic and genetic explanations for apparent resistance to HIV of highly exposed persistently seronegative commercial sex workers in Thailand. She also serves as the NCID coordinator for CDC genetics activities.

References

1. World Health Organization. Acquired immunodeficiency syndrome (AIDS)—November 20, 1996. Wkly Epidemiol Rec 1996; 48:361.
2. Steel CM, Ludlam CA, Beatson D, Peutherer JF, Cuthbert RJG, Simmonds P, et al. HLA haplotype A1 B8 DR3 as a risk factor for HIV-related disease. Lancet 1988;1:1185-8.
3. Kaslow RA, Carrington M, Apple R, Park L, Munoz A, Saah AJ, et al. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. Nat Med 1996;2:405-11.
4. McNeil AJ, Yap PL, Gore SM, Brettle RP, McCol M, Wyld R, et al. Association of HLA types A1-B8-DR3 and B27 with rapid and slow progression of HIV disease. QJM 1996;89:177-85.
5. Hill AVS. HIV and HLA: confusion or complexity? Nat Med 1996;2:395-400.
6. Malkovsky M. HLA and natural history of HIV infection. Lancet 1996;348:142-3.

[3]Centers for Disease Control and Prevention. Facts about CCR5 and protection against HIV-1 infection; 1997.

7. Rowland-Jones S, Sutton J, Ariyoshi K, Dong T, Gotch F, McAdam S, et al. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. Nature Medicine 1995;1:59-64.
8. Paxton WA, Martin SR, Tse D, O'Brient TR, Skurnick J, VanDevanter NL, et al. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposures. Nat Med 1996;2:412-7.
9. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR Horuk R, et al. Homozygous defect in HIV-1 co-receptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 1996;86:367-77.
10. Fowke KR, Nagelkerke NJD, Kimani J, Simonsen JN, Anzala AO, Bwayo JJ, et al. Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya. Lancet 1996;348:1347-51.
11. Stephens H, Beyrer C, Mastro T, Nelson KE, Klaythong R, Kunachiwa W, et al. HLA class I alleles in a cohort of HIV-1 exposed, persistently seronegative (HEPS) sex workers (CSWs) in Northern Thailand. In: Proceedings of the 3rd Conference on Retroviruses and Opportunistic Infections; 1996 January. Washington (DC): American Society for Microbiology; 1996.
12. Huang Y, Paxton WA, Wolinsky SM, Neumann AU, Zhang L, He T, et al. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nat Med 1996;2:1240-3.
13. Fowke K, Slaney LA, Simonsen JN, Nagelkerke N, Nath A, Anzala AO, et al. HIV-1 resistant prostitutes: an innate mechanism. In: Proceedings of the 1st National Conference on Human Retroviruses; Dec 12–16. Washington (DC): American Society for Microbiology; 1993; p. 82.
14. Plummer FA, Fowke K, Nagelkerke NDJ, Simonsen JN, Bwayo J, Ngugi E, et al. Evidence of resistance to HIV among continuously exposed prostitutes in Nairobi, Kenya. In: Abstracts of the 9th International Conference on AIDS; Berlin 1993 June 6–11; WS-A07-3. Sponsored by the International AIDS Society and World Health Organization.
15. Rowland-Jones SL, McMichael A. Immune responses in HIV-exposed seronegatives: have they repelled the virus? Curr Opin Immunol 1995;7:448-55.
16. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, et al. Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 1996;382:722-5.
17. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion of the CKR5 structural gene. Science 1996;273:1856-62.
18. Michael NL, Chang G, Louie LG, Mascola JR, Dondero D, Birx DL, et al. The role of viral phenotype and CCR-5 gene defects in HIV-1 transmission and disease progression. Nat Med 1997;3:338-40.
19. Zimmerman PA, Bucklerwhite A, Alkhatib G, Spalding T, Kubofcik J, Combadiere C. Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5—studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. Mol Med 1997;3:23-36.
20. Biti R, French R, Young J, Bennetts B, Stewart G. HIV-1 infection in an individual homozygous for the CCR5 deletion allele. Nat Med 1997;3:252-3.
21. O'Brien TR, Winkler C, Dean M, Nelson JAE, Carrington M, Michael NL, et al. HIV-1 infection in a man homozygous for CCR5 Δ32. Lancet 1997;349:1219
22. Theodorou I, Meyer L, Magierowska M, Katlama C, Rouzious C, Seroco Study Group. HIV-1 infection in an individual homozygous for CCR5Δ32. Lancet 1997;349:1219-20.
23. Eugenolsen J, Iverson AKN, Garred P, Koppelhus U, Pedersen C, Benfield TL, et al. Heterozygosity for a deletion in the CKR-5 gene leads to prolonged AIDS-free survival and slower CD4 T-cell decline in a cohort of HIV-seropositive individuals. AIDS 1997;11:305-10.
24. Garred P, Madson HO, Balslev U, Hofmann B, Gerstoft J, Svejgaard A. Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin. Lancet 1997;349:236-40.
25. Brinkman BMN, Keet IPM, Miedema F, Verweij CL, Klein M. Polymorphisms within the human tumor necrosis factor-α promoter region in human immuno-deficiency virus type 1-seropositive persons. J Infect Dis 1997;375:188-90.
26. Khoo SH, Pepper L, Snowden N, Hajeer AH, Vallely P, Wilkins EG, et al. Tumor necrosis factor c2 microsatellite allele is associated with the rate of HIV disease progression. AIDS 1997;11:423-8.
27. Murphy PM. Chemokine receptors: structure, function and role in microbial pathogenesis. Cytokine Growth Factor Rev 1996;7:47-64.
28. Napolitano M, Zingoni A, Bernardini G, Spinetti G, Nista A, Storlazzi C, et al. Molecular cloning of TER1, a chemokine receptor-like gene expressed by lymphoid tissues. J Immunol 1996;157:2759-63.
29. Miller LH. Impact of malaria on genetic polymorphism and genetic diseases in Africans and African Americans. Proc Natl Acad Sci U S A 1997;91:2415-9.
30. Tournamille C, Colin Y, Cartron JP, Le Van Kim C. Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in Duffy-negative individuals. Nat Genet 1995;10:224-8.
31. Pleskoff O, Treboute C, Brelot A, Heveker N, Seman M, Alizon M. Identification of a chemokine receptor encoded by human cytomegalovirus as a cofactor for HIV-1 entry. Science 1997;276:1874-8.
32. Cocchi F, DeVico AL, Garzine-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1α and MIPß as the major HIV-suppressive factors produced by CD8+ T cells. Science 1995;270:1811-5.
33. Deng HK, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, et al. Identification of a major co-receptor for primary isolates of HIV-1. Nature 1996;381:661-6.
34. Feng Y, Broder CC, Kennedy PE, Berger EA. HIV-1 entry cofactor—functional CDNA cloning of seven—transmembrane, G protein-coupled receptor. Science 1996;272:872-7.
35. Samson M, Labbe O, Mollereau C, Vassart G, Parmentier M. Molecular cloning and functional expression of a new human CC-chemokine receptor gene. Biochemistry 1996;35:3362-6.

36. Dragic T, Litwin V, Allaway GP, Martin SR, Huang YX, Nagashima KA, et al. HIV-1 entry into CD4(+) cells is mediated by the chemokine receptor CC-CKR-5. Nature 1996;381:667-73.
37. Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, et al. CC CKRS—A RANTES, MIP-1-α, MIP-1ß receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 1996;272:1955-8.
38. Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, et al. The ß-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell 1996;85:1135-48.
39. Doranz BJ, Rucker J, Yi YJ, Smyth RJ, Samson M, Peiper SC, et al. A dual-tropic primary HIV-1 isolate that uses fusin and the ß-chemokine receptors CKR-5, CKR-3 and CKR-2b as fusion cofactors. Cell 1996;85:1149-58.
40. He J, Chen Y, Farzan M, Choe H, Ohagen A, Gartner S, et al. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. Nature 1997;385:645-9.
41. Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. Proc Natl Acad Sci U S A 1997;94:1925-30.
42. Simmons G, Wilkinson D, Reeves JD, Dittmar MT, Beddows S, Weber J, et al. Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either lestr or CCR5 as coreceptors for virus entry. J Virol 1996;70:8355-60.
43. Zhang L, Huang Y, He T, Cao Y, Ho DD. HIV-1 subtype and second-receptor use. Nature 1996;383:768.
44. Wain-Hobson S. One on one meets two. Nature 1996;384:117-8.
45. Cocchi F, DeVico AL, Garzino-Demo A, Cara A, Gallo RC, Lusso P. The V3 domain of the HIV-1 gp 120 envelope glycoprotein is critical for chemokine-mediated blockade of infection. Nat Med 1996;2:1244-7.
46. Wu L, Gerard NP, Wyatt R, Choe H, Parolin C, Ruffing N, et al. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. Nature 1996;384:179-83.
47. Trkola A, Dragic T, Arthos J, Binley JM, Olson WC, Allaway GP, et al. CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR5. Nature 1996;384:184-7.
48. Rucker J, Samson M, Doranz BJ, Libert F, Berson JF, Yi Y, et al. Regions in ß-chemokine receptors CCR5 and CCR2b that determine HIV-1 cofactor specificity. Cell 1996;87:437-46.
49. Atchison RE, Gosling J, Monteclaro FS, Franci C, Digilio L, Charo IF, et al. Multiple extracellular elements of CCR5 and HIV-1: dissociation from response to chemokines. Science 1996;274:1924-6.
50. Lapham C, Ouyang J, Chandrasekhar B, Nguyen N, Dimitrov D, Golding H. Evidence for cell-surface association between fusin and the CD4-gp 120 complex in human cell lines. Science 1996;274:602-5.
51. Oravecz T, Pall M, Norcross MA. ß-Chemokine inhibition of monocytotropic HIV-1 infection. Interference with a postbinding fusion step. J Immunol 1996;157:1329-32.
52. Paxton WA, Dragic T, Koup RA, Moore JP. Perspective—research highlights at the Aaron Diamond AIDS Research Center—the beta-chemokines, HIV type 1 second receptors, and exposed uninfected persons. AIDS Res Hum Retroviruses 1996;12:1203-7.
53. Wu L, Paxton WA, Kassam N, Ruffing N, Rottman JB, Sullivan N, et al. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. J Exp Med 1997;185:1681-91.
54. Kaslow RA, Koup R, Zimmerman P, Dean M, Naik E, Enger C, et al. HLA scoring profile (HSP) and CCR5 deletion heterozygosity as predictors of AIDS in seroconverters. In: Proceedings of the 4th Conference on Retroviruses and Opportunistic Infections; Jan 22–26. Washington (DC): American Society of Microbiology: 1997; p. 69.
55. Combadiere C, Ahuja SK, Murphy PM. Cloning and functional expression of a human eosinophil CC chemokine receptor. J Biol Chem 1996;271:11034.
56. Daugherty BL, Siciliano SJ, DeMartino JA, Malkowitz L, Sirotina A, Springer MS. Cloning, expression, and characterization of the human eosinophil eotaxin receptor. J Exp Med 1996;183:2349-54.
57. Ponath PD, Qin S, Post TW, Wang J, Wu L, Gerard NP, et al. Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. J Exp Med 1996;183:2437-48.
58. D'Souza MP, Harden VA. Chemokines and HIV-1 second receptors. Nat Med 1996;2:1293-300.
59. New AIDS study reveal startling immunity data. Kolata G. The New York Times. 1996; September 27, 1996. p. A13 New York. The New York Times.
60. Kolata G. Geneticists seek to understand why disease genes spread. The New York Times 1996; Sect. B:5-9.
61. Easterbrook PJ, Chmiel JS, Hoover DR, Saah AJ, Kaslow RA, Kingsley LA, et al. Racial and ethnic differences in human immunodeficiency virus type 1 (HIV-1) seroprevalence among homosexual and bisexual men. The multicenter AIDS cohort study. Am J Epidemiol 1993;138:415-29.
62. Soto-Ramirez LE, Renjifo B, McLane MF, Marlink R, O'Hara C, Sutthent R, et al. HIV-1 Langerhans' cell tropism associated with heterosexual transmission of HIV. Science 1996;271:1291-3.

Resistance, Remission, and Qualitative Differences in HIV Chemotherapy

Denise E. Kirschner* and G.F. Webb†
*University of Michigan Medical School, Ann Arbor, Michigan, USA; and
†Vanderbilt University, Nashville, Tennessee, USA

To understand the role of qualitative differences in multidrug chemotherapy for human immunodeficiency virus (HIV) infection in virus remission and drug resistance, we designed a mathematical system that models HIV multidrug chemotherapy including uninfected CD4+ T cells, infected CD4+ T cells, and virus populations. The model, which includes the latent and progressive stages of the disease and introduces chemotherapy, is a system of differential equations describing the interaction of two distinct classes of HIV (drug-sensitive [wild type] and drug-resistant [mutant]) with lymphocytes in the peripheral blood; the external lymphoid system contributes to the viral load. The simulations indicate that to preclude resistance, antiviral drugs must be strong enough and act fast enough to drive the viral population below a threshold level. The threshold depends upon the capacity of the virus to mutate to strains resistant to the drugs. Above the threshold, mutant strains rapidly replace wild-type strains. Below the threshold, resistant strains do not become established, and remission occurs. An important distinction between resistance and remission is the reduction of viral production in the external lymphoid system. Also the virus population rapidly rebounds when treatment is stopped even after extended periods of remission.

Mathematical models provide a means to understand the human immunodeficiency virus (HIV)-infected immune system as a dynamic process. Models formulated as differential equations for the dynamic interactions of CD4+ lymphocytes and virus populations are useful in identifying essential characteristics of HIV pathogenesis and chemotherapy. Recent clinical studies have produced new insight into the dynamics of these virus populations during HIV infection (1-3). Turnover rates and lifespans of infected CD4+ T cells and virus have been identified by measuring their rates of change in patients undergoing strong antiviral mono-therapy. The determination of these rates showed that large numbers of CD4+ T cells and virus are gained and lost each day throughout the course of HIV infection (4,5); these findings have profoundly influenced strategies for therapy (6). It is now recognized that chemotherapeutic agents must strongly suppress viral production before rapidly appearing viral mutants evolve to drug resistance. Recent clinical trials have accomplished this goal by using combined drug therapy. Ongoing trials with combinations of drugs have shown sharp declines, in some patients, of viral counts to nondetectable levels within several weeks of treatment; these levels were sustained for 1 year or more (5,7,8). At the same time, CD4+ T-cell counts have risen markedly before gradually leveling off (5,7,8). This apparent remission of HIV infection offers hope for the chronic control or even eradication of HIV (6). The issue of stopping treatment after such extended periods of remission, however, is yet to be resolved (8).

Address for correspondence: Denise E. Kirschner, Department of Microbiology and Immunology, University of Michigan Medical School, 6730 Medical Science Building II, Ann Arbor, MI 48109-0620 USA; fax: 313-764-3562; e-mail: kirschne@umich.edu.

The Model

Our model of treatment distinguishes qualitatively two treatment outcomes indicated by clinical trials. The first is resistance. Examples of resistance for three-drug combined therapy are reported for completed clinical trials by Collier et al. (9). In these trials, there was on average an increase of CD4+ T-cell counts by approximately 30% (peaking at approximately 8 weeks and returning to baseline at approximately 40 weeks) and a decrease of plasma virus by approximately

70% (peaking at approximately 4 weeks and recovering to half baseline at approximately 40 weeks). The second treatment outcome is remission. Examples of remission are indicated in preliminary reports of ongoing clinical trials (5,7,8,10). In these trials, 1) plasma virus decreased sharply to nondetectable levels in 2 to 4 weeks, and these levels were sustained for periods of 1 year or more, and 2) CD4+ T-cell counts increased steadily by 100/mm^3 or more before gradually leveling off to below normal levels (this below normal recovery is believed to be due to an impaired production of new CD4+ T cells from the thymus and other sources [11,12]; this assumption is incorporated into the model).

The model consists of differential equations for the variables T(t) (the CD4+ T-cell population uninfected by virus at time t), $T_s(t)$ (the CD4+ T-cell population infected by drug-sensitive virus at time t), $T_r(t)$ (the CD4+ T-cell population infected by drug-resistant virus at time t), $V_s(t)$ (the drug-sensitive virus population at time t), and $V_r(t)$ (the drug-resistant virus population at time t). All these virus populations reside in the circulating blood, in which the values of uninfected CD4+ T cells and virus can be clinically measured. The assumptions of the model and its equations are given in the Appendix.

The model incorporates recent clinically determined dynamic information about the HIV-infected immune system. The essential elements are as follows. After an initial period of acute viremia in the first few weeks after seroconversion, CD4+ T-cell counts decline gradually from approximately 600 to 800/mm^3 to 0/mm^3 over approximately 10 years (11) (normal CD4+ T-cell counts are 800 to 1,200/mm^3). The decline of CD4+ T cells is more rapid early in the infection (13). Infected CD4+ T cells constitute 4% or less of the CD4+ T-cell population (14). The half-life of an infected CD4+ T cell is approximately 2 days (1-3,6). After the initial viremia, plasma virus increases from below 50/mm^3 to 1,000/mm^3 or more during the variable course of infection with a sharp increase toward the end of the symptomatic phase (11). The lifespan of a virus outside the cell is about 7.2 hrs (1-3).

A typical untreated disease course based upon CD4+ T-cell counts and viral level is simulated in Figure 1a,b (the initial period of viremia is not included in the model). The simulation in Figure 1a,b is in close agreement with a typical disease course (11). The initial virus level is determined by the model's parameters, which do not change throughout the course of the disease. This assumption is consistent with recent clinical findings that disease prognosis is correlated to a set-point of virus level established in each patient soon after the initial viremia, and viral levels and replication rates remain relatively stable after the set-point (5,8,15,16). In the model, different set-points are obtained by varying key parameter values.

In the model, treatment is incorporated as the reduction of two separate rates. The reduction of these rates provides treatment control variables corresponding to the intensity and velocity of drug action. The variables are the rate at which virus infects uninfected CD4+ T cells and the rate of virus influx into the plasma from the external lymphoreticular system. Reduction of this second rate is the most important for treatment outcome, since it is believed that as much as 98% of the virus in the circulating blood is contributed by the external lymphoid compartment (5,8,17). In the simulations, the dynamics in the lymphoid compartment are modeled as a viral source term rather than mechanistically, since limited data are available for this compartment (18). Models of combined plasma-lymph compartment dynamics will appear in future work. When treatment begins, the model assumes that a proportion of drug-sensitive virus mutates to drug-resistant virus. This proportion is also a treatment control variable corresponding to the combination of drugs used or the presence of genetic diversity at different disease stages (19).

The model distinguishes primarily between resistance and remission in the assumption of a threshold condition for the virus population in the plasma (and thus for the virus population in the lymphoid compartment). The threshold condition is incorporated into the rate that controls the contribution of drug-resistant virus from the external lymphoid compartment to the plasma. When treatment drives the plasma virus level below the threshold, the drug-resistant virus population does not emerge, and the drug-sensitive virus population falls to near 0. This threshold cannot be reached simply by gradually lowering the drug-sensitive virus population. Two additional factors must be considered: 1) when the virus population is above threshold, the high mutational capacity and short lifespan of the virus results in rapid production of drug-

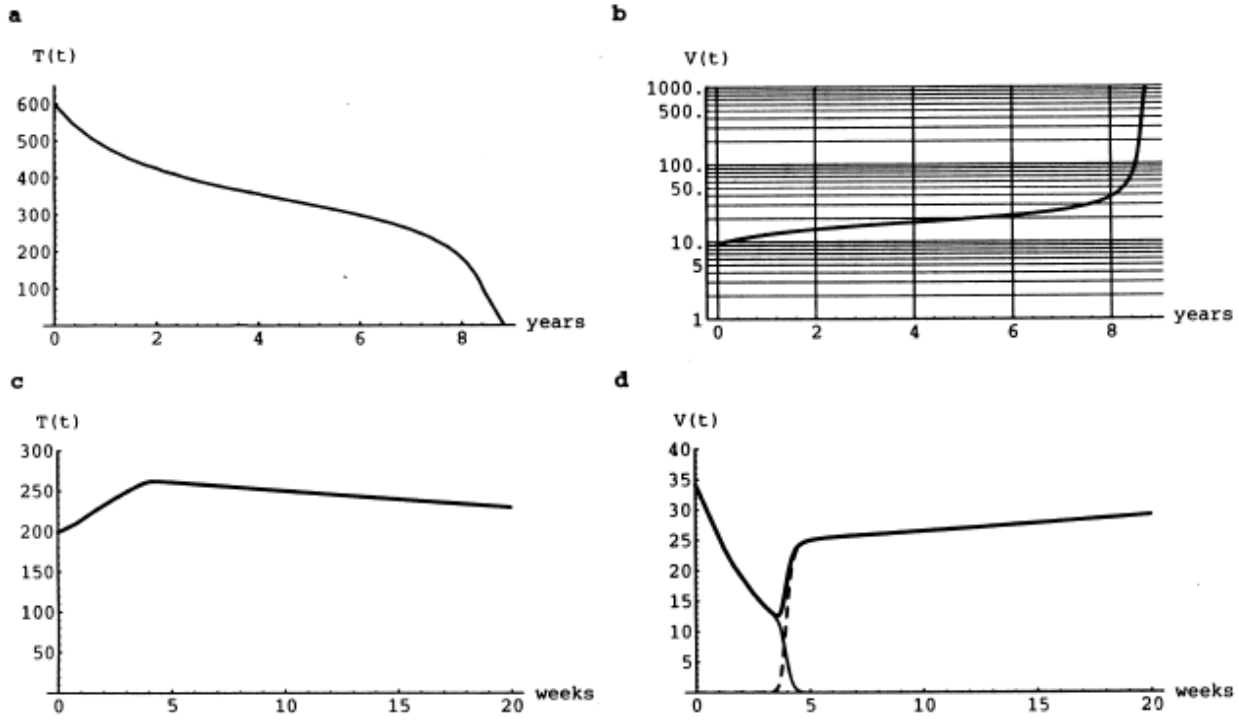


Figure 1a,b. A simulation of HIV dynamics for the model (A.1) - (A.3) with $T(0) = 600/mm^3$ and $V_s(0) = 10/mm^3$. The curves correspond to data in (11). The set-point of the virus is in the middle range (15) and corresponds to a typical disease progression of about 9 years. The contribution to the plasma virus from the external lymphoid compartment is more than 90%, as may be computed from equation (A.3). The curves T(t) and $V_s(t)$ are approximately inversely proportional, as may be seen from equation (A.3) (the inverse proportionality is specific to a given set-point). In c,d, a simulation of a combined drug treatment corresponds to data in (9). The treatment begins with the uninfected CD4+ T-cell count at $200/mm^3$, the infected CD4+ T-cell count at $9.5/mm^3$, and the virus level at $34/mm^3$ (these values are obtained from the simulation in a, b at 7.9 years). In d, the heavy line is the total virus population, the thin line is the wild-type virus population, and the dashed line is the drug-resistant virus population. Complete replacement of wild-type virus by resistant virus occurs by week 5. The treatment parameters are $c_1=.5$, $c_2=.025$, $c_3=.15$, and the resistance mutation parameter is $q=10^{-7}$.

resistant variants; and 2) as the virus population approaches the threshold, Darwinian competition gives competitive advantage to the resistant viral strain as the sensitive viral strain diminishes in fitness and in numbers.

To reach the threshold, the virus population must be brought down extremely fast before mutation and selection pressure allow resistant virus to propagate in the drug-altered environment. In the simulations, this rapid fall to the threshold can be achieved if treatment inhibits the rate of viral influx from the external lymphoid compartment sufficiently fast. The threshold value depends on the drugs used and the capacity of the virus to mutate against these drugs. In some patients, plasma levels were reduced by 99.9% or more, yet remission did not occur (1,2). In these cases, there may have been an extremely low threshold specific to the drugs used or a disproportionately lower suppression of virus in the lymphoid compartment than in the plasma.

The Simulations

Computer simulations of treatment are given in Figure 1c,d and Figures 2-5. The initial values at the start of treatment for the simulations are obtained from the simulation in Figure 1a,b. In all the simulations, the parameters are the same, except for the parameters controlling treatment and the resistance mutation.

In Figure 1c,d, resistance is simulated. The simulation corresponds to composite data given (9) for patients receiving the drug combination saquinavir, zidovudine, and zalcitabine (the first is a protease inhibitor and the other two are reverse transcriptase inhibitors). Despite an impressive increase in the CD4+ T-cell counts in Figure 1c (significantly higher than typically seen with zidovudine alone [20-23]), the reduction of viral influx from the external lymphoid compartment is not fast enough or strong enough to bring the virus below the threshold for remission. The viral level thus rebounds after a few weeks, and the T-cell population resumes a decline.

In Figure 2, resistance is simulated corresponding to data for patients receiving therapy with strong reverse transcriptase inhibitors (10). In these simulations, the reduction of the viral influx from the external lymphoid compartment is higher than in the simulation in Figure 1d, which means that the effect of the drug is stronger and the decrease of the virus level is faster. The resistance mutation parameter is also assumed to be higher than in Figure 1c,d, which means that resistance develops sooner. The exponential rates of increase of CD4+ T-cell counts in Figure 2a are inversely correlated to CD4+ T-cell starting values, as are the times to the appearance of resistance. The exponential rates of viral decay (Figure 2b), as indicated by the slopes of their logarithmic plots, are approximately parallel and thus not correlated to

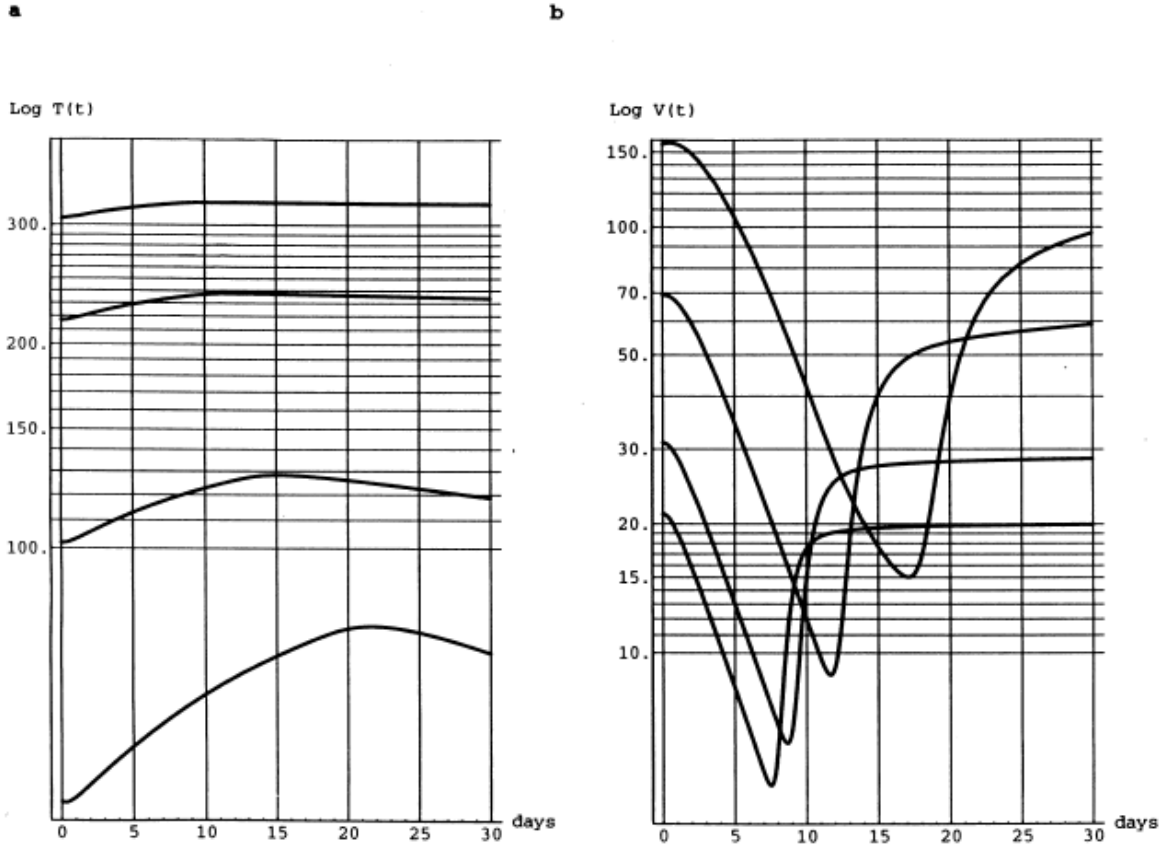


Figure 2. Four simulations corresponding to therapy (2). The simulations have a common viral set-point of disease progression with the treatment starting values T(0) = 306/mm^3 and $V_s(0)$ = 21/mm^3 (obtained from Figure 1a,b at 5.8 years), T(0) = 217/mm^3 and $V_s(0)$ = 31/mm^3 (obtained from Figure 1a,b at 7.7 years), T(0) = 100/mm^3 and $V_s(0)$ = 69/mm^3 (obtained from Figure 1a,b at 8.4 years), and T(0) = 43/mm^3 and $V_s(0)$ = 156/mm^3 (obtained from Figure 1a,b at 8.6 years). The rates of exponential increase in Figure 2a (approximately .03, .02, .01, .005) are inversely correlated to starting CD4+ T-cell counts, and the exponential rates of decay in Figure 2b (all about -.2) are not correlated to starting viral levels (different viral set-points would give different values for the parallel slopes) (1,2). The lack of correlation of viral decay rates is an indication of slower clearance of wild-type virus in the external lymphoid compartment. The time to the downward spike in Figure 2b is correlated to starting viral levels (1). The treatment parameters c_1=2.0, c_2=.17, c_3=.15 and the resistance mutation parameter $q=10^{-6}$ are the same in all four simulations.

treatment starting values. This lack of correlation has been reported in clinical data (1,2).

In the simulations in Figure 2, the lack of correlation can be explained by the failure of the treatment to suppress rapidly enough the viral production caused by the external lymphoid system. As this production is suppressed at faster and faster rates, the viral exponential decay rates approach the actual loss rates of the plasma virus, which in this model are correlated to the CD4+ T-cell levels. The decay rates (Figure 2b) do not yield the actual half-life of free virus, which is shorter. The difference is due to the incomplete inhibition of the external lymphoid viral production. The exponential rate of viral decay in patients undergoing treatment is claimed to correspond to the decay of noninfectious virus produced by CD4+ T cells infected after treatment begins by infectious virus present before treatment begins (where it is assumed that after treatment begins, all newly produced virions are noninfectious) (3). It is claimed that the reciprocal of the viral decay rate is the average lifespan of infected CD4+ T cells (3). The model considered here has a different interpretation of the effects of treatment, since the prouction of virus from the external lymphoid compartment is not immediately blocked by treatment and thus influences the viral decay rate.

In Figure 3, simulations are given with the treatment parameter corresponding to suppression of virus influx from the lymphoid compartment higher than in Figure 1c,d and the mutation parameter lower than in Figure 2. In Figure 3a, remission is achieved, but in Figure 3b,c,d, it is not (the threshold value is indicated by the horizontal lines). The concurrence of strong suppression of the lymphoid virus compartment, lower resistance mutation parameter,

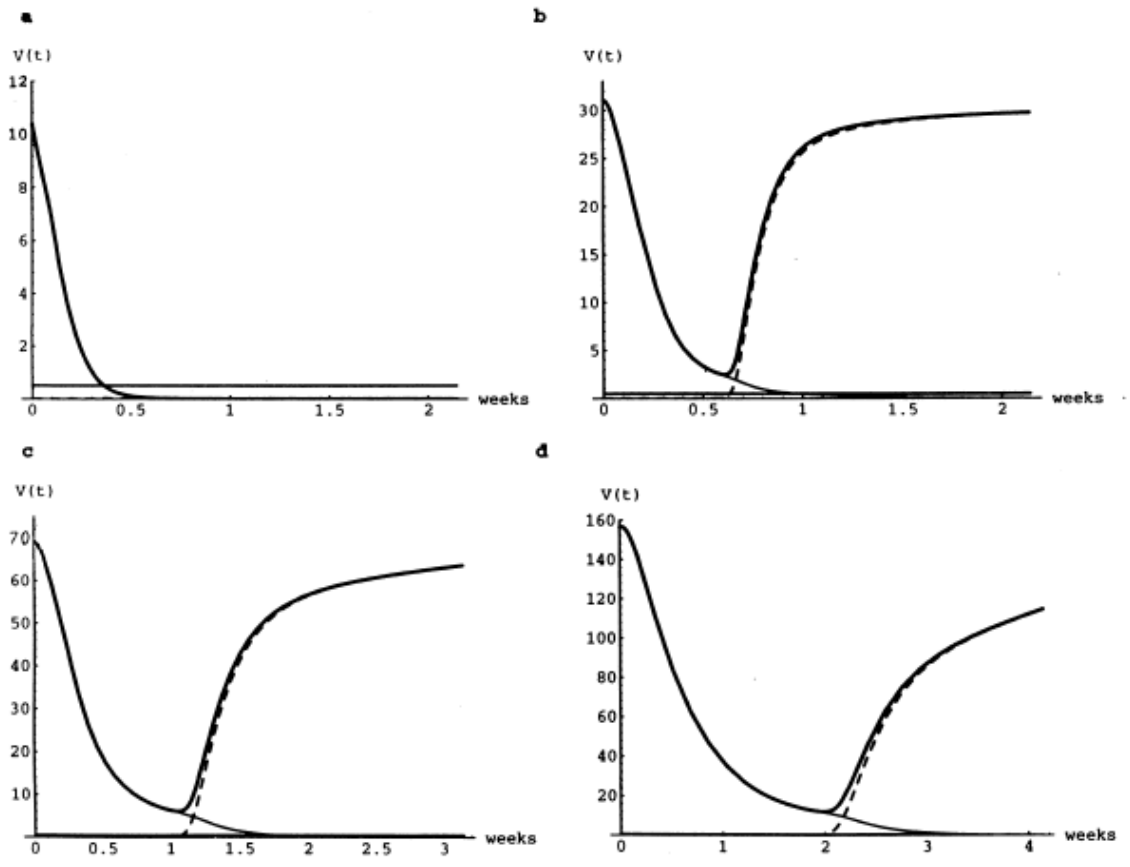


Figure 3. Treatment simulations for four starting viral levels. The simulations have a common viral set-point of disease progression, and the treatment starting values are from Figure 1a,b. For all four simulations, the treatment parameters are c_1=2.0, c_2=1.0, c_3=.1, the resistance mutation parameter is $q=10^{-7}$, and the threshold value is V_0=.5 (indicated by the horizontal line). The lowest starting viral level achieves remission (a), while the other three develop resistance.

and lower viral starting value allows the remission threshold to be reached (Figure 3a).

In Figure 4, treatment is simulated with an even higher value of the parameter corresponding to suppression of virus production from the external lymphoid compartment. Remission is achieved for the two lowest viral starting levels. As in Figure 2a, the exponential rates of increase of CD4+ T cells are inversely correlated to CD4+ T-cell starting values (an explanation in terms of the relative rates of changes in the differential equations for the CD4+ T-cell population is given in the Appendix). The exponential rates of viral decay (Figure 4b), however, are inversely correlated to increasing values of starting viral levels (in contrast with Figure 2b). When drug inhibition of virus in the lymph compartment is very high, the plasma viral clearance rate during treatment approaches the plasma viral clearance rate before treatment. In our models, it is assumed that the plasma viral clearance rate before treatment depends on CD4+ T-cell levels. The inverse correlation of plasma viral clearance rates during treatment to viral levels at the start of treatment is thus an indicator of higher viral clearance from the lymphoid compartment (an explanation in terms of the relative rates of change in the differential equation for the virus population is given in the Appendix).

In Figure 5a, treatment data for a patient receiving zidovudine, didanosine, and lamivudine are simulated (18; Figure 1d). This treatment induces a remission, even though the plasma virus does not fall below a nondetectable level. The plasma viral decay is approximately three times as fast as the lymph viral decay (18). This difference is incorporated into the treatment parameters for the simulation in Figure 5a. The two-phase plasma viral decay process (Figure 5a) matches the data (18) and is a strong indication that the rate of plasma viral decay is influenced by the slower rate of decay in the lymph system.

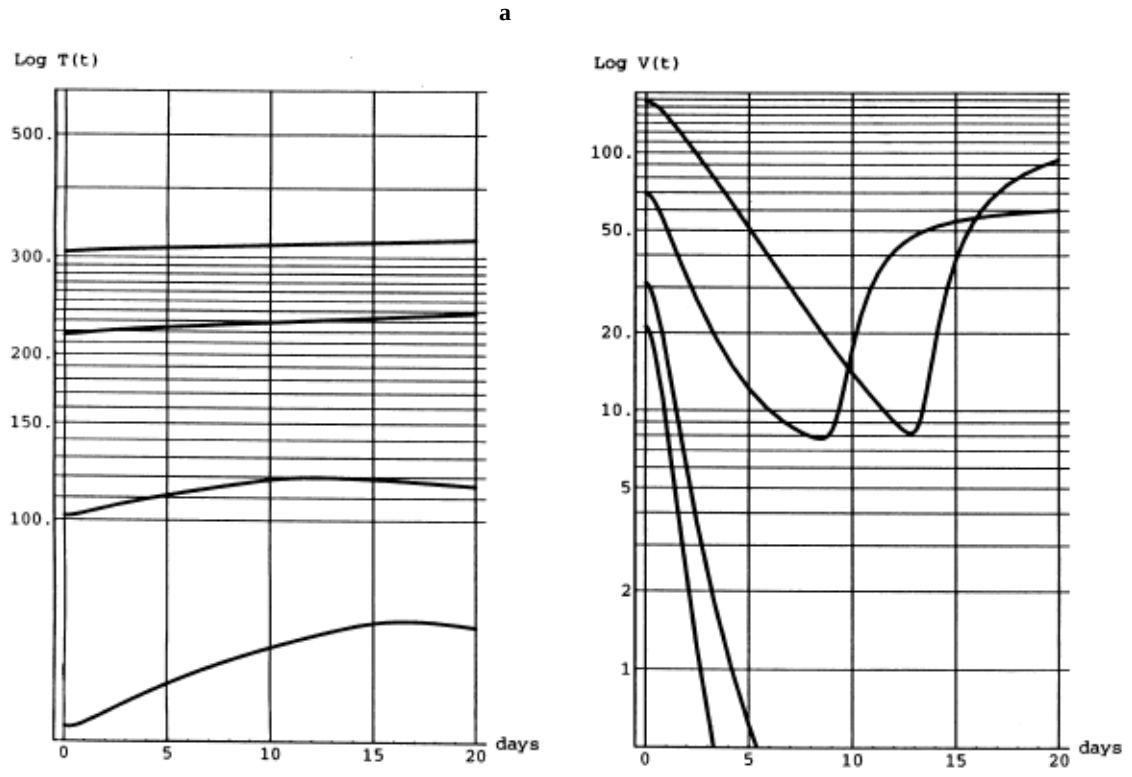


Figure 4. Four treatment simulations having a common viral set-point of disease progression. The treatment starting values are as in Figure 2. For all four simulations, the treatment parameters are c_1=2.0, c_2=2.0, c_3=.05, the resistance mutation parameter is $q=10^{-8}$, and the threshold value is V_0=2.0. Remission is achieved for the two lowest viral starting values, but the other two develop resistance. The viral exponential decay rates are -1.4, -.93, -.51, and -.26, which are inversely correlated to the viral starting values (an indication of rapid suppression of virus in the external lymphoid compartment).

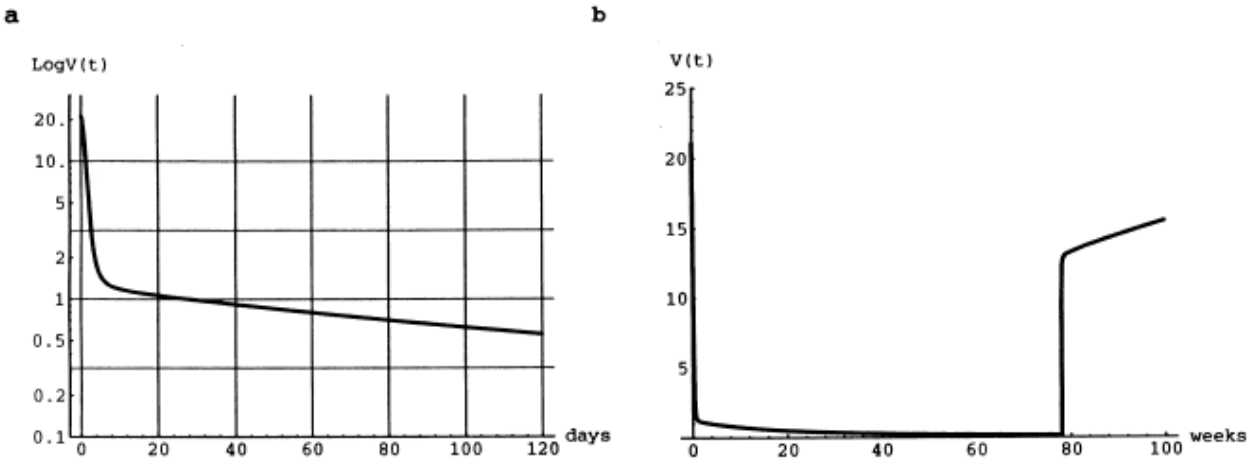


Figure 5. 5a simulates combined drug treatment data reported for a patient (18; Figure 1d). The treatment begins with the uninfected CD4+ T-cell count at 306/mm^3, the infected CD4+ T-cell count at 10/mm^3, and the virus level at 21/mm^3 (these values are obtained from the simulation in Figure 1a, b at 5.75 years). The treatment parameters are c_1=2.0, c_2=1.0, c_3=.15, the resistance threshold value is V_0=3.0, and the resistance mutation parameter is q=10^{-7}. Resistance does not develop, and the therapy results in remission. The plasma viral level shows a two-phase exponential decay, which is attributed to a slower drug-induced inhibition of virus in the lymphoid compartment. In 5b, the treatment simulation in 5a is continued for 78 weeks and then stopped. The virus population rebounds rapidly when treatment stops.

In Figure 5b, the treatment simulation (Figure 5a) is stopped at 78 weeks, whereupon the drug-sensitive virus population rebounds sharply (8). This simulation is consistent with the report of a patient undergoing combined therapy who sustained nondetectable levels of virus for 78 weeks and upon voluntarily stopping treatment experienced high levels of virus in the blood within 1 week (3). In this simulation the virus would rapidly become reestablished even after much longer treatment. The resurgence of the virus population in the simulation is due to the capacity of the virus to grow very quickly from extremely low levels, which is due to the incomplete drug-induced inhibition of the external lymphoid viral source (18). This incomplete inhibition can be attributed to the presence of latently infected CD4+ T cells in the lymphoid compartment. Resumption of treatment in the simulation could again induce a remission. The resurgence of virus when treatment is stopped (Figure 5b) would hold also in all the simulations of remission because the 0 viral level is unstable; and if the virus is not suppressed by drugs, it rapidly grows from even very low levels. This instability of the 0 viral level results from the large viral influx from the lymph system, which is required to produce the characteristic dynamics of HIV throughout its entire progression.

Conclusions

Although computer models of HIV therapy are no substitute for clinical trials, they can bring into focus essential elements of the dynamic processes involved. The treatment simulations presented here identify the following qualitative dynamic elements involved in resistance and remission: 1) remission can occur if the viral production in the external lymphoid tissues is suppressed below a threshold level; 2) drug action must be strong enough and fast enough to drive the virus population to the threshold before resistant virus appears and propagates; 3) combination therapy or early treatment lowers the capacity of the virus to mutate to resistant strains and thus forestalls their emergence until the threshold is reached; and 4) stopping treatment even after an extended period of remission may result in a rapid rebound of the virus population.

The remission threshold is an abstract construct, and its quantitative value is relative to the capacity of the virus to mutate against a specific drug regimen. In the simulations, the threshold divides resistance and remission outcomes, and the dynamic developments in the first few days and weeks of drug administration are crucial in determining the outcome of therapy. Remission over extended time, however,

may require continuing treatment to suppress low-level viral replication in the lymphoid tissues. The presence of even low-level viral production due to latently infected CD4+ T cells allows the possibility for the eventual evolution of drug-resistant viral strains.

Appendix

For the model without treatment, it is assumed that only drug-sensitive virus, uninfected CD4+ T cells, and CD4+ T cells infected by drug-sensitive virus are present. The equations for the model without treatment are as follows (24):

(A.1): $dT(t)/dt = S(t) - \mu_T T(t) + p_1(t)T(t)V_s(t) - k_s V_s(t)T(t)$

(A.2): $dT_s(t)/dt = k_s V_s(t)T(t) - \mu_{Ti} T_s(t) - p_2(t)T_s(t)V_s(t)$

(A.3): $dV_s(t)/dt = p_3(t)T_s(t)V_s(t) - k_v T(t)V_s(t) + G_s(t)$

In (A.1) S(t) represents the external input of uninfected CD4+ T cells from the thymus, bone marrow, or other sources. It is assumed that there is a deterioration of this source as the viral level increases during the course of HIV infection. The form of this source is $S(t) = S_1 - S_2 V_s(t)/(B_s+V_s(t))$, where B_s is a saturation constant (the various saturation constants in the model are designed to adjust the rate parameters to large changes in the population levels during disease progression or treatment). In (A.1) μ_T is the death rate of uninfected CD4+ T cells whose average lifespan is $1/\mu_T$ (25). In (A.1) the term $p_1(t)\, T(t)\, V_s(t)$ represents CD4+ T-cell proliferation in the plasma due to an immune response that incorporates both direct and indirect effects of antigen stimulation ($p_1(t) = p_1/(C+V_s(t))$, where C is a saturation constant). This term accounts for the above normal turnover of CD4+ T cells (other forms for this production have been used, including a logistic approach [26]). The form assumed here idealizes the growth mechanisms of CD4+ T cells, since subpopulations of antigen specific CD4+ T cells are not modeled. In (A.1) k_s is the infection rate of CD4+ T cells by virus (it is assumed that the rate of infection is governed by the mass action term $k_s V_s(t)\, T(t)$). In the absence of virus the CD4+ T-cell population converges to a steady state of S_1/μ_T.

In (A.2) there is a gain term $k_s V_s(t)\, T(t)$ of CD4+ T cells infected by drug-sensitive virus, a loss term $\mu_{Ti}\, T_s(t)$ due to the death of these cells independent of the virus population, and a loss term $p_2(t)\, T_s(t)\, V_s(t)$ dependent on the virus population due to bursting or other causes (where $p_2(t) = p_2/(C_i+V_s(t))$ and C_i is a saturation constant). The dependence of the loss term $p_2(t)\, T_s(t)\, V_s(t)$ on $V_s(t)$ allows for an increased rate of bursting of infected cells as the immune system collapses and fewer of these cells are removed by CD8+ T cells.

In (A.3) the virus population is increased by the term $p_3(t)\, T_s(t)\, V_s(t)$, where $p_3(t) = p_3/(C_i+V_s(t))$. This term corresponds to the internal production of virus in the blood. The dependence of this term on $T_s(t)$ allows for a decreased rate of viral production in the plasma when the infected CD4+ T-cell population in the plasma collapses. Since most of the plasma virus is contributed by the external lymph source, the plasma virus population still increases steeply at the end stage of the disease. In (A.3) the virus population is decreased by the loss term $k_v\, T(t)\, V_s(t)$, which represents viral clearance. In (A.3) there is a source of virus from the external lymphoid compartment, which is represented by the term $G_s(t) = G_s V_s(t)/(B+V_s(t))$ (B is a saturation constant). This term accounts for most of the virus present in the blood (8).

The lifespans of infected CD4+ T cells and virus can be computed from the terms in (A.2) and (A.3) during the asymptomatic period of infection (when the rates of population increase are almost balanced by the rates of population decrease). The loss terms in (A.2) yield an average infected CD4+ T-cell lifespan of $1/\mu_{Ti} + p_2\, V_s(t)/(C_i+V_s(t))$, which decreases from $= 1/\mu_{Ti}$ to $1/(\mu_{Ti}+p_2)$ as $V_s(t)$ increases. The loss term in (A.3) yields an average virus lifespan of $1/(k_v T(t))$, which increases from $1/(k_v T(0))$ as T(t) decreases.

The equations for the model with treatment are as follows:

(A.4): $dT(t)/dt = S_0(t) - \mu_T T(t) + p_1(t)T(t)V(t) - (\eta_1(t)k_s V_s(t) + k_r V_r(t))\, T(t)$

(A.5): $dT_s(t)/dt = \eta_1(t)\, k_s V_s(t)\, T(t) - \mu_{Ti}\, T_s(t) - p_2(t)\, T_s(t)\, V(t)$

(A.6): $dT_r(t)/dt = k_r\, V_r(t)\, T(t) - \mu_{Ti}\, T_r(t) - p_2(t) T_r(t)\, V(t)$

(A.7): $dV_s(t)/dt = (1-q)p_3(t)T_s(t)V(t) - k_v T(t)V_s(t) + \eta_2(t)\, G_s V_s(t)/(B+V(t))$

(A.8): $dV_r(t)/dt = p_3(t)\, T_r(t)\, V(t) + q\, p_3(t)\, T_s(t)\, V(t) - k_v\, T(t)\, V_r(t) + G_r(V(t))V_r(t)/(B+V(t))$

In the model, treatment inhibits (with a delay) new infections of CD4+ T cells and inhibits (with a delay) the influx of virus from the external source. In equations (A.4) - (A.8) $V(t) = V_s(t) + V_r(t)$ is the total virus population at time t,

and its inclusion in the rate coefficients results in competition between the sensitive and resistant viral strains. In these equations, treatment is modeled by the decreasing functions $\eta_1(t) = \exp(-c_1 t)$ (which inhibits the rate at which uninfected CD4+ T cells become infected) and $\eta_2(t) = \text{maximum}\{\exp(-c_2 t), c_3\}$ (which inhibits the influx of virus from the external lymphoid compartment). The parameters c_1, c_2, and c_3 control the speed and strength of the drug-induced inhibitions. The form of the treatment function $\eta_1(t)$ produces an eventual complete inhibition of infection of CD4+ T cells in the plasma but does not do so immediately upon treatment (1-3). The form of the treatment function $\eta_2(t)$ produces a delayed and incomplete suppression of viral influx from the external lymphoid system (18). Treatment does not affect the drug-resistant virus or the CD4+ T cells infected by drug-resistant virus.

When treatment begins, it is assumed that the source term of CD4+ T cells in equation (A.4) has the value $S_0(t) = \text{minimum}\{ S_0 , S_1 - S_2 V(t)/(B_s+V(t))\}$, where S_0 is the value of the source of CD4+ T cells when treatment is started (S_0 is obtained from the source function S(t) in the model without treatment). This assumption means that the source of CD4+ T cells does not increase once treatment begins but may decrease if the virus population later increases because of the development of resistance or the cessation of treatment.

In the model, it is assumed that there is no significant level of background resistant virus present to substantially affect the dynamics before treatment begins. After treatment begins, drug-resistant virus does become significant and is introduced into the virus population as a proportion q of the drug-sensitive virus population (19). It is not assumed that drug administration induces resistant mutations, but only that it gives selective advantage to them. The value of q corresponds to the capacity of resistant variants to mutate (larger q corresponds to monotherapy and smaller q to combined therapy). It is assumed that the external input of drug-resistant virus from the lymphoid compartment is controlled by the threshold function $G_r(V)$, where $G_r(V) = 0$ if V is less than the threshold value V_0 and $G_r(V) = G_s$ if V is greater than V_0. This assumption means that the capacity of the resistant virus to become established requires that the total virus population level remain above the threshold V_0.

The lack of correlation of the slopes in Figure 2b to starting CD4+ T-cell counts in Figure 2a can be explained in terms of equation (A.7). When treatment starts at time t_0, $G_s/(B+V(t_0)) \approx k_v T(t_0)$ (since the virus population is changing very slowly before treatment starts and the major source of virus present is due to the external source). After treatment starts, $dV_s(t)/dt \approx - \rho(t) V_s(t)$, where $\rho(t) = c_2(t) G_s/(B+V(t)) - k_v T(t)$. If $c_2(t) \approx 1$ (which corresponds to slow clearance of the external compartment), then $\rho(t) \approx 0$ and $\rho(t)$ does not have a strong dependence on $T(t_0)$ (as in Figure 2b). If $c_2(t) \approx 0$ (which corresponds to rapid clearance of the external lymphoid compartment), then $\rho(t) \approx k_v T(t)$ and thus shows a strong dependence on $T(t_0)$ (as in Figure 4b). A similar argument using equation (A.4) shows that when $c_1(t) \approx 0$ (as in Figures 2a and 4a), then the exponential rates of increase in CD4+ T-cell counts are inversely correlated to treatment CD4+ T-cell starting values.

The models described in this paper have evolved from earlier models by the authors (24,26-28). A major goal of the present work is to align the model simulations with an expanding base of data for HIV dynamics. The construction of the present models is based in part on theoretical assumptions about the rate changes of the interacting populations and in part on simulation of their known dynamic properties. Another major goal of the present work is to derive insight into the qualitative distinctions between monotherapy resistance and combined-therapy remission. In the model (A.4)-(A.8), this distinction resides in the mutation parameter q, which corresponds to the capacity of resistant virus to arise as a proportion of sensitive virus when the total virus population is above the threshold value V_0. When q is large (monotherapy), the total virus population does not fall below V_0, and resistant virus becomes established. When q is small (combined therapy) and the total virus population is brought below V_0 sufficiently fast in the first days and weeks of treatment, the resistant virus population cannot grow.

The models of this paper differ from earlier models (1-3). The models here describe disease progression, whereas others (1-3) describe short intervals of treatment from presumed dynamic steady states. The models here describe dynamics in the plasma, whereas others (1,3) describe dynamics in the total body. The models of this paper distinguish between the behavior of virus in the plasma and in the lymph system. In the models here, the virus increases steeply in the

plasma but saturates in the lymph system. The assumption of a large saturating external source of virus to the plasma is required in this model for the simulation of data.

The models of this paper also assume that the viral clearance rate depends on the CD4+ T-cell level, whereas other models (1-3) assume that this rate is constant. This last assumption is required in our models to obtain the dynamics of disease progression. This assumption is reasonable in understanding how the virus population can increase steeply in the plasma as the CD4+ T-cell population in the plasma collapses. If the viral clearance rate in the plasma is independent of CD4+ T-cell levels, the steep increase of plasma virus (as much as 100-fold) at disease end would have to result from increased production. But the CD4+ T-cell population in the plasma collapses to near 0 so that this population cannot account for the high viral increase. In the models here, this steep increase of plasma virus results from the collapse of the immune response (which means that the plasma viral clearance rate should depend on CD4+ T-cell levels) and from a continuing influx of virus from the saturated external lymph source.

We provide a list of parameter values for the models with and without treatment (Table).

Table. Parameter values for the models

Parameters and Constants			Values
μ_T	=	mortality rate of uninfected CD4+ T cells	0.005/day
μ_{Ti}	=	mortality rate of infected CD4 + T cells	0.25/day
k_s	=	rate CD4+ T cells are infected by sensitive virus	0.0005 mm^3/day
k_r	=	rate CD4+ T cells are infected by resistant virus	0.0005 mm^3/day
k_v	=	rate of virus loss due to the immune response	0.0062 mm^3/day
p_1	=	production rate of uninfected CD4+ T cells	0.025/day
p_2	=	production rate of infected CD4+ T cells	0.25/day
p_3	=	production rate of virus in the blood	0.8/day
G_s	=	external lymphoid sensitive virus source constant	41.2/mm^3 day
G_r	=	external lymphoid resistant virus source constant	specified in text
V_0	=	threshold value for remission	specified in figure legends
q	=	proportion of drug-resistant virus produced from wild-type virus	specified in figure legends
C	=	half saturation constant of uninfected CD4+ T cells	47.0/mm^3
Ci	=	half saturation constant of infected CD4+ T cells	47.0/mm^3
B	=	half saturation constant of external virus input	2.0/mm^3
B_s	=	half saturation constant of CD4+ T-cell source	13.8/mm^3
S_1	=	source of CD4+ T cells in absence of the disease	4.0/mm^3 day
S_2	=	reduction constant of CD4+ T-cell source	2.8/mm^3 day
c_1	=	treatment parameter for suppression of the rate of CD4+ T-cell infection by virus	specified in figure legends
c_2	=	treatment parameter for suppression of the rate of virus contributed by the external lymphoid compartment	specified in figure legends
c_3	=	treatment parameter for maximal suppression of virus contributed by the external lymphoid compartment	specified in figure legends
η_1	=	treatment function for inhibition of the rate at which virus infects uninfected CD4+ T cells	specified in text
η_2	=	treatment function for inhibition of the rate of virus influx from the external lymphoid system virus	specified in text

Acknowledgment

This work was supported under grant numbers DMS 9596073 and DMS 9631580 of the National Science Foundation.

Dr. Kirschner's research is in computational microbial pathogenesis in the Microbiology and Immunology Department, University of Michigan Medical School. Her work focuses on understanding the mechanisms for disease progression for such pathogens as HIV and *Helicobacter pylori*. She also studies the role of chemotherapy in disease dynamics as well as treatment strategies for these infections. Her recent work explores the role of the thymus in pediatric HIV infection.

Dr. Webb's research is in mathematical models of population dynamics; it includes theoretical investigations of differential equations that arise in models of biomathematics as well as computational investigations of specific models of biological populations, including models of the HIV-infected immune system, tumor growth, the spread of epidemics, and the blood production system.

References

1. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 1995;373:123-6.
2. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 1995,373:117-22.
3. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho D. HIV-1 Dynamics in vivo: clearance rate, infected cell lifespan, and viral generation time. Science 1996;271:1582-6.
4. Piatak M, Saag MS, Yang LC, Clark SJ, Kappes JC, Luk KC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science 1993;259:1749-54.
5. Richman DD. HIV therapeutics. Science 1996;272:1886-7.
6. Coffin JM. HIV population dynamics in vivo: implications for genetic variation, pathogenesis and therapy. Science, 1995;267:483-9.
7. Stephenson J. New anti-HIV drugs and treatment strategies buoy AIDS researchers. JAMA 1996;275:579-80.
8. Carpenter CJ, Fischl MA, Hammer SM, Hirsch MS, Jacobsen DM, Katzenstein DA, et al. Antiretroviral therapy for HIV infection. JAMA 1996;276:146-54.
9. Collier AC, Coombs RW, Schoenfeld DA, Bassett RL, Timpone J, Baruch A, et al. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. N Engl J Med 1996;334:1011-7.
10. Cohen J. Shooting for the moon with drugs. Science 1996;273:302.
11. Pennisi E, Cohen J. Eradicating HIV from a patient: not just a dream? Science 1996;272:1884.
12. Grody WW, Fligiel S, Naeim F. Thymus involution in the acquired immunodeficiency syndrome. Am J Clin Pathol 1985;84:85-95.
13. Philips AN, Sabin CA, Mocroft A, Janossy G. Antiviral therapy. Nature 1995;375:195.
14. Embretson J, Zupancic M, Ribas JL, Burke RA, Racz P, Tenner-Racz K, Haase AT. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 1993;362:359-62.
15. Ho D. Viral counts in HIV infection. Science 1996;272:1124-5.
16. Mellors JW, Rinaldo CR, Gupta P, White RM, Todd JA, Kingsely LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996;272:1167-70.
17. Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, et al. HIV infection is active and progressive in lymphoid during the clinically latent stage of disease. Nature 1996;362:355-8.
18. Lafeuillade A, Poggi C, Profizi N, Tamalet C, Costes O. Human immunodeficiency virus type I kinetics in lymph nodes compared with plasma. J Infect Dis 1996;174:404-7.
19. McLean A, Nowak M. Competition between AZT sensitive and AZT resistant strains of HIV. AIDS 1992;6:71-9.
20. Fischl MA, Richmann DD, Hansen N, Collier AC, Carey JT, Para MF, et al. The safety and efficiency of AZT in the treatment of subjects with mildly symptomatic HIV type 1. Ann Intern Med 1990;112:727-37.
21. Graham NMH, Zeger SL, Park LP, Vermund SH, Detels R, Rinaldo CR, Phair JP. The effects on survival of early treatment of HIV infection. N Engl J Med 1992;326:1037-42.
22. Hamilton JD, Hartigan PM, Simberkoff MS, Day PL, Diamond GR, Dickinson GM, et al. A controlled trial of early vs late treatment with AZT in symptomatic HIV infection. N Engl J Med 1992;326:437-43.
23. Montaner JSG, Singer J, Schechter MT. Clinical correlates of in vitro HIV-1 resistance to zidovudine. Results of the Multicentre Canadian AZT trial. AIDS 1993;7:189-95.
24. Kirschner DE, Webb GF. A model for treatment strategy in the chemotherapy of AIDS. Bull Math Biol 1996;58:367-91.
25. Trough DF, Sprent J. Lifespan of lymphocytes. Immunol Res 1995;14:1-14.
26. Perelson AS, Kirschner DE, DeBoer R. The dynamics of HIV infection of CD4+ T cells. Math Biosci 1993,114:81-125.
27. Kirschner DE, Webb GF. Effects of drug resistance on monotherapy treatment of HIV infection. Bull Math Biol 1997.
28. Kirschner DE, Webb GF. A mathematical model of combined drug therapy of HIV infection. Journal of Theoretical Medicine 1997. In press.

Emerging Foodborne Diseases

S.F. Altekruse, M.L. Cohen, and D.L. Swerdlow
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

The epidemiology of foodborne diseases is rapidly changing. Recently described pathogens, such as *Escherichia coli* O157:H7 and the epidemic strain of *Salmonella* serotype Typhimurium Definitive Type 104 (which is resistant to at least five antimicrobial drugs), have become important public health problems. Well-recognized pathogens, such as *Salmonella* serotype Enteritidis, have increased in prevalence or become associated with new vehicles. Emergence in foodborne diseases is driven by the same forces as emergence in other infectious diseases: changes in demographic characteristics, human behavior, industry, and technology; the shift toward a global economy; microbial adaptation; and the breakdown in the public health infrastructure. Addressing emerging foodborne diseases will require more sensitive and rapid surveillance, enhanced methods of laboratory identification and subtyping, and effective prevention and control.

Foodborne diseases have a major public health impact (Table 1; 1). In the United States, each year foodborne illnesses affect 6 to 80 million persons, cause 9,000 deaths, and cost an estimated 5 billion U.S. dollars (2). The epidemiology of foodborne diseases is rapidly changing as newly recognized pathogens emerge and well-recognized pathogens increase in prevalence or become associated with new food vehicles (Table 2). In addition to acute gastroenteritis, many emerging foodborne diseases may cause chronic sequelae or disability. Listeriosis, for example, can cause miscarriages (3) or result in meningitis in patients with chronic diseases (3). Toxoplasmosis is an important cause of congenital malformation (4), and *Escherichia coli* O157:H7 infection is a leading cause of hemolytic uremic syndrome, the most common cause of acute kidney failure in children in the United States (5). Salmonellosis can cause invasive disease (6) or reactive arthritis (7), and campylobacteriosis can lead to Guillain-Barré syndrome, one of the most common causes of flaccid paralysis in the United States in the last 50 years (8).

The factors contributing to the emergence of foodborne diseases are changes in human demographics and behavior, technology and industry, and international travel and commerce; microbial adaptation; economic development and land use; and the breakdown of public health measures (9). We describe selected foodborne pathogens, factors influencing their emergence, and possible controls.

Table 1. Estimated number of illnesses and deaths per year caused by infection with selected foodborne bacterial pathogens, United States (1-2)

Pathogen	Cases (10^3)	Deaths (10^3)	Foods
Campylobacter jejuni	4,000	0.2-1	Poultry, raw milk, untreatedwater
Salmonella (nontyphoid)	2,000	0.5-2	Eggs, poultry, meat, fresh produce, other raw foods
Escherichia coli O157:H7	725	0.1-0.2	Ground beef, raw milk, lettuce, untreated water, unpasteurized cider/apple juice
Listeria monocytogenes	1.5	0.25-0.5	ready-to-eat foods (e.g. soft cheese, deli foods, pâté)
Vibrio species	10	0.05-0.1	Seafood (e.g. molluscan, crustacean shellfish) raw, undercooked, cross-contaminated.

Address for correspondence: Sean F. Altekruse, Mail Stop A-38, Centers for Disease Control and Prevention, Atlanta, GA, USA 30333; fax: 404-639-2212; e-mail: sba8@ciddbd1.em.cdc.gov.

Table 2. Selected outbreaks in the United States 1988–1997, associated with emerging foodborne pathogens and factors for the emergence of these pathogens

Pathogen/outbreak	Location(s)	Year	Factors in emergence	Reference
Hepatitis A Frozen strawberries	MI	1997	international travel and commerce technology and industry	28
Salmonella Typhimurium DT 104 Farm visit	NE	1996	microbial adaptation	48
Cyclospora cayetanensis Guatemalan raspberries	Multistate, Canada	1996	international travel and commerce	25
Salmonella Enteritidis PT 4 Egg-containing foods	CA	1995	international travel and commerce technology and industry	44
Salmonella Enteritidis Mass-distributed ice cream	Multistate	1994	technology and industry	34
Norwalk-like virus Gulf Coast oysters	LA	1994	economic development and land use	53
Escherichia coli O157:H7 Fast-food chain hamburgers	Multistate	1993	technology and industry breakdown of public health measures	54
Escherichia coli O157:H7 Raw apple cider	MA	1991	human demographics and behavior technology and industry	15
Vibrio cholerae O1, El Tor Thai coconut milk	MD	1991	international travel and commerce human demographics and behavior	39
Trichinella spiralis Undercooked pork	IA	1990	international travel and commerce human demographics and behavior	40
Salmonella Chester Sliced cantaloupe	Multistate	1989	international travel and commerce human demographics and behavior	22
Yersinia enterocolitica Pork chitterlings	GA	1988	human demographics and behavior	41

Selected Foodborne Pathogens of Public Health Importance

Salmonella Serotype Enteritidis

Nontyphoidal salmonellosis is one of the most commonly reported infections in the United States. The doubling of salmonellosis incidence in the last two decades has accompanied modern food industries' centralized production and large-scale distribution. The most prevalent serotypes, *Salmonella* serotype Enteritidis (SE), *Salmonella* Typhimurium, and *Salmonella* Heidelberg, account for most human salmonellosis in the United States.

Some serotypes of *Salmonella*, such as *S.* Enteritidis, have specific animal reservoirs and are primarily transmitted by specific foods (10). Reflecting a worldwide trend, in the United States, the proportion of *Salmonella* isolates that were SE increased from 6% in 1980 to 25% in 1995 (Figure 1) (CDC, unpub. data). Between 1985 and 1991 in the United States, grade A shell eggs were implicated in 82% of SE outbreaks with known vehicles (10).

SE's ability to cause ovarian infections in egg-laying hens, thus contaminating the contents of intact shell eggs, has been important in the transmission of SE among humans and hens (11).

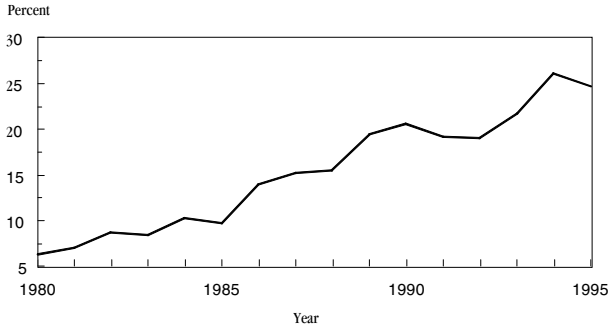


Figure 1. *Salmonella* serotype Enteritidis as a percentage of all *Salmonella* isolates reported in the United States, 1980–1995. Source: Centers for Disease Control and Prevention

SE can be transmitted vertically from breeding flocks to egg-laying hens, which in turn produce contaminated eggs (10,11). Once the organism is present in a flock, the infection is difficult to eliminate because transmission is sustained by environmental sources including rodents and manure.

Campylobacter jejuni

Campylobacter jejuni, an emerging foodborne pathogen not recognized as a cause of human illness until the late 1970s, is now considered the leading cause of foodborne bacterial infection (12). An estimated four million *C. jejuni* infections occur each year in the United States; most sporadic infections are associated with improper preparation or consumption of mishandled poultry products (12). Incidence of campylobacteriosis is particularly high among young men. The high incidence of disease in this group may reflect poor food preparation skills (12). Most *C. jejuni* outbreaks, which are far less common than sporadic illnesses, are associated with consumption of raw milk or unchlorinated water (12).

The Guillain-Barré syndrome, an acute paralytic illness that may leave chronic deficits, may follow *Campylobacter* infections (8). In a multicenter study of 118 patients with Guillain-Barré syndrome in the United States, 36% had serologic evidence of *C. jejuni* infection in the weeks before neurologic symptoms developed(8).

E. coli O157:H7

E. coli O157:H7 was first recognized as a human pathogen in 1982 when two outbreaks in the United States were associated with consumption of undercooked hamburgers from a fast-food restaurant chain (13). The pathogen has since emerged as a major cause of bloody and nonbloody diarrhea, causing as many as 20,000 cases and 250 deaths per year in the United States (2,5). Outbreaks have been reported in Canada, Japan, Africa, the United Kingdom, and elsewhere. In addition to causing bloody diarrhea, *E. coli* O157:H7 infection is the most common cause of the hemolytic uremic syndrome, the leading cause of acute kidney failure in children in the United States. The syndrome is associated with long-term complications; 3% to 5% of patients with hemolytic uremic syndrome die, and approximately 12% have sequelae including end-stage renal disease, hypertension, and neurologic injury (5). Consumption of ground beef (13), lettuce (14), raw cider (15), raw milk, and untreated water have been implicated in outbreaks, and person-to-person transmission is well documented (5).

Vibrio vulnificus

In the late 1970s, *Vibrio vulnificus* was recognized to cause an usually severe syndrome of foodborne *V. vulnificus* infection called primary septicemia. *V. vulnificus* primary septicemia generally affects people with underlying disease, particularly liver disease. Patients become ill within 7 days after eating raw molluscan shellfish. Tracebacks implicate shellfish harvested from warm water areas. The symptoms may include shock and bullous skin lesions and may quickly progress to death. Most reported shellfish-associated *V. vulnificus* infections are fatal (16).

Listeria monocytogenes

Since the early 1980s, foodborne transmission has been recognized as a major source of human listeriosis (3). Listeriosis can cause stillbirths, miscarriages, meningitis, or sepsis in immunocompromised hosts. Case-fatality rates as high as 40% have been reported during outbreaks (3). Outbreaks have been associated with ready-to-eat foods, including cole slaw, milk probably contaminated after pasteurization, pâté, pork tongue in jelly, and soft cheese made with inadequately pasteurized milk (3). The U.S. Department of Agriculture and U.S. Food and Drug Administration established zero tolerance policies for *L. monocytogenes* in foods in 1989. From 1989 to 1993, the food industry launched efforts to reduce *Listeria* contamination in processed foods, and dietary recommendations were established and publicized for persons at increased risk for invasive listeriosis. During this 4-year interval, the incidence of listeriosis declined by 40% in nine surveillance areas across the United States (17).

Factors Contributing to the Emergence of Foodborne Diseases

Human Demographics

Because of demographic changes in industrialized nations, the proportion of the population with heightened susceptibility to severe foodborne infections has increased. In the United States, a growing segment of the population is immunocompromised as a consequence of infection with human immunodeficiency virus (HIV), advancing

age, or underlying chronic disease. Reported rates of salmonellosis, campylobacteriosis, and listeriosis were higher among HIV-infected persons than among those not infected with HIV (6). *Salmonella* (and possibly *Campylobacter*) infections are more likely to be severe, recurrent, or persistent in this population (6). Furthermore, extraintestinal disease caused by *Salmonella* and *L. monocytogenes* infection is more likely to be reported in HIV-infected persons than in the general population (6).

During the 20th century, the median age of the U.S. population steadily increased (18), a trend that is accelerating. (Figure 2). The elderly are at increased susceptibility to foodborne infections. In a series of seven SE outbreaks in nursing homes, for example, 10% of residents who became ill were hospitalized, and 7% died (19). In the general population, SE hospitalizations and death rates are much lower (10).

Advances in medical technology (e.g., organ transplantation and cancer therapy) have extended the life expectancy of persons with chronic diseases, thus increasing the proportion of the population with heightened susceptibility to severe foodborne illness. In the 1970s, for example, the 5-year survival rate for Hodgkin lymphoma was approximately 50%; by 1985, the 5-year survival rate approached 80%. The survival rate for all cancers combined also increased (20).

Human Behavior

Changes in food consumption have brought to light unrecognized microbial foodborne hazards. Fresh fruit and vegetable consumption, for example, has increased nearly 50% from 1970 to 1994 (21). Fresh produce is susceptible to contamination during growth, harvest, and distribution. The surface of plants and fruits may be contaminated by human or animal feces. Pathogens on the surface of produce (e.g., melons) can contaminate the inner surface during cutting and multiply if the fruit is held at room temperature (22). In the United States from 1990 to 1997, increased consumption of fresh produce may have contributed to a series of foodborne outbreaks associated with foods such as sliced cantaloupe (22), green onions (23), unpasteurized cider (15), fresh squeezed orange juice (24), lettuce (14), raspberries (25), alfalfa sprouts (26), sliced tomatoes (27), and frozen strawberries (28).

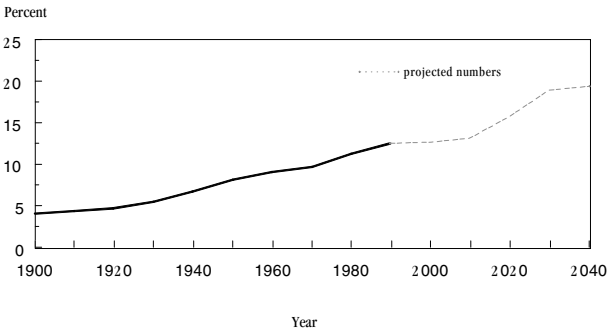


Figure 2. Percentage of U.S. population over 65 years of age, 1990–2040 (projected). Source: U.S. Bureau of Census

The percentage of spending on food eaten away from home has increased during recent decades (29). Fast-food restaurants and salad bars were rare 50 years ago but are primary sites for food consumption in today's fast-paced society (29). Outbreaks outside the home account for almost 80% of reported outbreaks in the United States in the 1990s (30). This figure may reflect the fact that these outbreak settings are most likely to be recognized and, therefore, reported to health officials; however, such food venues may also contribute to foodborne disease. Through practices such as the pooling of eggs, holding of hazardous foods at temperatures that permit amplification of low-dose pathogens, incomplete cooking of foods such as hamburgers, and cross-contamination of cooked foods.

Behavioral changes leading to foodborne infections are further complicated by decreased opportunities for food safety instruction both in school and at home. Health educators in secondary schools emphasize prevention of other important health concerns (e.g., HIV infection, obesity) over consumer safety issues including food safety education (31). In addition, because of two-income families and increased eating away from home, fewer opportunities may exist to pass food safety information from parent to child (29).

Changes in Industry and Technology

The trend toward greater geographic distribution of products from large centralized food processors carries a risk for dispersed outbreaks. When mass-distributed food products are intermittently contaminated or contaminated at a low level, illnesses may appear sporadic rather than part of an outbreak (32).

Industry consolidation and mass distribution of foods may lead to large outbreaks of foodborne disease. In 1985, an outbreak of salmonellosis

associated with contaminated milk from a large midwestern dairy was estimated to have resulted in approximately 250,000 illnesses (33). A nationwide outbreak of *Salmonella* serotype Enteritidis of similar magnitude occurred in 1994 when ice cream premix was transported in tanker trucks that had not been thoroughly sanitized after transporting raw liquid egg (34).

The trend toward larger markets and consolidation of industry has exacerbated the SE problem in another way. Changes in egg production have adversely affected infection control in poultry flocks (35). In 1945, a typical hen house contained 500 birds. By 1995, many houses contained 100,000 hens, and multiple houses were often linked by common machinery (35), resulting in large flocks with common risk profiles. Large-scale distribution of shell eggs from infected flocks has caused outbreaks in which contaminated eggs were distributed in many states over a period of months (10).

Changes in Travel and Commerce

International travel has increased dramatically during the 20th century. Five million international tourist arrivals were reported worldwide in 1950, and the number is expected to reach 937 million by 2010 (Figure 3; 36). Travelers may become infected with foodborne pathogens uncommon in their nation of residence, thus complicating diagnosis and treatment when their symptoms begin after they return home. In 1992, for example, an outbreak of cholera caused 75 illnesses in international airline passengers; 10 persons were hospitalized, and one died (37). Pathogens may also be carried home to infect nontravelers. (38).

As the diversity of foods in the marketplace has increased, illnesses have been associated with internationally distributed foods. In 1992, an outbreak of epidemic cholera in Thai immigrants living in Maryland was caused by coconut milk imported from Thailand (39). In 1996, 1,465 cases of infection with *Cyclospora cayetanensis* were reported by 20 states, the District of Columbia, and two Canadian provinces. The investigation implicated raspberries from Guatemala (25).

In the mid-1990s, half to one and one-half million immigrants were admitted to the United States each year. Some reports of foodborne illnesses involve transmission by foods consumed primarily by immigrant groups. Outbreaks of

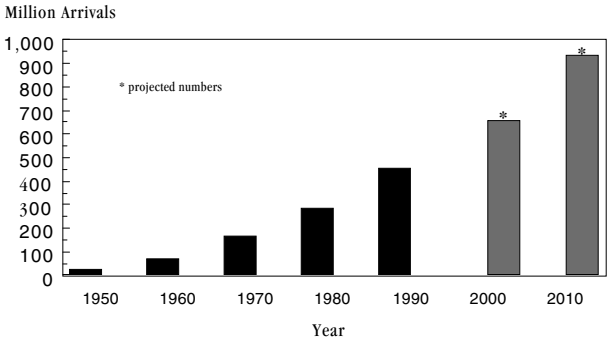


Figure 3. Global international tourist arrivals, in millions per year. 1950–2010 (projected). Source: World Tourism Organization

trichinosis have become relatively rare in the United States because cooking pork thoroughly has become a widespread cultural practice. An exception occurred in 1990, when Laotian immigrants in Iowa prepared and ate undercooked pork, a traditional food, in celebration of a wedding (40). Other reports involve foods consumed by ethnic populations. *Yersinia enterocolitica* outbreaks are also rare, but several have occurred in inner-city African-American communities and were associated with preparation and consumption of pork intestines (41). The epidemiology of human brucellosis in California has shifted from an occupational disease of animal husbandry to a foodborne disease most frequently affecting Hispanics who often while abroad consume raw milk and cheeses made with raw milk (42).

Microbial Adaptation

Environmental Conditions

Natural selection is a key process in the emergence of pathogens (11). Microbes adapt to have an advantage in unfavorable environments (e.g., heat and acidity) (43). SE phage type (PT) 4 may have developed traits that enable it to rapidly replace closely related SE phage types in egg-laying poultry environments (43). During the 1980s, for example, SE PT 4 became the predominant phage type in humans and poultry in Europe and caused a marked increase in human illnesses. SE PT 4 was rare in the United States before 1994 when SE PT 4 emerged in southern California, resulting in a fivefold increase in reported human SE infection (44). In 1995, a similar increase in SE PT 4 infection was reported in Utah.

Antimicrobial Resistance

The therapeutic use of an antimicrobial agent, in human or animal populations, creates a selective pressure that favors survival of bacterial strains resistant to the agent. Antimicrobial-resistant strains of *Salmonella* have become increasingly prominent (45). In the United States, the percentage of antimicrobial resistant *Salmonella* infections increased from 17% of isolates in the late 1970s to 31% in the late 1980s (45). Compared with patients with susceptible infections, patients with antimicrobial-resistant infections are more likely to require hospitalization and to be hospitalized for longer periods (45).

During the 1990s, *Salmonella* serotype Typhimurium Definitive Type 104 (DT 104) emerged in the United Kingdom. By 1995, DT 104 was the second most common cause of human salmonellosis in England and Wales; more than 3,800 isolates were reported from humans in that year alone (46). Ninety percent of all DT 104 isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline (R-type ACSSuT). Strains of *S*. Typhimurium DT 104 with resistance to trimethoprim and ciprofloxacin have also emerged in the United Kingdom. Surveillance for DT 104 infections in the United Kingdom indicated high hospitalization and mortality rates with this organism compared with infections caused by other *Salmonella* serotypes. In one study more than 10% of patients with multidrug-resistant DT 104 infection required hospitalization, and more than 3% died (46). In the United Kingdom, illness has been associated with contact with farm animals and with consumption of foods, including beef, pork sausages, and chicken. The organism has been isolated primarily from cattle but also from poultry, sheep, and pigs (46).

S. Typhimurium DT 104 is rapidly emerging in the United States. A study of *S*. Typhimurium isolates from the Pacific Northwest indicated that 43% of human isolates obtained in 1994 had R-type ACSSuT compared with 4% in 1989 (47). Among cattle isolates of *S*. Typhimurium from the Pacific Northwest obtained before 1986, none had this R-type, compared with 13% of isolates obtained between 1986 and 1991, and 64% of isolates obtained between 1992 and 1995 (47). When 25 isolates from either human or cattle sources in the Pacific Northwest with R-type ACSSuT were phage-typed, all were *S*. Typhimurium DT 104 (47). The characteristic resistance pattern (R-type ACSSUT) was present in 32% of human *S*. Typhimurium isolates tested in 1996. The first confirmed outbreak of *S*. Typhimurium DT 104 in the United States occurred in Nebraska in 1996 (48).

Another example of an antimicrobial-resistant foodborne pathogen is fluoroquinolone-resistant *C. jejuni*, which has increased in Europe since the early 1990s (49). The emergence of human infections has been temporally associated with the approval of fluoroquinolones for veterinary use in Europe.

Economic Development and Land Use

In the United States, food animals generate over 1.6 billion tons of manure per year (50). On large-scale production facilities, manure disposal is a growing problem. Without disposal, manure may serve as a reservoir for *Salmonella*, *C. jejuni*, and other farm pathogens.

The shift from a cold season oyster harvest in the Gulf of Mexico to a year-round harvest (51) is a change in resource use associated with the emergence of *V. vulnificus*. *V. vulnificus* primary septicemia has a summer seasonality, and most tracebacks implicate raw oysters from the Gulf of Mexico (52). Although the annual oyster harvest from the U.S. Gulf of Mexico has not changed since the 1930s, the percentage of oysters harvested during summer months increased from 8% of the annual harvest in 1970 to 30% in 1994 (51). The disposal of feces in oyster beds by oyster harvesters with gastroenteritis has been implicated in shellfish-associated Norwalk-like virus outbreaks, including one in Louisiana in 1994 (53).

Breakdown of the Public Health Infrastructure

Many public health agencies operate with extremely limited resources. The consequent breakdown in public health infrastructure increases the potential for underreporting of foodborne infections (54). In the mid-1990s, for example, 12 states had no personnel dedicated to foodborne disease surveillance (54), largely because of budget restrictions at the state and local levels. When the infrastructure for infectious diseases surveillance is compromised, recognition of outbreaks is jeopardized (9,54).

Prevention and Control

The prevention of foodborne disease depends on careful food production, handling of raw products, and preparation of finished foods.

Hazards can be introduced at any point from farm to table. Technologies are available to prevent many foodborne illnesses. Just as the 20th century's revolution in food sanitation and hygiene (including refrigeration, chlorination of drinking water, pasteurization of milk, and shellfish monitoring) was a consequence of applied technologies, industrial engineering can hold the key to food safety in the 21st century. Among technologies that merit evaluation are chlorination of drinking water sources for food animals (55); sanitary slaughter and processing of meat (56), poultry (56), and seafood (57); irradiation (58); and other microbial reduction steps for raw agricultural commodities.

When monitoring and control technologies are systematically applied to food production to prevent foodborne illnesses, the program is said to use a Hazard Analysis Critical Control Point (HACCP) process (56,57). Such programs require food industries to identify points in food production where contamination may occur and target resources toward processes that may reduce or eliminate foodborne hazards. In the 1990s, programs were implemented by meat (56), poultry (56), and seafood (57) industries and federal regulatory agencies. In these programs, industry takes the lead for the control of foodborne hazards, and regulatory agencies maintain oversight.

Preparers of meals are the last critical control point before foods reach the table. Interventions to promote safe food preparation practices are needed (59). Food preparers can reduce the risk of foodborne diseases with a few practical food-handling precautions. Thorough heating of potentially hazardous foods kills pathogens, and refrigeration prevents their multiplication. Cross-contamination of foods can be avoided by separating cooked and raw foods and preventing contamination of cooked foods by drippings from raw foods. Foodworkers should wash hands, cutting boards, and contaminated surfaces as warranted to prevent cross-contamination. Consumers can reduce the risk of foodborne infections by avoiding high-risk foods, such as runny eggs, hamburgers that are pink at the center, and raw shellfish.

Each link in the production, preparation, and delivery of food can be a hazard to health. While technologies designed to improve the safety of the food supply hold promise, changes in food processing, products, practices, and people will continue to facilitate the emergence of foodborne pathogens into the next century. Foodborne disease surveillance provides a basis for detecting disease and identifying points at which new strategies are needed to protect the food supply.

Dr. Altekruse is a veterinary epidemiologist with the U.S. Food and Drug Administration, assigned as a liaison officer to CDC's Foodborne and Diarrheal Diseases Branch. His research activities include surveillance for noncholera *Vibrio* infections.

References

1. Helmick CG, Griffin PM, Addiss DG, Tauxe RV, Juranek DD. Infectious diarrheas. In: Everhart JE, editor. Digestive diseases in the United States: epidemiology and impact. Bethesda (MD): National Institutes of Health, 1994; Publication No. 94-1447. p. 85-120.
2. Council for Agricultural Science and Technology. Foodborne pathogens: risks and consequences. Ames (IA): The Council; 1994. Task Force Report No. 122.
3. Schuchat A, Swaminathan B, Broome CV. Epidemiology of human listeriosis. Clin Microbiol Rev 1991;4:169-83.
4. Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, Eskild A, Eng J. Risk factors for *Toxoplasma gondii* infection in pregnancy. Am J Epidemiol 1996;144:405-12.
5. Boyce TG, Swerdlow DL, Griffin PM. *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. N Engl J Med 1995;333:364-8.
6. Altekruse SF, Hyman FH, Klontz KC, Timbo BT, Tollefson LK. Foodborne bacterial infections in individuals with the human immunodeficiency virus. South Med J 1994;87:169-73.
7. Swerdlow DL, Lee LA, Tauxe RV, Bean NH, Jarvis JQ. Reactive arthropathy following a multistate outbreak of *Salmonella typhimurium* infections [abstract]. Proceedings of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1990 Oct 21-24; Atlanta, GA. Washington (DC): American Society for Microbiology; 1990. Abstract No. 916.
8. Mishu B, Ilyas AA, Koski CL, Vriesendorp F, Cook SD, Mithen FA, et al. Serologic evidence of previous *Campylobacter jejuni* infection in patients with the Guillain-Barré syndrome. Ann Intern Med 1993;118:947-53.
9. Institute of Medicine. Emerging infections: microbial threats to health in the United States. Washington (DC): National Academy Press; 1992.
10. Mishu B, Koehler J, Lee LA, Rodrigue D, Hickman-Brenner F, Blake P, et al. Outbreaks of *Salmonella enteritidis* infections in the United States, 1985-1991. J Infect Dis 1994;169:547-52.
11. Snoeyenbos GH, Smyzer CF, Van Roekel H. *Salmonella* infections of the ovary and peritoneum of chickens. Avian Dis 1969;13:668-70.

12. Tauxe RV. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin I, Tompkins S, Blaser M, editors. *Campylobacter jejuni*: Current Status and Future Trends. Washington (DC): American Society for Microbiology; 1992.
13. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JB, Davis BR, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med 1983;308:681-5.
14. Ackers M, Mahon B, Leahy E, Damrow T, Hutwagner L, Barrett T, et al. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption, Western Montana [abstract]. Proceedings of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1996 Sep 15-18; New Orleans, LA. Washington (DC): American Society for Microbiology; 1996:257. Abstract No. K43.
15. Besser RE, Lett SM, Weber T, Doyle MP, Barrett TJ, Wells JG, et al. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA 1993;269:2217-20.
16. Blake PA, Merson MH, Weaver RE, Hollis DG, Heublin PC. Disease caused by a marine *Vibrio*. Clinical characteristics and epidemiology. N Engl J Med 1979;300:1-5.
17. Tappero JW, Schuchat A, Deaver KA, Mascola L, Wenger JD. Reduction in the incidence of human listeriosis in the United States. Effectiveness of prevention efforts? JAMA 1995;273:1118-22.
18. Bureau of the Census, U.S. Department of Commerce. 1990 Census of population. General population characteristics. Washington (DC): U.S. Government Printing Office; 1990; CP-1-1 20240.
19. Levine WC, Smart JF, Archer DL, Bean NH, Tauxe RV. Foodborne disease outbreaks in nursing homes, 1975 through 1987. JAMA 1991;266:2105-9.
20. Miller BA, Ries LAG, Hankey BF, Kosary CL, Harras A, Devesa SS, Edwards BK, editors. SEER Cancer Statistics Review 1973-1990. Bethesda (MD): National Cancer Institute; 1991. NIH Publication 93-789.
21. Bureau of the Census, U.S. Department of Commerce. Per capita utilization of selected commercially produced fresh fruits and vegetables: 1970 to 1994. Statistical Abstract of the United States, 116th Edition. Washington (DC): U.S. Government Printing Office; 1996. p. 148.
22. Reis AA, Zaza S, Langkop C, Tauxe RV, Blake PA. A multistate outbreak of *Salmonella chester* linked to imported cantaloupe [abstract]. Proceedings of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1990 Oct 21-24; Atlanta, GA. Washington (DC): American Society for Microbiology; 1990:38.
23. Cook KA, Boyce T, Langkop C, Kuo K, Swartz M, Ewert D, et al. Scallions and shigellosis: a multistate outbreak traced to imported green onions [abstract]. Proceedings of the 44th Annual Conference of the Epidemic Intelligence Service; 1995 March 27–31; Atlanta, GA. Atlanta (GA): Centers for Disease Control and Prevention; 1995:36.
24. Cook KA, Swerdlow D, Dobbs T, Wells J, Puhr N, Hlady G, et al. Fresh-squeezed *Salmonella*: an outbreak of *Salmonella hartford* associated with unpasteurized orange juice—Florida [abstract]. Proceedings of the 45th Annual Epidemic Intelligence Service Conference; 1990 Apr 22-26; Atlanta, GA. Atlanta (GA): Centers for Disease Control and Prevention; 1996:38.
25. Herwaldt BL, Ackers M-L, and the Cyclospora Working Group. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. N Engl J Med 1997;336:1548-56.
26. Mahon BE, Ponka A, Hall W, Komatsu K, Dietrich SE, Siitonen A, et al. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. J Infect Dis 1997;175:876-82.
27. Wood RC, Hedberg C, White K, MacDonald K, Osterholm M. A multi-state outbreak of *Salmonella javiana* infections associated with raw tomatoes [abstract]. Proceedings of the 40th Annual Conference of the Epidemic Intelligence Service; 1991 April 8–12; Atlanta, GA. Atlanta (GA): Centers for Disease Control and Prevention; 1991:69.
28. Hepatitis A associated with consumption of frozen strawberries—Michigan, March 1997. MMWR Morb Mortal Wkly Rep 1997;46:288,295.
29. Manchester A, Clauson A. 1994 spending for food away from home outpaces food at home. Food Review 1995;18:12-5.
30. Bean NH, Goulding JS, Lao C, Angulo FJ. Surveillance for foodborne disease outbreaks—United States, 1988-1991. CDC Surveillance Summaries, October 25, 1996. MMWR Morb Mortal Wkly Rep 1996;45(SS-5):1-66.
31. Collins JL, Small ML, Kann L, Pateman BC, Gold RS, Kolbe LJ. School health education. J Sch Health 1995;65:302-11.
32. Killalea D, Ward LR, Roberts D, de Louvois J, Sufi F, Stuart JM, et al. An outbreak of *Salmonella agona* infection in England and the United States caused by contamination of a ready-to-eat savoury snack. BMJ 1996;313:1105-7.
33. Ryan CA, Nickels MK, Hargrett-Bean NT, Potter ME, Endo T, Mayer L, et al. Massive outbreak of antimicrobial-resistant salmonellosis traced to pasteurized milk. JAMA 1987;58:3269-74.
34. Hennessy TW, Hedberg CW, Slutsker L, White KE, Besser-Wiek JM, Moen ME, et al. A national outbreak of *Salmonella enteritidis* infections from ice cream. N Engl J Med 1996;334:1281-6.
35. Bell D. Forces that have helped shape the U.S. egg industry: the last 100 years. Poultry Tribune. 1995:30-43.
36. Paci E. Exploring new tourism marketing opportunities around the world. Proceedings of the Eleventh General Assembly of the World Tourism Organization; 1995 Oct 15; Cairo, Egypt. Cairo, Egypt: World Tourism Organization; 1995.
37. Eberhart-Phillips J, Besser RE, Tormey MP, Feikin D, Araneta MR, Wells J, et al. An outbreak of cholera from food served on an international aircraft. Epidemiol Infect 1996;116:9-13.
38. Finelli L, Swerdlow D, Mertz K, Ragazzoni H, Spitalny K. Outbreak of cholera associated with crab brought from an area with epidemic disease. J Infect Dis 1992;166:1433-6.
39. Taylor JL, Tuttle J, Pramukul T, O'Brien K, Barrett TJ, Jolbitado B, et al. An outbreak of cholera in Maryland associated with imported commercial coconut milk. J Infect Dis 1993;167:1330-5.
40. Stehr-Green JK, Schantz PM. Trichinosis in Southeast Asian refugees in the United States. Am J Public Health 1986;76:1238-9.

41. Lee LA, Gerber AR, Lownsay DR, Smith JD, Carter GP, Puhr ND, et al. *Yersinia enterocolitica* O:3 infections in infants and children associated with household preparation of chitterlings. N Engl J Med 1990;322:984-7.
42. Chomel BB, DeBess EE, Mangiamele DM, Reilly KF, Farver TB, Sun RK, et al. Changing trends in the epidemiology of human brucellosis in California from 1973 to 1992: a shift toward foodborne transmission. J Infect Dis 1994;170:1216-23.
43. Humphrey TJ, Slater E, McAlpine K, Rowbury RJ, Gilbert RJ. *Salmonella enteritidis* phage type 4 isolates more tolerant of heat, acid, or hydrogen peroxide also survive longer on surfaces. Appl Environ Microbiol 1995;61:3161-4.
44. Passaro DJ, Reporter R, Mascola L, Kilman L, Malcolm G, Rolka H, et al. Epidemic illness caused by *Salmonella enteritidis* infection in Los Angeles County: the predominance of phage type 4. West J Med 1996;165:126-30.
45. Lee LA, Puhr ND, Maloney EK, Bean NH, Tauxe RV. Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989-1990. J Infect Dis 1994;170:18-34.
46. Threlfall EJ, Hampton MD, Schofield SL, Ward LR, Frost JA, Rowe B. Epidemiological application of differentiating multiresistant *Salmonella typhimurium* DT 104 by plasmid profile. Commun Dis Rep CDR Rev 1996;6:R155-9.
47. Besser TE, Goldoft M, Gay CC. Emergence of *Salmonella typhimurium* DT 104 in humans and animals in the Pacific Northwest. Proceedings of the 51st Annual Northwestern International Conference on Diseases in Nature Communicable to Man; Seattle, WA; 1996 Aug 11–14. Hosted by Washington State Department of Health.
48. Multidrug-resistant *Salmonella* Serotype Typhimurium—U.S., 1996. MMWR Morb Mortal Wkly Rep 1997;46:308-10.
49. Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T, Mouton RP. Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J Antimicrob Chemother 1991;27:199-208.
50. Natural Resources Conservation Service, U.S. Department of Agriculture, Animal manure management. Washington (DC): U.S. Department of Agriculture; 1995; Issue Brief 7. p. 1-6.
51. National Marine Fisheries Service. Monthly oyster landings by region. Silver Spring (MD): U.S. Department of Commerce, National Oceanic and Atmospheric Administration; 1995.
52. Centers for Disease Control and Prevention. *Vibrio vulnificus* infections associated with eating raw oysters—Los Angeles. MMWR Morb Mortal Wkly Rep 1996;45:621-4.
53. Kohn MA, Farlet TA, Ando T, Curtis M, Wilson SA, Jin Q, Monroe SS, et al. An outbreak of Norwalk virus gastroenteritis associated with eating raw oysters. Implications for maintaining safe oyster beds. JAMA 1995;273:466-71.
54. Berkelman RL, Bryan RT, Osterholm MT, LeDuc JW, Hughes JM. Infectious disease surveillance: a crumbling foundation. Science 1994;264:368-70.
55. Kapperud G, Skjerve E, Hauge K, Lysaker A, Aalmen I, Ostroff SM, et al. Epidemiological investigation of risk factors for *Campylobacter* colonization in Norwegian broiler flocks. Epidemiol Infect 1993;111:45-55.
56. Pathogen Reduction; Hazardous Analysis and Critical Control Point (HACCP) Systems, 9 C.F.R. 304, 308, 310, 320, 327, 381, 416, 417 (1996).
57. Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products, 21 C.F.R. Pt. 123, 1240 (1995).
58. Steele JH, Engel RE. Radiation processing of foods. J Am Vet Med Assoc 1992;201:1522-9.
59. Altekruse SF, Street DA, Fein SB, Levy AS. Consumer knowledge of foodborne microbial hazards and food-handling practices. Journal of Food Protection 1996;59:287-94.

DDT, Global Strategies, and a Malaria Control Crisis in South America

Donald R. Roberts, Larry L. Laughlin, Paul Hsheih, and Llewellyn J. Legters
Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA

Malaria is reemerging in endemic-disease countries of South America. We examined the rate of real growth in annual parasite indexes (API) by adjusting APIs for all years to the annual blood examination rate of 1965 for each country. The standardized APIs calculated for Brazil, Peru, Guyana, and for 18 other malaria-endemic countries of the Americas presented a consistent pattern of low rates up through the late 1970s, followed by geometric growth in malaria incidence in subsequent years. True growth in malaria incidence corresponds temporally with changes in global strategies for malaria control. Underlying the concordance of these events is a causal link between decreased spraying of homes with DDT and increased malaria; two regression models defining this link showed statistically significant negative relationships between APIs and house-spray rates. Separate analyses of data from 1993 to 1995 showed that countries that have recently discontinued their spray programs are reporting large increases in malaria incidence. Ecuador, which has increased use of DDT since 1993, is the only country reporting a large reduction (61%) in malaria rates since 1993. DDT use for malaria control and application of the Global Malaria Control Strategy to the Americas should be subjects of urgent national and international debate. We discuss the recent actions to ban DDT, the health costs of such a ban, perspectives on DDT use in agriculture versus malaria control, and costs versus benefits of DDT and alternative insecticides.

Malaria is reemerging in most disease-endemic countries of South America (Figure 1). Even though disease incidence is increasing, the level of increase is undefined. More importantly, the reasons for the increase in malaria rates after decades of successful disease control have not been assessed. Herein we will show how rapidly malaria is increasing, examine the patterns of resurgent malaria in relationship to the Global Malaria Control Strategy (1), and test the hypothesis that increased malaria is due to decreased spraying of homes with DDT. Also, we will discuss recent actions to ban DDT, the health costs of such a ban, perspectives on DDT use in agriculture versus malaria control, and costs versus benefits of DDT and alternative insecticides.

Revised Estimates of Malaria Rates in South America

The Pan American Health Organization has been compiling and reporting malaria data since 1959. These data are used to compute the annual parasite index (API) and the annual blood examination rate (ABER). API is the number of positive slides per 1,000 population. For each country, API can be viewed as a measure of numbers of cases detected and numbers of cases treated. API is based on composite data derived from both active and passive case detection; e.g., 63% of all blood slides taken in the Americas during 1994[1] were from passive case detection (2).

API is commonly used to compare amounts of malaria within geographically or temporally distinct human populations. The formula for

Address for correspondence: Donald R. Roberts, DPMB, USUHS, 4301 Jones Bridge Rd., Bethesda, MD 20814; fax: 301-295-3860; e-mail: droberts@usuhsb.usuhs.mil.

[1] This percentage was obtained by adding the number of slides from passive case detection reports and from voluntary collaborators and then dividing the sum by the number of slides acquired through active case detection.

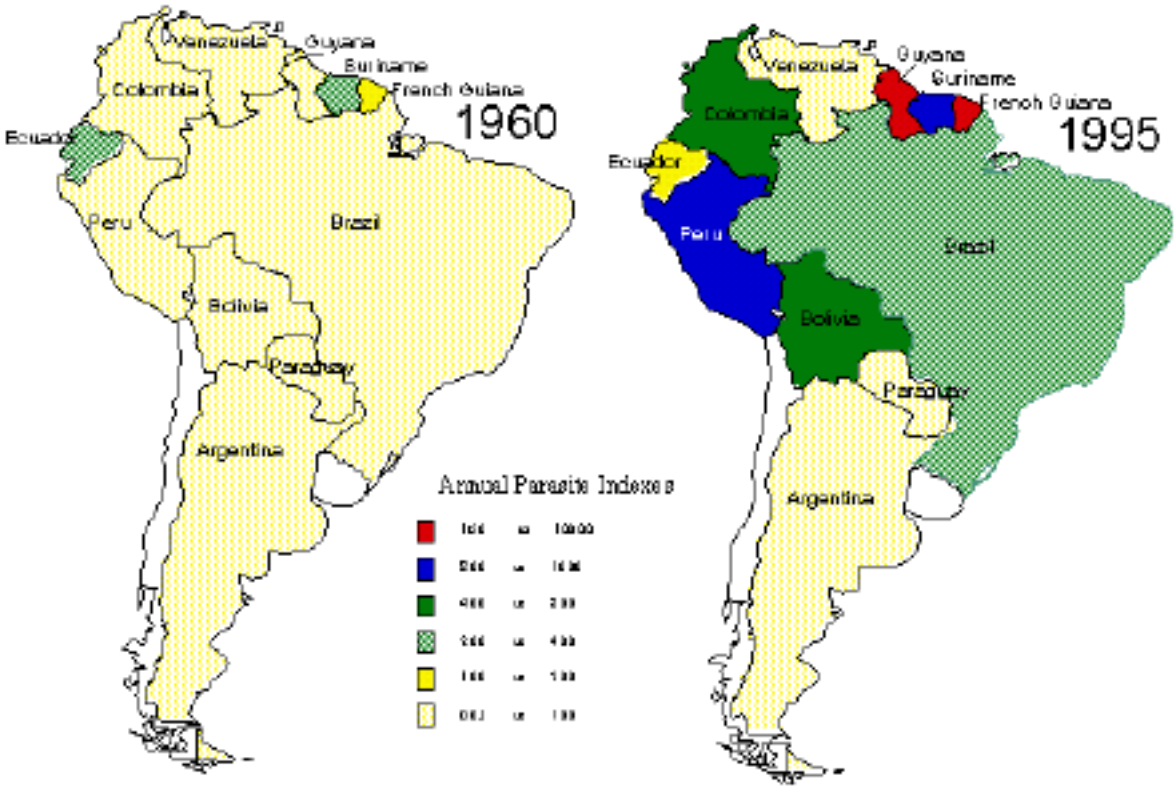


Figure 1. Distribution of malaria in South America (2-5). Color codes correspond to annual parasite indexes as reported by the Pan American Health Organization.

calculating API is

API for year x = 1,000 (number of positive slides/total population)

The number of positive slides (numerator of the API formula) for a given year is a function of the slide positivity rate (which indicates intensity of malaria within the environment) and the total number of slides examined. The total number of slides examined is used to calculate ABER, which indicates case detection effort. The formula for calculating ABER is

ABER for year x = 100 (number of slides examined/total population)

ABER represents the number of slides examined per 100 population.

The general pattern presented by conventional APIs for Brazil (2-4) is presented in Figure 2. Our analysis shows that population growth, combined with decreased numbers of slides examined, underestimates upward trends in malaria cases.

The population of Brazil has grown continuously (Figure 2). The number of blood slides examined each year increased from 1965 to

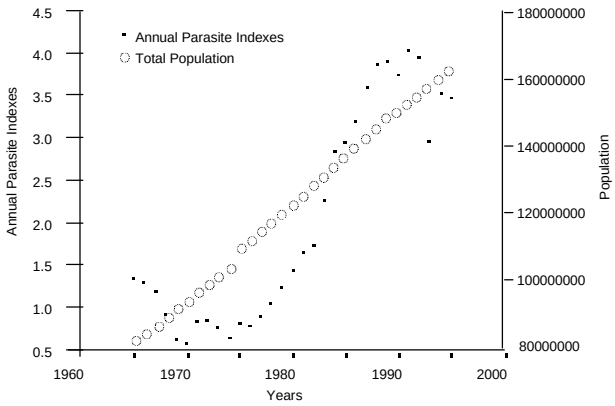


Figure 2. Annual parasite indexes and population growth, Brazil, 1965-1995 (2-5). A graphical representation of data compiled by the Pan American Health Organization.

1985, and then started an erratic decline (Figure 3). Slide positivity rates progressively increased after 1979 (Figure 3). Variable numbers of slides examined, combined with population growth, resulted in ABER values from 2.1 to 2.55 until the mid-1980s; after 1985, ABERs steadily declined (Figure 4).

Given the quantitative components of API, a comparison of indexes is meaningful only if numbers of slides examined relative to the population base are comparable across years. To illustrate, a population of 4,000,000 (with 200,000 slides examined and 60,000 malaria-positive slides) equates to a 30% slide-positivity rate and an API of 15. However, if 400,000 slides are examined, the API will be 30. This example shows how sensitive API is to the number of slides examined.

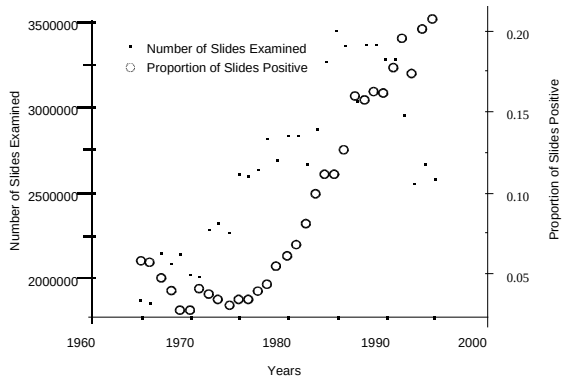


Figure 3. Slides examined and proportions positive for malaria, Brazil, 1965–1995 (2-5). Points correspond to data compiled by the Pan American Health Organization.

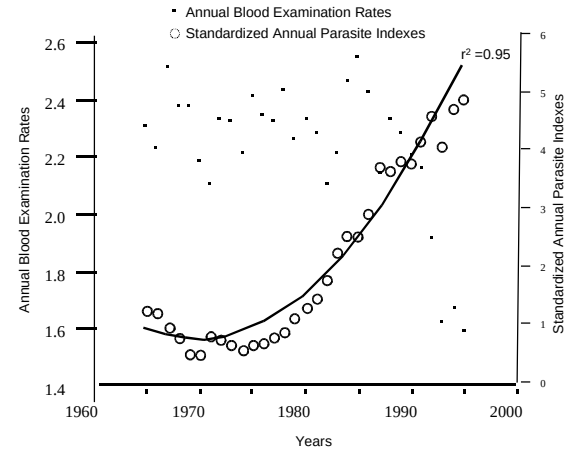


Figure 4. Annual blood examination rates and standardized annual parasite indexes, Brazil, 1965–1995 (2-5). The standardized APIs were adjusted to a common sample size across years (the annual blood examination rate of 1965). Original data for calculating the standardized APIs were obtained from Pan American Health Organization reports (2-5).

We recalculated APIs for Brazil by using the slide-positivity rate for each year and a standardized ABER of 2.31 (ABER for Brazil in 1965). The number of slides examined for each year was recalculated as follows:

Number of slides examined/year = (2.31/100)(total population)

Using this formula for each year, we multiplied the original proportion of positive slides for each year by the revised estimate of total number of slides examined. This calculation provided a uniform estimate of malaria-positive slides. We then divided the estimate of malaria-positive slides by the population of Brazil for each year in the series. These quotients, multiplied by 1,000, produced APIs that were standardized for sampling effort (ABER).

When the derived or standardized API is plotted (Figure 4), the pattern of increasing quantities of malaria is very different from Figure 2. The pattern in Figure 4 shows stable or small yearly increases in malaria rates from 1965 to the late 1970s. After 1978, APIs increased fivefold through 1995. Similar relationships were found with years versus standardized indexes for falciparum and vivax malarias (not shown). Standardized APIs were also developed for Peru and Guyana (2-4). Like Brazil, Peru and Guyana show geometric growth in numbers of malaria cases (Figure 5)(also for falciparum and vivax malarias [not shown]).

It might be argued that the appearance of increasing malaria is an artifact of increasing reliance on passive case detection. Such an argument is equally valid against conventional APIs since the sources of blood smears are the same for both the conventional and standardized indexes. We concede that increased use of passive case detection contributes to increasing slide-positivity rates; however, passive case detection is not sufficient to account for the magnitude or consistency of increases described in Figures 4

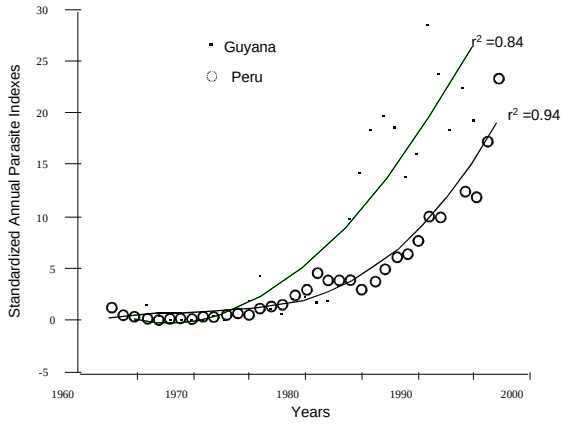


Figure 5. Standardized annual parasite indexes, Peru (1959–1995) and Guyana (1960–1995). The original data were derived from Pan American Health Organization reports (2-5). The APIs were adjusted to a common sample size across years (the annual blood examination rate of 1965).

and 5. Using Brazil as an example, more than 35% of slides were taken through passive case detection in 1972.[2] In 1991, 32.8% of all slides in Brazil were obtained through passive case detection (5). Clearly, the 17-year pattern of increasing malaria is not due to increased reliance on passive case detection.

The Global Malaria Control Strategy

The policies and strategies of the World Health Organization, the Pan American Health Organization, and national donor agencies contributed to the successful control of malaria from the late 1940s to the late 1970s (6-8). However, the policies and strategies of these organizations have changed (8). In 1979, the World Health Organization Expert Committee on Malaria (9) developed a new malaria control strategy with four tactical variants. Variants 1 and 2 included no organized vector control measures. Variant 3 included limited vector control, and only variant 4 included, for highly qualified countries, countrywide vector control. The goal for variant 4 was specified as eradication, not sustained malaria control. The new strategy was adopted by the 31st World Health Assembly as resolution WHA 31.45 (9). During this assembly, the Director-General of World Health Organization stressed the importance of including curative and preventive services, including control of infectious diseases (malaria control), in the framework of primary health care. In 1985, the 38th World Health Assembly adopted resolution WHA 38.24, which recommended that malaria control be developed as an integral part of the national primary health care system (8). In October 1992, the Ministerial Conference adopted the Global Malaria Control Strategy that had been developed at World Health Organization interregional meetings in 1991 and 1992 (1). The Global Malaria Control Strategy calls for deemphasis of vector control and emphasizes case detection and treatment. The first conclusion and recommendation of the report is that malaria control should be fully integrated into general health services and should reflect socioeconomic development objectives. The World Health Assembly resolutions and committee reports document, from 1979 to the present, general, and sometimes specific, policy decisions that promote case detection and treatment and deemphasize residual spraying for national malaria control programs.

Failure to maintain control over malaria most likely results from failures in the functions of interventions or from failures to make proper application of interventions. Although DDT resistance is often posed as a reason for malaria control failure, resistance of vector populations to DDT is not widespread in South America (10).

There are two common interventions for reducing malaria transmission within human populations. One potential intervention is case detection and treatment. The second is spraying insecticide on house walls to prevent malaria transmission inside the houses.

DDT and the Reemergence of Malaria

Field studies of DDT action against malaria vectors provide dramatic evidence of reduced human-vector contact (11-13). However, the effect of DDT against human malaria cannot be definitively answered by vector studies alone. Consequently, we studied the effect of DDT on malaria rates with regression models to look at the interactive effects of home-spray and malaria rates across years of malaria control activities.

[2] Statistics drawn from a U.S. Agency for International Development review in 1973-1974 of the malaria eradication program in Brazil and based on regions that were in the attack phase. There was greater reliance on active and epidemiologic surveys in such areas than in areas of consolidation or maintenance. Therefore, the overall percentage of slides derived from passive case detection was undoubtedly higher than 35%.

Once missing data were factored into the analyses, 28 and 32 years of malaria control data were used in separate tests for Brazil and Ecuador, respectively. Brazil was selected because it has sustained a robust malaria control program with progressive decreases in numbers of houses sprayed with DDT (Figure 6). Ecuador also reported progressive decreases in numbers of houses sprayed with DDT. However, Ecuador also reported great vacillations in spray rates (Figure 6), which were accompanied by variations in malaria rates. Data presented in Pan

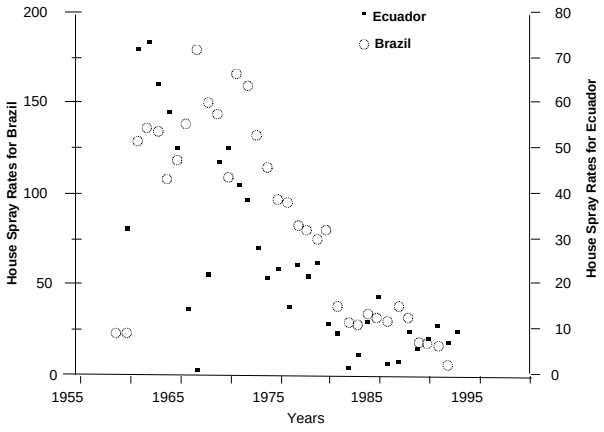


Figure 6. House-spray rates (HSRs) with DDT, Brazil and Ecuador, 1959–1993. Points correspond to data compiled by the Pan American Health Organization (2-5).

American Health Organization reports (2-5) were used in these analyses; i.e., malaria rates were not standardized by a fixed ABER.

Preliminary tests with a normalized API variable as the dependent variable and house-spray rates (HSRs), time, and ABER as independent variables showed statistically significant negative relationships between HSR and API values. However, the normalized response variables did not fulfill the requirement of constant variance, and the model exhibited problems of autocorrelation.

The problems with data variance and autocorrelation were solved for Brazil data by performing analyses with the following regression model:

$$\gamma_t = \alpha + ß_1\gamma_{t-1} + ß_2 X_{t-1} + \varepsilon$$

The γ represents an API value and t is time (year). Given that γ_t represents API for year t, then γ_{t-1} represents API for the preceding year. This component of the formula represents a lag year API value. The α is intercept, and ß is a parameter (slope) of the γ regression line. The HSR is from the year preceding γ_t, represented by X_{t-1}. Last, $ß_2$ is slope of the HSR regression line, and ε is the residual effect not accounted for by the API and HSR variables. This formula captures the idea that API plus HSR of one year can be used to accurately predict the API of the following year.

The analysis of variance of 28 years of data from Brazil produced an excellent fit of the regression model ($F = 354$; df=(2,26); $p < 0.0001$). The adjusted r^2 for the regression analysis was 0.96. Parameter estimate for γ_{t-1} was 0.74, and this relationship was highly significant ($p < 0.0001$). The parameter estimate for X_{t-1} was -0.0174; it was also highly significant ($p < 0.0004$). A test for autocorrelation was performed by correlation analysis of residuals versus lag time residuals. The Pearson Correlation Coefficient test sta-tistic was used to test the null hypothesis that ρ =O. The p value was 0.87 (not statistically significant), so we accepted the null hypothesis of no autocorrelation.

The problems of variance and autocorrelation were solved for data from Ecuador by performing regression analyses with the following model:

$$\gamma_t = \alpha + ß_1\gamma_{t-1} + ß_2(\log(X_{t-1})) + \varepsilon$$

The only difference between the Brazil and Ecuador models was a log transformation of the lag HSR values. A log transformation of lag HSR values reduced variation in this variable and produced a better fit of the whole model.

The regression analysis of 32 years of data from Ecuador produced an excellent fit of the regression model ($F = 45.6$; df=(2,30); $p < 0.0001$). The adjusted r^2 for the regression analysis was 0.73. Parameter estimate for γ_{t-1} was 0.58, and this relationship was highly significant ($p < 0.0001$). The parameter estimate for $\log(X_{t-1})$ was -1.197; it was also highly significant ($p < 0.0001$). The test for autocorrelation produced a p value of 0.51 (not statistically significant), and again we accepted the null hypothesis of no autocorrelation.

These highly predictive models showed the powerful relationship between DDT-sprayed houses and malaria rates. We documented that a high API in combination with a high HSR is predictive of a lower API the following year. Alternatively, when low APIs are combined with low HSRs, malaria rates are higher the following year.

Therefore, when large numbers of houses are sprayed with DDT, malaria rates decline and when fewer houses are sprayed, malaria rates increase.

Eliminating DDT for Malaria Control

Countries are banning or reducing the use of DDT because of continuous international and national pressures against DDT (e.g., the International Pesticide Action Network is "...working to stop the production, sale, and use..." of DDT [14]) and aggressive marketing tactics of producers of more expensive alternative insecticides. It has become easier for political pressures to succeed given the global strategy to deemphasize use of the house-spray approach to malaria control. A recent agreement of the North American Commission on Environmental Cooperation for eliminating the production and use of DDT in Mexico within the next 10 years[3] is the latest development in the campaign to eliminate DDT.

The Health Costs of Abandoning DDT

There is a cost in abandoning DDT for malaria control. This cost is seen in the results of malaria control programs from 1993 to 1995. We can get a uniform picture of events from 1993 to 1995 by standardizing malaria rates according to size of population at risk for malaria in each country (3,4). Since there were variations in this population variable for the 3 years, we took the population estimates for the midyear interval, 1994, as the basis for adjusting malaria rates for 1993 and 1995 (2). Each country was also characterized according to its reported use of DDT for malaria control in 1993 through 1995 (2-4).

As shown in Figure 7, countries that discontinued their house-spray programs reported large increases in malaria rates. Countries that reported low or reduced HSRs also reported increased malaria. Only Ecuador reported increased use of DDT and greatly reduced malaria rates.

The Use of DDT in Agriculture versus Malaria Control

In 1993, North, Central, and South American countries used 1,172,077 kg of DDT to spray house walls (4). While this may seem to be a large amount of insecticide, it actually represents less than 6% of the DDT used in the United States alone in 1968 (15). More than 795 kg of DDT might be used to treat a mere 0.4 km^2 (100 acres) of cotton during a growing season.[4] This amount of DDT would be sufficient to treat more than 1,692 houses. At four to five persons per house, spraying 1,692 houses translates into protection for as many as 8,460 persons. Since rural households are the primary candidates for house spraying, the 1,692 houses would be spread over a very large area. If a household of five persons is used, for example, significant levels of malaria control could be obtained for all populations at moderate to high risk for malaria transmission in Guyana by spraying only 17,000 houses during a single spray cycle. This level of treatment for the whole country of Guyana, covering an area of 215,000 km^2, is roughly equal to the amount of

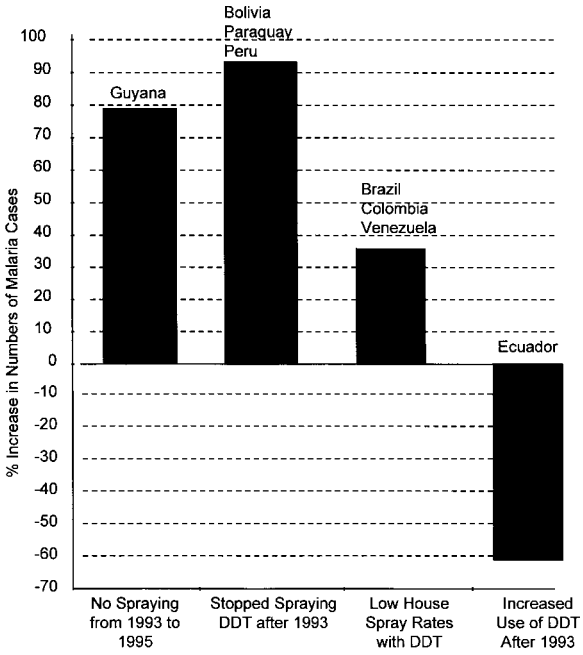


Figure 7. Increases in annual parasite indexes for four categories of countries, South America, 1993–1995. For each country, the populations at moderate to high risk for malaria were adjusted to midyear (1994) values. Data were derived from reports of the Pan American Health Organization (2-5).

[3] The Commission for Environmental Cooperation (CEC) is a North American environment commission established by a North American Free Trade Agreement side agreement. The CEC draft agreement entitled "North American Regional Action Plan on DDT, Task Force on DDT and Chlordane," dated October 10, 1996, calls for the elimination, distribution, and use of DDT for malaria control in Mexico in 10 years.

[4] Recommended weekly treatments of 0.9-1.36 kg (2-3 pounds) of DDT per 0.004 km^2 (1 acre) of cotton. Using a 7-week period and a treatment of 1.13 kg (2.5 pounds) per 0.004 km^2, 340 kg (1.750 pounds) of DDT is required for 0.4 km^2 (100 acres) of cotton.

DDT that might be used to spray only 4 km^2 (1,000 acres) of cotton during a single growing season. These statistics demonstrate the differences between DDT for agriculture and DDT for malaria control. On a landscape scale, a sprayed house represents an infinitesimally small spot treatment of a closed and protected environment (the house). DDT is relatively insoluble in water, so even when a house collapses and decays, DDT will not easily move from the house site.

Cost versus Benefit

Based on statistics compiled in 1978,[5] costs of chemicals for protecting a person showed malathion to be five times more expensive than DDT. In evaluating the cost of case treatment versus insecticide spraying, it is important to weigh the fact that a treated person will probably return to sleep in the very house where a potentially infectious blood meal was served to malaria vector mosquitoes the night(s) before diagnosis and treatment. Even with treatment and cure, persons can become reinfected by mosquitoes that fed on them before treatment. Indeed, a person can be reinfected and undergo curative treatment repeatedly over a period of a few months. While DDT residues do not provide complete protection from malaria transmission, they do provide variable but significant levels of protection for months after walls are sprayed.

A study of DDT alternatives for malaria control in Ecuador showed that the cost of other insecticides was many times higher than the cost of $1.44 to spray one house per year with DDT (16). The prohibitive cost of DDT alternatives has been a problem in malaria control (16). From 1986 to 1988, Mexico evaluated DDT alternatives in its national malaria program but discontinued their use because of unfavorable responses and high costs (17). High costs and downward trends in foreign aid suggest that many countries cannot afford the switch to DDT alternatives.

In 1994, the U.S. Agency for International Development allocated only $850,000 for malaria control in the Americas, compared with $4.13 million for malaria vaccine research (2). National malaria control budgets in the Americas declined 27% from 1994 to 1995, and loans and grants declined by 29%. With economic downsizing and reduced levels of foreign aid from industrialized countries (3), there is little likelihood that more money will be allocated for more expensive insecticides.

Discussion and Conclusions

Malaria is reemerging in disease-endemic countries. We have shown the patterns of real growth in malaria rates for Brazil, Peru, and Guyana. Figure 8 shows a similar pattern of growth in malaria rates for 18 other countries of the Americas. Figure 8 also depicts the relationships of increased malaria incidence to changing global strategies for malaria control. There is no inference of causation between changing policies and malaria increases. In fact, the HSRs were declining (as illustrated in Figure 8) even before global strategies were changed. However, it certainly seems that the new strategies are not producing a desirable outcome.

We have used two regression models to show

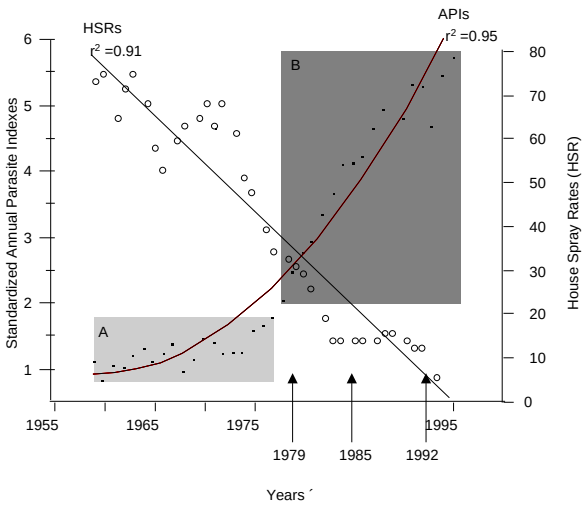


Figure 8. Standardized annual parasite indexes for 21 countries of the Americas, 1959–1995. Major changes in global malaria control strategies are depicted with arrows along the x axis (WHA 31.45 for 1979; WHA 38.24 for 1985; and the Global Malaria Control Strategy for 1992). Statistical data were derived from reports of the Pan American Health Organization (2-4). Block A represents a period of malaria control by spraying adequate numbers of houses with insecticide residues (primarily DDT). Block B represents a period of increasing malaria as the house-spray rates declined below effective levels. Open circles represent house-spray rates and solid squares represent standardized annual parasite indexes.

[5] World Health Assembly document A31/19, 1978.

that as numbers of DDT-sprayed houses declined, malaria incidence increased. The period from 1959 to 1978 can be characterized as a period of insecticide-controlled malaria. The period from 1979 to 1995 can be characterized as a period of decreased use of residual spraying and geometric growth in malaria incidence. Other factors contribute to resurgent malaria, but none would appear to equal the influence of decreases in the house-spray programs.

Public health researchers in the United States helped initiate the use of DDT for malaria control in 1943 (19). Today, DDT is still needed for malaria control. If the pressure to abandon this effective insecticide continues, unchanged or declining health budgets, combined with increasingly expensive insecticides and rising operational costs, will result in millions of additional malaria cases worldwide.

DDT should be produced and distributed for governments to use in malaria control only. Use of this insecticide should not be abandoned unless its known detrimental health effects are greater than the effects of uncontrolled malaria on human health.

The multifaceted issues of DDT use for malaria control (e.g., ecologic damage, human carcinogenicity, and pesticide resistance) and the applicability of the Global Malaria Control Strategy to the Americas should be the subject of intensive national and international debate. We are now facing the unprecedented event of eliminating, without meaningful debate, the most cost-effective chemical we have for the prevention of malaria. The health of hundreds of millions of persons in malaria-endemic countries should be given greater consideration before proceeding further with the present course of action.

Dr. Roberts is professor, Department of Preventive Medicine/Biometrics, Uniformed Services University of the Health Sciences (USUHS), Bethesda, MD. His research focuses on modeling of malaria control methods and on the applications of remote sensing and geographic information systems to malaria control in the Americas.

References

1. World Health Organization. Implementation of the global malaria control strategy. World Health Organ Tech Rep Ser 1993; no. 839.
2. Pan American Health Organization. Status of malaria programs in the Americas. XLIII Report. Washington (DC): PAHO; 1995.
3. Pan American Health Organization. Status of malaria programs in the Americas. XLIV Report. Washington (DC): PAHO; 1996.
4. Pan American Health Organization. Status of malaria programs in the Americas. XLII Report. Washington (DC): PAHO; 1994.
5. Pan American Health Organization. Status of malaria programs in the Americas. XL Report. Washington (DC): PAHO; 1991.
6. World Health Organization. Sixth Report, Expert Committee on Malaria. World Health Organ Tech Rep Ser 1957; No. 123.
7. Brown AWA, Haworth J, Zahar AR. Malaria eradication and control from a global standpoint. J Med Entomol 1976;13:1.
8. Gilles HM, Warrell DA. Bruce-Chwatt's essential malariology. Boston: Edward Arnold; 1993.
9. World Health Organization. Seventeenth Report, WHO Expert Committee on Malaria. World Health Organ Tech Rep Ser 1979; No. 640.
10. World Health Organization. Resistance of vectors of disease to insecticides. World Health Organ Tech Rep Ser 1980; No. 655.
11. Roberts DR, Andre RG. Insecticide resistance issues in vector-borne disease control. Am J Trop Med Hyg 1994;50:21.
12. Roberts DR, Alecrim WD. Behavioral response of *Anopheles darlingi* to DDT-sprayed house walls in Amazonia. Bull Pan Am Health Organ 1991;25:210-7.
13. Rozendaal JA. Behavioral responses of *Anopheles darlingi* in Suriname to DDT residues on housewalls. J Am Mosq Control Assoc 1989;5:351-8.
14. Pan American Health Organization. PAHO Environmental Series 1993;12:53.
15. Agricultural Stabilization and Conservation Service. Pesticide review. United States Department of Agriculture; 1968.
16. Arellano F. Uso de insecticidas en el programa de control de la malaria del Ecuador. Estrategia aconsejada. In: Comité Rotario Interdistrital Andino de Lucha Contra la Malaria, editors. Nuevas estrategias contra la malaria. Quito, Ecuador: Rotario Interdistrital Andino de Lucha Contra la Malaria; 1990.
17. Bruce-Chwatt LJ. The cost of malaria and of its control in relation to socio-economic realities. In: Study Group on Malaria in the Americas, editors. Washington (DC): Pan American Health Organization, 1977.
18. Pan American Health Organization. Empleo de insecticidas en salud pública Estados Unidos Mexicanos, 1957-1987. In Proceedings of "Taller sobre resistance," PAHO meeting 1988 18-29 Julio, Guatemala.
19. Gahan JB, Travis BV, Morton FA, Lindquist AW. DDT as a residual-type treatment to control *Anopheles quadrimaculatus*, practical tests. J Econ Entomol 1945;38:223-35.

Emerging and Reemerging Helminthiases and the Public Health of China

**Peter J. Hotez,* Feng Zheng,† Xu Long-qi,† Chen Ming-gang,†
Xiao Shu-hua,† Liu Shu-xian,† David Blair,‡
Donald P. McManus,§ and George M. Davis¶**

*Yale University School of Medicine, New Haven, Connecticut, USA;
†Chinese Academy of Preventive Medicine, Shanghai, PR China;
‡James Cook University of North Queensland, Townsville, Australia;
§Queensland Institute of Medical Research, Brisbane, Australia; and
¶The Academy of Natural Sciences, Philadelphia, Pennsylvania, USA

Despite great strides in their control throughout the People's Republic of China, helminth infections remain an important public health problem. The Institute of Parasitic Diseases of the Chinese Academy of Preventive Medicine, under the guidance of the Chinese Ministry of Health, completed a nationwide survey of more than 1 million people that showed the high prevalence and intensity of intestinal nematode infections; prevalence can sometimes exceed 50% in the Yangtze River valley provinces. *Schistosoma japonicum* is also a major cause of illness in this region. Attempts to control Chinese helminthic diseases with conventional anthelminthic drugs have been partially thwarted by high posttreatment rates of reinfection. Recently, several new human trematode pathogens have been identified. Novel approaches to chemoprophylaxis and vaccination may alleviate the public health problem caused by Chinese helminths. However, recombinant helminth vaccine development will depend on first cataloguing the extensive genetic diversity of Chinese helminths and candidate vaccine antigens. Evidence from biogeography, genetics, and systematics suggests that the genetic diversification of Chinese helminths and their vectors is an ongoing evolutionary process that began 12 million years ago near the convergence of major Asian river systems. Construction of the Three Gorges Super Dam on the Yangtze River may promote the emergence and reemergence of new helminths and their snail vectors.

Since the early 1900s, hundreds of millions of Chinese living near the Yangtze River have been at high risk for infection with helminths (1,2). Aggressive campaigns to control schistosomiasis among the peasants in Hunan Province and elsewhere in South China were a cornerstone of Chairman Mao's "patriotic health campaigns" (3). During the 1950s and 1960s, entire populations in schistosomiasis-endemic areas were mobilized against *Oncomelania* snails and recruited to drain rivers and ditches; millions of people were treated with anthelminthics (3). As a result, the number of schistosome-infected persons was reduced from 10 million in 1955 to 1.52 million in 1989 (4); today fewer than 1 million people harbor schistosomes of the *Schistosoma japonicum* complex. Similarly, lymphatic filariasis caused by *Wuchereria bancrofti* and *Brugia malayi* has been reduced by selective or reduced mass chemotherapy control programs, which have included the use of salt medicated with diethylcarbamazine (4). By the year 1994, all 864 filariasis-endemic counties and cities in the country had reached the criterion for effective control of filariasis (less than 1% microfilaria rate in every village) through check-up. Although great strides have been made over the last 40 years in controlling helminths in China, many helminthiases remain public health problems, particularly in the Yangtze River valley provinces (Anhui, Hubei, Hunan, Jiangxi, Jiangsu, Sichuan, Yunnan). The application of modern biotechnology to their study suggests that Chinese helminths are reemerging.

Address for correspondence: Feng Zheng, Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, 207 Rui Jin Er Lu, Shanghai 200025, P.R. China; fax: 86-21-6-4332670; e-mail: zfeng@fudan.ihep.ac.cn or tmrc@fudan.ihep.ac.cn.

Intestinal Nematode Infections in the Yangtze River Basin

In a nationwide effort to assess the prevalence of parasitic diseases, the Chinese Academy of Preventive Medicine and the Ministry of Health initiated the largest human parasite survey ever undertaken. From 1988 to 1992, 1,477,742 persons were examined in 2,848 study sites in 726 sampled counties (5-8). China's 30 provinces were divided into sectors; the counties and townships in each sector were then grouped into high, intermediate, and low on the basis of socioeconomic parameters (including levels of sanitation, income, and literacy) (8). All long-term residents in each randomly selected site (5,8,9) were asked to submit a fecal specimen for examination (8); compliance was at least 90%. Fecal specimens were collected at village offices or township health centers and examined by personnel trained specifically to participate in the nationwide survey.

The identification and quantitation of helminth eggs was determined by the Kato-Katz thick-smear technique on all slides positive for hookworm and *Trichuris*, but in only one slide of every 10 *Ascaris*-positive slides (8). To examine eggs of *Enterobius vermicularis*, adhesive transparent tape was applied to the perianal area on children under 12 years of age (5,8). To ensure quality control for fecal examinations, random checks of selected slides were made throughout the study. Specimens of previously unidentified or questionable samples were referred to either a provincial institute of parasitic diseases or to the Institute of Parasitic Diseases in Shanghai. Data from the study were ultimately transferred to a VAX computer for processing and analysis (8).

Survey results are summarized in Tables 1-3. An estimated 707 million Chinese (62.6%) were found to harbor one or more parasites (5-8). The overall infection rate for most parasites was higher in females than in males (7). A high number of Chinese were infected with the "unholy trinity" of intestinal nematodes, *Ascaris lumbricoides*, *Trichuris trichiura,* and hookworms *Ancylostoma duodenale* and *Necator americanus*—an estimated 531 million, 212 million, and 194 million cases, respectively (8; Table 1). Of the 374,753 children under 12 years of age tested for *E. vermicularis*, 26.4% were infected (8). Intestinal nematode infections were particularly abundant in the agriculturally intensive Yangtze River provinces (Table 2, Figure 1). In addition, more than 60% of the South China Sea island population of Hainan Province were infected with one or more intestinal nematodes (10-12). A significant percentage of infected persons had moderate and heavy intestinal nematode infections, although most had light infections (Table 3). This pattern of aggregated distribution has been encountered in other disease-endemic areas (8). High hookworm prevalence with clinical disease was found throughout Hainan and the rural areas of the Yangtze River

Table 1. Prevalence of major intestinal helminths in China*

Helminth	Number of Cases (millions)
Ascaris lumbricoides	531 ± 8
Trichuris trichiura	212 ± 10
Hookworm	194 ± 7
Clonorchis sinensis	4.1 ± 0.4
Fasciolopsis buski	1.9 ± 0.3
Taenia spp.	1.3 ± 0.2
Hymenolepis spp.	0.7 ± 0.1
Heterophyidae	0.2 ± 0.1

*Based on analysis of 1,477,742 fecal examinations and a Chinese population of 1.13 billion people.

Table 2. Prevalence of soil-transmitted intestinal nematode infections in the Yangtze River provinces*

Province	No. Fecal Exams	% Infection *Ascaris*	*Trichuris*	hookworm
Anhui	54,392	46.4 ± 1.9	17.4 ± 1.7	33.4 ± 1.9
Hubei	53,382	39.5 ± 2.2	18.3 ± 1.4	8.8 ± 1.1
Hunan	63,794	67.7 ± 1.3	20.2 ± 1.3	22.9 ± 1.4
Jiangsu	63,699	39.5 ± 1.7	27.3 ± 1.8	21.8 ± 1.5
Jianxi	52,079	71.1 ± 1.4	17.1 ± 1.7	17.6 ± 1.3
Sichuan	97,222	68.4 ± 1.2	30.4 ± 1.3	40.9 ± 1.9
Yunnan	53,061	59.6 ± 1.7	27.3 ± 2.2	19.3 ± 2.2
Zhejiang	55,284	60.0 ± 1.7	40.3 ± 2.3	28.2 ± 1.8

*Modified from a table in reference 8.

Table 3. Intensities of soil-transmitted intestinal nematode infections (based on eggs per gram [epg])[a]

Helminth	No. Fecal Exams	Infection Level (%) Light	Moderate	Heavy
Ascaris lumbricoides[b]	170,252	77.0	21.1	1.8
Trichuris trichiura[c]	247,020	94.6	5.2	0.2
Hookworm[d]		75.4	21.9	2.7

[a]Based on data provided in reference 8.
[b]*Light* = <5,000 epg; *Moderate* = 5,000 to < 50,000 epg; *Heavy* = > 50,000 epg
[c]*Light* = <1,000 epg; *Moderate* = 1,000 to < 10,000 epg; *Heavy* = > 10,000 epg
[d]*Light* = <400 epg; *Moderate* = 400 to < 3,000 epg; *Heavy* = > 3,000 epg

provinces, where adequate moisture and warm temperatures ensure survival of hookworm larvae in the soil. By comparison, *Ascaris* and *Trichuris* eggs withstand greater extremes of moisture and temperature, which may explain why ascariasis and trichuriasis are also endemic in urban settings and other harsh environments usually unfavorable for hookworm transmission (13,14).

The survey linked all three intestinal nematodes to foodborne infections, which in China are caused by the common agricultural practice of using uncomposted human manure for fertilizing crops such as corn, sweet potatoes, mulberry trees, and bananas (15-18); foodborne nematode infections are commonly transmitted by the ingestion of *Ascaris* eggs on sugar cane or *A. duodenale* hookworm infective larvae on raw vegetables (19). Vegetable garden farmers have the highest prevalence of hookworm infection (31.0%) (5,8). Vegetable growers also have high rates of *Ascaris* (36.7%) and *Trichuris* (47.9%) infections (8).

Intestinal nematodes affect maternal-child health. Schoolchildren have the highest prevalence and intensity of ascariasis (48.5%) (5,8), and all three major intestinal nematodes retard the intellectual and physical growth of children. Approximately 66 million women 15 to 49 years of age, many with underlying dietary iron-deficiency anemia, harbor hookworms (8). Cases of infantile hookworm caused by *A. duodenale* were frequent; children with infantile hookworm failed to thrive and had diarrhea, melena, and extreme pallor resulting from hemoglobin concentrations below 6 g/dl (20,21). Illness caused by heavy hookworm infection in infants was high (20,21). Among the hypothetical sources of infantile hookworm infection is the use of contaminated soil-filled diapers or the ingestion of contaminated breast milk (21).

Current efforts to control intestinal nematode infections with conventional anthelminthic chemotherapeutic agents (e.g., albendazole) or combination therapy with either pyrantel pamoate and oxantel or mebendazole and levamisole have been only moderately successful. All regimens reduce the number of worms (22,23). However, without altering ancient agricultural practices and improving sanitation, reinfection to pretreatment

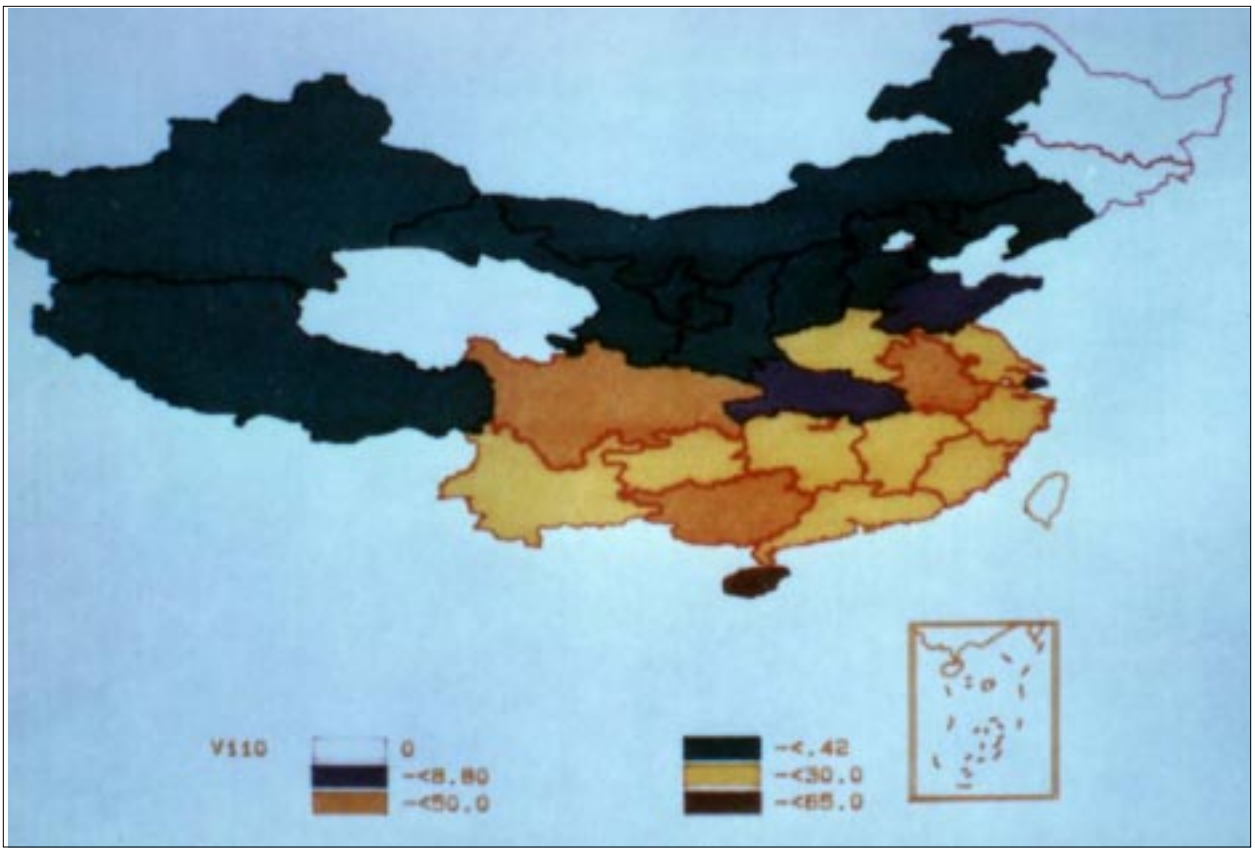


Figure 1. Distribution and prevalence of human hookworm infection in China.

levels may occur within 6 months (24). Moreover, the benzimidazole anthelminthics have shown low efficacy against *N. americanus* infections in China and have stimulated the migration of adult *Ascaris* worms. The Institute of Parasitic Diseases and Yale University have embarked on a basic research program to evaluate genetically engineered polypeptides from hookworms as recombinant subunit vaccines as an alternative control approach (25). The goal is to develop a recombinant vaccine that reduces worm levels below the disease-causing threshold. One recombinant molecule from the hookworm *Ancylostoma caninum* has shown promise at reducing worm levels during preclinical testing in mice (25,26).

Emerging Foodborne Helminthic Zoonoses

Some foodborne helminthic zoonoses are on the rise in China (17). Outbreaks of trichinellosis have increased in Yunnan Province over the last 10 years, in part because of the consumption of raw pork (called "oru" or "shengpi") by some residents (27). The seroprevalence of antibodies to *Trichinella spiralis* in this region averages 13.5%, and there is evidence that trichinellosis has spread from the southwestern province of Yunnan to other parts of China (5).

Among the foodborne trematode zoonoses, several previously undescribed human infections have been reported, including those caused by *Echinostoma angustitestis, Centrocestus formosanus, Echinochasmus fujianensis,* and *Echinochasmus liliputanus* (5,28,29). Clonorchiasis and paragonimiasis remain highly endemic in China. Many of the *Clonorchis sinensis* infections are in southern China, where nearly 3 million Cantonese (Guangdong Province) are infected because of their raw fish consumption (30). High prevalence rates are also found among Korean minority populations in the northeastern provinces of Heilongjiang, Jilin, and Liaoning (30). Among the reservoir hosts are mammals such as dogs, cats, pigs, and rats. Chronic *C. sinensis* infection in childhood has been recently linked to delayed physical growth and development, while chronic infection in adulthood is associated with cholangiocarcinoma (30). Paragonimiasis is estimated to occur in 1 million Chinese, and approximately 100 million Chinese are at risk for infection. The study of Chinese *Paragonimus* infections is in its infancy, having been eclipsed by a comparatively larger amount of investigative work in schistosomiasis. However, preliminary studies suggest that the taxonomy of these human parasites will be extensively revised.

The number and identity of species infecting humans in China is being investigated through molecular phylogenetic studies at the Institute of Parasitic Diseases, in collaboration with the Academy of Natural Sciences and James Cook University. Approximately 25 species, of which some are likely synonyms, have been recorded in China. *Paragonimus westermani* and *Paragonimus skrjabini* are most commonly associated with human infection. The former species produces the classical lung-fluke infection, while juveniles of the latter rarely mature in humans but instead undergo extrapulmonary migrations. Some Chinese investigators ascribe *P. skrjabini* to a distinct genus—*Pagumogonimus*. To complicate matters, both *P. westermani* and *P. skrjabini* probably represent complexes of species that differ in clinical importance and biologic properties. Several subspecies of diploid sexually reproducing *P. westermani* have been proposed on the basis of their clinical picture (31). In addition, a triploid parthenogenetic variety is common in northeastern China, where it is a serious pathogen. Almost nothing is known about strains and species within the *P. skrjabini* complex. However, novel findings may soon be made. For instance, cercariae of *P. skrjabini* have been reported from no fewer than 23 snail families distributed among three tribes and two subfamilies of the Pomatiopsidae throughout southern China (32). Barring misidentifications, these records suggest that *P. skrjabini* must be unique among digeneans in its lack of host specificity. A more likely explanation is that a complex of different, as yet unrecognized, *Paragonimus* species occurs in these regions (32,33). Also, studies of snail hosts of these parasites are being initiated; most species transmitting *P. skrjabini* have not been described, and many others are known only from shells and radulae. Snail hosts of *P. westermani* in China have never been studied in terms of modern systematics.

Schistosomiasis: Novel Control Strategies

Asian schistosomiasis is caused by blood flukes of the *S. japonicum* complex (*S. japonicum, S. mekongi,* and *S. malayensis).* Only *S. japonicum* has been described in China, although identification of unique Chinese strains based on molecular genetic data suggests emergence of

new members of this complex (34). Also found in China, *S. sinensium*, which may occupy its own taxonomic complex, may ultimately be shown to infect humans. Although the national control program through aggressive efforts over the last 30 years has reduced the number of infected persons and cattle, schistosomiasis japonica remains a major public health threat in China (35). The number of infected persons and cattle is estimated at just under 1 million and 70,000, respectively (35). The major endemic foci are in the marsh and lake regions of southern China and the Yangtze River Basin (Hubei, Hunan, Jianxi, and Anhui Provinces), which account for 86% of the cases. Sixty to 100 million persons are at risk in these schistosomiasis-endemic areas. The *Oncomelania* snail (the intermediate host)-infested areas have been reduced to 3.64 billion m^2 in the lakes region and mountainous territories in Yunnan and Sichuan Provinces. Aside from intestinal, liver, and central nervous system damage from heavy infections with *S. japonicum*, ample evidence has also documented the negative impact of moderate infections on childhood growth and development.

The present policy to control Chinese schistosomiasis relies primarily on two approaches: large-scale chemotherapy with praziquantel (produced in China since 1978) and snail control with molluscicides and environmental modification. Although praziquantel can reduce the incidence of severe forms of the disease, it does not prevent reinfection and may not reverse many of the hepatosplenic complications of heavy *S. japonicum* infections. Three novel schistosomiasis control programs (using qinghaosu, bromoacetamide, and vaccine) are under investigation at the Institute of Parasitic Diseases.

On the basis of studies suggesting that derivatives of the antimalarial drug qinghaosu (artemisinin) are also effective in animal schistosome infections (36), human clinical investigations have been initiated with oral artemether (37). In field studies conducted in a disease-endemic area in the southern Dongting Lake region of Hunan Province, residents who frequently contacted infested water were pretreated with praziquantel and then, in a randomized pattern, received either artemether or a placebo control (37). Approximately 1 month after the final dose, patients who received artemether had significantly reduced rates of newly acquired schistosomiasis. Further studies are under way to evaluate artemether for schistosomiasis chemoprophylaxis.

Bromoacetamide, a new type of molluscicide, was synthesized and developed in China during the early 1980s and is now being produced (35). The lethal dose of bromoacetamide is 1 ppm for *Oncomelania* snails—approximately one tenth that required when pentachlorophenate, a traditional molluscicide, is used (35). At 1 ppm, bromoacetamide has been shown to be nontoxic to fish.

A third control strategy—schistosome vaccination—is also being developed at the Institute of Parasitic Diseases, in collaboration with the Queensland Institute of Medical Research. Injection of mice and pigs with a recombinant *S. japonicum* 26 kDa glutathione-S-transferase (Sjc26GST) vaccine induced a pronounced antifecundity effect after experimental infection with a Chinese strain of *S. japonicum* (38,39). This finding is important because the eggs released from female schistosomes cause both the pathogenic sequence leading to clinical schistosomiasis and transmission of the infection. Encouraged by these results, the Institute of Parasitic Diseases and the Queensland Institute of Medical Research vaccinated water buffaloes, a principal animal reservoir host for *S. japonicum* in southern China. Results suggest that immunization of animal reservoir hosts might reduce schistosomiasis transmission to humans. Field studies to test this hypothesis are in progress.

One concern about the development of recombinant antigen-based vaccines is the marked antigenic diversity of different geographic isolates of Chinese schistosomes (40). The genetic diversity of Chinese schistosomes probably parallels an often underappreciated diversity of *Oncomelania* snail vectors. By both classical and molecular phylogenetic techniques, the Academy of Natural Sciences has provided evidence for the coevolution of *S. japonicum* with their snail vectors (41). The coevolution of *Schistosoma* and snail hosts involves reciprocal selective pressures affecting the genetic diversification of both parasites and snails (41). These data may explain the high degree of snail host specificity compared with very loose mammalian host specificity for *S. japonicum*. Thus, through the careful genetic analysis of host snails, the emergence of *S. mekongi* within the *S. japonicum* complex was predicted years before it could be confirmed by using parasites from clinical specimens (42). A similar

analysis led to the discovery in China of a separate triculine snail-transmitted *S. sinensium* complex (43), which may yet reveal new species of human schistosomes. The discovery of a new triculine-transmitted schistosome species may be easily overlooked since *Oncomelania* (transmitting *S. japonicum*) is frequently sympatric with triculine snails and a schistosome infection could erroneously be identified as schistosomiasis japonica. Indeed, members of the *S. japonicum* species complex have worm and egg morphologic characteristics that are virtually identical qualitatively. If snail evolution is a predominant driving force behind the evolution of the trematode parasite, then the marked genetic variation observed among populations of the genus *Oncomelania,* as it evolved down emerging river systems from Xizang (Tibet) and Yunnan Provinces, China, will drive the evolution of new and emerging schistosomes.

Impact of the Three Gorges Super Dam on Human Transmission

Superimposed on the preexisting coevolutionary forces driving schistosomes and snails is a human-made intervention of enormous scale. Begun in 1994 and scheduled to be completed by the year 2009, the Three Gorges Super Dam, which rises to a height of 180 m on the Yangtze, will affect Hubei and Sichuan Provinces by creating a reservoir area of 50,700 km^2 and submerging more than 220 counties extending from Yichang County, Hubei Province, in the east to Jiangjin and Hechuan Counties, Sichuan Province, in the west (Figure 2). The reservoir will displace an estimated 1.4 million people. Several of the environmental changes created by the dam and the resultant reservoir will affect both *Oncomelania* populations and schistosomiasis transmission. 1) After the dam is completed, the perennial high water level in the reservoir region will elevate the groundwater level and contribute to the breeding and propagation of *Oncomelania* spp. One site of special concern is the Jianghan Plain, Hubei Province. 2) Alluvial land and beaches will appear near many of the Yangtze River tributaries and provide beds suitable for the development of snails; immigrants to the area exploiting these new environments for aquaculture and agriculture will be exposed to schistosomes. 3) The sand deposits and beach areas of Dongting and Boyang lakes will be altered in a way that may either increase or decrease snail breeding. 4) For the first time in history, lateral canals may permit easy distribution of *Oncomelania* snails around the gorges from Sichuan to Hubei and downstream.

Investigations by the Chinese Academy of Sciences and the Water Conservancy Committee of the Yangtze River have resulted in programs to evaluate the distribution of *Oncomelania* snails; the infection rates among children ages 7 to 14 years; changes in census and immigration; and alterations in soil composition, vegetation, rainfall, and topographic features, to identify high risk areas for schistosomiasis transmission. Observation sites will be established in selected schistosomiasis-endemic administrative villages (of approximately 10,000 residents) located midstream and downstream from the Yangtze River—two village sites will be in Jiangsu Province and four in Hunan, Hubei, Jiangxi, and Anhui Provinces. Data will be collected on census, socioeconomic status, daily activities, duration and frequency of water contact, and schistosomiasis infection rates among the residents and domestic animals. In addition, two or three beaches will be selected at each observation site to measure the change in monthly water level, the duration of beach submersion, changein beach vegetation, soil type and water content, snail density and range, and infected-snail density and range.

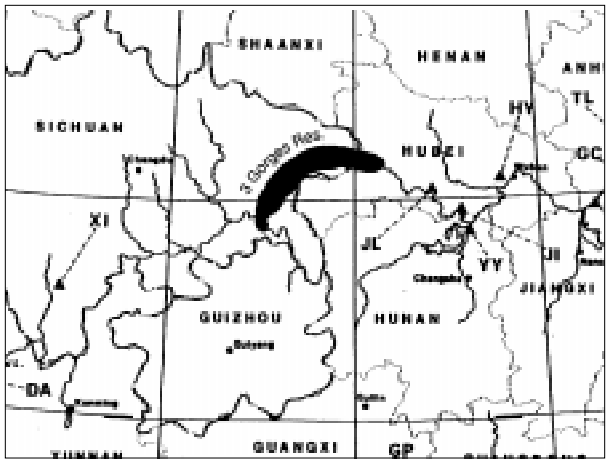


Figure 2. Projected size and location of Three Gorges Reservoir in Sichuan and Hubei Provinces resulting from the Three Gorges Super Dam construction.

Conclusions

Chinese helminthiases are intimately connected to the nation's social and ecologic fabric. Traditional agricultural and foodhandling practices account for the high prevalence of ascariasis, hookworm infection, paragonimiasis, and schistosomiasis near the Yangtze River. Increasing evidence also suggests that the major helminthiases are intricately woven into the relationships between families and between mothers and their children (44-47). China is undergoing major social changes due to economic reforms which, in some rural areas, have not improved the access to healthcare (48). Major ecologic changes are anticipated as a consequence of the Three Gorges Super Dam project. The Institute of Parasitic Diseases of the Chinese Academy of Preventive Medicine is now poised to examine the impact of these huge changes on the genetic diversity of helminths and their snail vectors. This information will be used to design novel helminth control measures for specific geographic regions of China.

Acknowledgments

The collaboration between the Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine and the Academy of Natural Sciences and Yale University School of Medicine is supported by 1 P50 AI39461 (Tropical Medicine Research Center) from the National Institutes of Health. Further support was obtained through NIH AI32726, a March of Dimes Clinical Research Grant, and an Established Investigator Grant from the American Heart Association. The Chinese nationwide survey of human parasites was conducted while Prof. Yu Sen-hai (World Health Organization, Geneva) was director of the Institute of Parasitic Diseases.

Dr. Hotez is associate professor of epidemiology and public health and pediatric infectious diseases, Yale University School of Medicine. He heads a laboratory group that develops chemically defined vaccines for helminth infections, including hookworm infection. Dr. Hotez is also assistant program director for the Tropical Medicine Research Center on Emerging Helminthiases in the People's Republic of China, sponsored by the National Institutes of Health.

References

1. Faust EC, Meleney HE. Studies on schistosomiasis japonica, with a supplement on the molluscan hosts of the human blood fluke in China and Japan, and species liable to be confused with them. American Journal of Hygiene, Monogr Ser No 3 1924; Baltimore: Johns Hopkins Press.
2. Cort WW, Grant JB, Stoll NR, et al. Researches on hookworm in China. American Journal of Hygiene, Monogr Ser No 7 1926; Baltimore: Johns Hopkins University Press.
3. Oksenberg MC. Chinese politics and the public health issue. In: Bowers JZ, Purcell EF, editors. Medicine and society in China. New York: Josiah Macy Foundation, 1974;128-61.
4. Yu Senhai. Control of parasitic diseases in China, current status and prospects. Chin Med J 1996;109:259-65.
5. Yu S-H, Xu L-Q, Jiang Z-X, Xu S-H, Han J-J, Zhu Y-G, et al. Special report, nationwide survey of human parasites in China. Southeast Asian J Trop Med Public Health 1994;25:4-10.
6. Yu SH, Xu LQ, Jiang ZX, Xu SH, Han JJ, Zhu YG, et al. Report on the first nationwide survey of the distribution of human parasites in China. 1. Regional distribution of parasite species. Chinese Journal of Parasitology and Parasitic Diseases 1994;12:241-7.
7. Xu LQ, Jiang ZX, Yu SH, Xu S, Huang D, Yang SX, et al. Nationwide survey of the distribution of human parasites in China—infection with parasite species in human population. Chinese Journal of Parasitology and Parasitic Diseases 1995;1-7.
8. Xu L-Q, Yu S-H, Jiang Z-X, Yang J-L, Lai C-Q, Zhang X-J, et al. Soil-transmitted helminthiases: nationwide survey in China. Bull World Health Organization 1995;73:507-13.
9. Cocharne WG. Sampling techniques, 3rd ed. New York: John Wiley and Sons 1977;66-91.
10. Xu F, Wu R, Chen J, Guo R. Investigation on human parasite distribution in Hainan Province. Chinese Journal of Parasitology and Parasitic Diseases [special issue] 1994;12:27-30.
11. Chen JZ, Xu FS, Wu RZ, Guo RN, Xing YZ, Huang DL, et al. Investigation on hookworm infection in Hainan Province. Chinese Journal of Parasitology and Parasitic Diseases [special issue] 1994;12:148-51.
12. Chen JZ, Xu FS, Wu RZ, Guo RN, Xing YZ, Huang DL, et al. Investigation and analysis of intestinal parasite infections in rural children in Hainan Province. Chinese Journal of Parasitology and Parasitic Diseases [special issue] 1994;12:157-9.
13. Ma X, Cai L, Fu Y, Huan D. Influence of some factors on prevalence of intestinal parasites in Shanghai. Chinese Journal of Parasitology and Parasitic Diseases [special issue] 1994:12:30-8.
14. Ma X-B, Cai L, Fu Y-F, Huang D-S. Present status of intestinal parasite infections in Pudong new area of Shanghai. Chinese Journal of Parasitology and Parasitic Diseases 1991;9-11.2
15. Yang JL, Yang HM, Wang YK, Zhang LL, Yu H, Zhang BX, et al. A survey of epidemiological factors in hookworm, *Ascaris* and *Trichuris* infections. Chinese Journal of Parasitology and Parasitic Diseases [special issue] 1994;12:145-7.
16. Xu XR, Cheng YZ, Lin AQ, Guo ZF, Hong L, Zhang XB, et al. Investigation of hookworm infection in banana planting field in Zhangzhou City, Fujian Province. Chinese Journal of Parasitology and Parasitic Diseases 1991;9:50-2.
17. Xu LQ, Jiang ZX, Yu SH, Xu S, Chang J, Wu ZX, et al. Characteristics and recent trends in endemicity of human parasitic diseases in China. Chinese Journal of Parasitology and Parasitic Diseases 1995;13:214-7.

18. Han JJ, Liu C, Liu CY, Wan ZY, Zheng DF, et al. Epidemiological aspects of human parasitoses in Sichuan province. Chinese Journal of Parasitology and Parasitic Diseases [special issue] 1994;12:8-16.
19. Lin J-X, Lin A-Q, Chen B-J, Xu X-R. An investigation of acute *Ascaris* infection resulted from eating sugarcane in children. Chinese Journal of Parasitology and Parasitic Diseases 1991;9:48-50.
20. Wu C-S, Ni F-Y, Cheng W-J, Wang C-H. Hookworm disease in infants: report of eight cases (with two cases of leukemoid reaction due to infant hookworm disease). Acta Parasitol Sinica 1966;3:138-40.
21. Yu S-H, Jiang Z-X, Xu L-Q. Infantile hookworm disease in China. A review. Acta Tropica 1995;265-70.
22. Xu LQ, Jiang ZX, Yu SH, Ding XM, Bin XH, Yang HF, et al. Treatment of soil-transmitted helminth infections by anthelminthics in current use. Chinese Jour-nal of Parasitology and Parasitic Diseases 1992;10:95-9.
23. Chen ZZ, Fang YY, Huo LC, Liu MZ, Gu SH, Chen XQ, et al. Efficacy of albendazole medicated-salt on treatment of intestinal helminth infections. Chinese Journal of Parasitology and Parasitic Diseases [special issue] 1994;12:122-4.
24. Albonico M, Smith PG, Ercole E, et al. Rate of reinfection with intestinal nematodes after treatment of children with mebendazole or albendazole in a highly endemic area. Trans R Soc Trop Med Hyg 1995;89:538-41.
25. Hotez PJ, Hawdon JM, Ghosh K, Cappello M, Jones BF, Volvovitz F, et al. Molecular approaches to vaccinating against hookworm disease. Pediatr Res 1996;40:515-21.
26. Ghosh K, Hawdon JM, Hotez PJ. Vaccination with alum-precipitated ASP-1 protects mice against challenge infections with infective hookworm (*Ancylostoma caninum*) larvae. J Infect Dis 1996;174:1380-3.
27. Yang H-M. Studies on epidemiology and control of trichinosis in Yunnan Province. Chinese Journal of Parasitology and Parasitic Diseases 1991;9:79-82.
28. Chen YZ, Xu XL, Chen BJ, Guo ZF, Zhen HY, Lin SS, et al. First report on human infection of *Centrocestus formosanus* (in Fujian). Chinese Journal of Parasitology and Parasitic Diseases 1991;9:273.
29. Chen YZ, Lin JX, Fang YY, Lin AQ, Chen BJ, Chin J, et al. Discovery on infections of *Echinostoma angustitestis* in humans. Chinese Journal of Zoonoses 1992;8:7-8.
30. Chen M, Lu Y, Hua X, Mott KE. Progress in assessment of morbidity due to *Clonorchis sinensis* infection: a review of recent literature. Tropical Disease Bulletin 1994;91:R7-65.
31. Xu Z-B. Studies on clinical manifestations, diagnosis and control of paragonimiasis in China. Southeast Asian J Trop Med Public Health 1991;22[supplement]:345-8.
32. Lei C, Wang P. Analysis on pathogencity of *Paragonimus westermani* with diploid form in Lanting of Shaoxing County, Zhejiang Province. Chinese Journal of Parasitology and Parasitic Diseases [special issue] 1994;12:209-11.
33. Davis GM, Chen C-E, Kang Z-B, Liu Y-Y. Snail hosts of *Paragonimus* in Asia and the Americas. Biomed Environ Sci 1994;7:369-82.
34. He YX, Hu YQ, Yu QF, Tang ZJ. Characteristics of different isolates of *Schistosoma japonicum* from China in the final hosts. Southeast Asian J Trop Med Public Health 1991;22:240-1.
35. Chen M-G. Control and research on schistosomiasis in China. Chin Med J (Engl) 1995;108:643-6.
36. Xiao SH, You JQ, Yang YQ, Wang CZ. Experimental studies on early treatment of schistosomal infection with artemether. Southeast Asian J Trop Public Health 1995;26:306-18.
37. Xiao SH, Shi ZG, Zhuo SJ, Wang CZ, Zhang ZG, Chu B, et al. Field studies on the preventive effect of oral artemether against schistosomal infection. Chin Med J (Engl) 1996;109:272-5.
38. Liu S, Song G, Xu Y, Wen Y, McManus DP. Immunization of mice with recombinant Sjc26GST induces a pronounced anti-fecundity effect after experimental infection with Chinese *Schistosoma japonicum*. Vaccine 1995;13:603-7.
39. Liu SX, Song GC, Xu YX, Yang W, McManus DP. Anti-fecundity immunity induced in pigs vaccinated with recombinant *Schistosoma japonicum* 26 kDa glutathione-S-transferase. Parasite Immunol 1995;17:335-40.
40. Xue H-C, Qiu L-S, He Y-X, Zhang Y-H, Zhu C-W. SDS-PAGE protein pattern and its antigenicity analysis of different isolates of *Schistosoma japonicum* in China. Chin Med J (Engl)1994;107:25-9.
41. Davis GM. Evolution of prosobranch snails transmitting Asian Schistosoma: coevolution with Schistosoma: a review. Prog Clin Parasitol 1993;3:145-204.
42. Davis GM, Kitikoon V, Temcharoen P. A monograph on *Lithoglyphopsis aperta*, the snail host of Mekong River schistosomiasis. Malacologia 1976;15:241-78.
43. Davis GM, Greer G. A new genus and two new species of triculinae and the transmission of a malaysian mammalian *Schistosoma* sp. Proceedings of the Academy of Natural Sciences of Philadelphia 1980;132:245-76.
44. Xu LQ, Jiang ZX, Yu SH, Xu SH, Chen SL, Lin JX, et al. Status and strategy of parasitosis control in preventive health care in China. Chinese Journal of Parasitology and Parasitic Diseases 1995;13:264-8.
45. Xu LQ, Jiang ZX, Yu SH, Xu SH, Meng N, Gan YC, et al. Nationwide survey of the distribution of parasites in China—the characteristics and rules of geographic distribution of human helminth infection. Chinese Journal of Parasitology and Parasitic Diseases 1995;13:99-103.
46. Han J-J, Liu C-H, Yin G-Y, Zheng D-F. Human intestinal parasite infections and their family aggregation in Sichuan. Chinese Journal of Parasitology and Parasitic Diseases 1991;9:76-9.
47. Li BJ, Li YB, Ge FT, Shang LJ, Hou FL, Liu HB, Yu M. Analysis on family clustering of human intestinal parasitic infections in Hebei Province. Chinese Journal of Parasitology and Parasitic Diseases [special issue] 1994;12:215-6.
48. Hsiao WCL, Liu Y. Economic reform and health—lessons from China. N Engl J Med 1996;335:430-2.

Vancomycin-Resistant Enterococci Outside the Health-Care Setting: Prevalence, Sources, and Public Health Implications

L. Clifford McDonald, Matthew J. Kuehnert, Fred C. Tenover, and William R. Jarvis
Centers for Disease Control and Prevention, Atlanta, Georgia

Although nosocomial acquisition and subsequent colonization of vancomycin-resistant enterococci (VRE), an emerging international threat to public health, has been emphasized in the United States, colonization among nonhospitalized persons has been infrequently documented. In contrast, in Europe, colonization appears to occur frequently in persons outside the health-care setting. An important factor associated with VRE in the community in Europe has been avoparcin, a glycopeptide antimicrobial drug used for years in many European nations at subtherapeutic doses as a growth promoter in food-producing animals. In Europe, evidence suggests that foodborne VRE may cause human colonization. Although avoparcin has never been approved for use in the United States, undetected community VRE transmission may be occurring at low levels. Further studies of community transmission of VRE in the United States are urgently needed. If transmission with VRE from unrecognized community sources can be identified and controlled, increased incidence of colonization and infection among hospitalized patients may be prevented.

Vancomycin-resistant enterococci (VRE), first reported in Europe in 1988, are emerging as a global threat to public health (1). The incidence of VRE infection and colonization among hospitalized patients has increased rapidly in the last 7 years. From 1989, the year VRE was first identified in the United States, through 1993, the proportion of enterococcal isolates resistant to vancomycin reported to the National Nosocomial Infections Surveillance System increased 20-fold (2). Infection with VRE may be associated with increased mortality (3,4), and no effective antimicrobial therapy is available for many VRE (5,6).

Multiple factors predispose a person to infection with VRE, but colonization precedes most infections (7). In the United States, nosocomial transmission of VRE from patient to patient has been emphasized (3,7-10). Although VRE introduced into hospitals by colonized patients from VRE-endemic settings has been reported (8,9), it is unclear how VRE is first introduced into most U.S. hospitals.

Although no data so far support significant acquisition and transmission of VRE outside the health-care setting in the United States, a growing number of reports from Europe suggest that colonization with VRE frequently occurs in the community (11-15). Reports from Europe also have suggested that VRE exist elsewhere in the environment, including animal feces and human foods of animal origin (15-23). Additional evidence supports the transmission of VRE to persons in contact with these sources, resulting in an increased human reservoir of VRE colonization (15,16,22). If VRE is frequently introduced into health-care settings from community sources, its control will require community-based initiatives, unlike measures used to control nosocomial pathogens.

This review summarizes the existing evidence for community acquisition and transmission of VRE outside the health-care setting in Europe, relates these findings to the epidemiology

Address for correspondence: Dr. William R. Jarvis, Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, Mail Stop E-69, Atlanta, GA 30333; fax: 404-639-6459; e-mail: wrj1@cdc.gov.

of VRE in the United States, discusses steps to stem community transmission of VRE, and identifies research needs.

Animal and Community Reservoirs of VRE in Europe

VRE were first reported outside the health-care setting in 1993 when vancomycin-resistant *Enterococcus faecium* was recovered from waste water samples collected from sewage treatment plants in urban areas of England (16) and in small towns in Germany (17). The following year, Bates et al. recovered VRE from livestock feces and from uncooked chicken samples purchased from retail outlets (18). Klare et al. also have found VRE in manure samples from pig and poultry farms in Germany and suggested a possible relationship between the recovery of these organisms and the use of avoparcin, a glycopeptide antimicrobial drug used as a livestock feed additive in many European countries (19). The association between the recovery of VRE from food animals, especially poultry, and the use of avoparcin at subtherapeutic doses for growth promotion has now been confirmed by epidemiologic studies in Denmark (20,21), Norway (22), and the Netherlands (15).

The link between VRE colonization of animals used in food production and human VRE colonization was first suggested by Bates et al., who recovered VRE with identical ribotypes from retail chicken carcasses and humans (17). VRE has also been recovered from poultry and pork collected from slaughterhouses and retail outlets in Germany (19,23), Norway (22), and the Netherlands (15). More recently, a higher prevalence of VRE colonization was found among persons who worked on turkey farms or in turkey processing plants than among urban residents in the Netherlands (15). Because of the heterogeneity of pulsed-field gel electrophoresis patterns encountered in each population and the contrasting similarity of patterns found between individual turkey farmer/slaughterer isolates and animal isolates, the investigators concluded that "dissemination of VRE from animal origin via the food chain seems likely" (15).

The presence of the *vanA*, *vanX*, and *vanR* genes in VRE isolates examined from the feces of pigs and poultry in Denmark (21) suggests that a gene cluster similar to that found on Tn*1546*, the transposon responsible for high-level vancomycin resistance in human isolates (24,25), is responsible for resistance among these animal isolates. Regardless of whether individual clones of VRE are transmitted through the food chain or whether transfer of a transposable genetic element (i.e., Tn*1546*) from VRE-colonized animals to humans occurs, evidence points to a similarity between organisms present in these two populations and suggests transmission of vancomycin resistance between the microbial flora of human and animal species.

If VRE from poultry, swine, and other food-producing animals play a role in human colonization and infection, a significant level of VRE colonization may be found among persons not associated with the health-care setting. In Europe, VRE have been detected among persons outside the health-care setting in several studies (11-14). VRE were isolated from the stool of three (2%) of 184 persons in Oxford, England (11), 7 (17%) of 40 persons living in Charleroi, Belgium, without recent exposure to a health-care setting (12), and 22 (3.5%) of 636 patients cultured within 2 days of entering a hospital in Belgium (13). A community survey in the Netherlands performed on 200 outpatient stool samples submitted from patients with symptoms of diarrhea demonstrated a prevalence of VRE colonization of 2%, a rate similar to the 1%-3% prevalence among hospital inpatients (14). In another survey, 13 (11%) of 117 urban residents in the Netherlands harbored VRE (15).

VRE Outside the Health-Care Setting in the United States

Human VRE colonization outside the health-care setting has not been demonstrated in the United States. No VRE was found in two community prevalence surveys designed specifically to detect VRE (26,27). The number of persons studied, however, remains small (304), and the culture methods used may not have been the most sensitive for detecting small numbers of organisms (26,28).

Other lines of epidemiologic evidence in the United States support the possible existence of community VRE transmission. Although several hospital outbreaks of VRE caused by a limited number of genetic clones have been reported (9,26), polyclonal outbreaks have been observed in other instances (3,10,29,30). Delayed detection of VRE in the hospital population or the presence of a highly mobile element such as Tn*1546* transmitting vancomycin resistance between genetically

dissimilar enterococci could explain this finding. Another plausible but unproven explanation is that a larger community reservoir containing genetically diverse VRE exists. Additional evidence is provided by reports of VRE among patients in medically isolated communities (i.e., isolated population centers served by few inpatient health-care facilities that infrequently receive outside patient transfers) in the United States (L.C. McDonald, unpub. data) and the recent finding of VRE colonization among patients from the community within the first 24 hours of admission to a hospital intensive care unit (31). Finally, the existence of enterococci possessing high-level resistance to aminoglycosides in a community prevalence survey (32) provides evidence that antimicrobial-resistant enterococci can disseminate outside health-care settings in the United States.

If community transmission is occurring in the United States, potential vehicles of such transmission include human or animal food. Antimicrobial resistance in enterococci has been more prevalent in farm animals exposed to antimicrobial drugs (33,34). Although high-level aminoglycoside–resistant enterococci have been recovered from chicken prepared in the cafeteria of a hospital where VRE was endemic (35), isolation of VRE with the VanA phenotype from animals or human foods of animal origin have not been reported. Recovery of VRE with the VanA phenotype from dog food sold in the United States (36) and evidence from Europe suggesting that VRE may be prevalent in household pets (cats and dogs) (37, 38) with a genotype common to both human and pet hosts (37) suggests another mode of community transmission. Finally, transmission of VRE from a recently discharged patient to a family member suggests that household contact, including food preparation, may lead to community transmission in the United States (39).

Implications

If community transmission is important in the global spread of VRE, factors leading to its emergence in this setting must be examined, and measures must be taken to control transmission. In response to data linking the use of avoparcin with the emergence of VRE in the food chain and potential transmission to humans, Denmark (1995) and Germany (1996) imposed bans on the use of avoparcin at subtherapeutic doses in food animals for growth promotion (12,40). This action by two member states has been followed recently by a European Union-wide ban on avoparcin (41). Avoparcin has never been licensed for use in the United States or Canada because of its carcinogenic potential; illegal use, however, has been reported (42).

Feed additive manufacturers have resisted proposals to ban the use of avoparcin at subtherapeutic doses for food animal growth promotion in Europe (43,44). Opponents of the ban have suggested that the relatively low incidence of human VRE infections in European countries where avoparcin has been used for many years, compared with the high rate in the United States, where avoparcin is not used, is inconsistent with the hypothesis that avoparcin is a major factor in the emergence of VRE (43). However, profound differences may exist between the United States and European countries in the amount of glycopeptides used in health-care settings.

Vancomycin use in U.S. hospitals has increased dramatically in the past 10 to 15 years (45,46) because of a variety of factors, including increases in the incidence of methicillin-resistant staphylococci, prosthetic device–related infections, *Clostridium difficile* colitis, and inappropriate use of the drug. Although vancomycin and other glycopeptide use in European health-care settings has not been similarly documented, this marked increase in human glycopeptide use is thought to be primarily a U.S. phenomenon (43). Because the use of vancomycin and other antimicrobial drugs is an important risk factor for human VRE infection (3,7), if glycopeptides were prescribed in European hospitals at levels common in U.S. hospitals, there might be an even greater incidence of VRE infections in Europe. Likewise, given the suspected greater use of glycopeptides in U.S. hospitals, if community carriage of VRE were to increase in the United States, there might be an even greater incidence of VRE infections in U.S. hospitals.

Although the exact role of human antimicrobial use in the transmission of VRE is not known, observations from an animal model (in which mice were orally administered VRE and became only transiently colonized unless simultaneously exposed to antimicrobials [47]) support an important role for antimicrobial drugs in establishing persistent colonization. Antimicrobial drugs used in health-care settings may alter bowel flora, rendering patients more susceptible to colonization by VRE transmitted from other colonized or infected patients. Epidemiologic

evidence of foodborne VRE transmission in the community suggests that antimicrobial drugs may predispose hospitalized patients to colonization with ingested VRE. Contamination of a patient's food may occur during consumption by a variety of mechanisms, including contamination with VRE from the hands of the patient or health-care worker. In areas where VRE is also found in the animal food supply, contamination may also occur during processing by contact with VRE from the bowel flora of the food animal.

In addition to predisposing patients to colonization, some antimicrobial drugs appear to increase the number of enterococci in the stool (48) and may therefore increase the number of VRE in the stool to a level where colonization can become more readily detected by culture methods. Either of these effects could explain the findings of Van der Auwera et al.(49), who described changes in the bowel flora of 22 healthy human volunteers in Belgium who were administered oral glycopeptides (teicoplanin or vancomycin) for 3 weeks in 1989. None had VRE recovered from their stool before exposure to the oral glycopeptide; whereas after such exposure, VRE was recovered from 64% of the volunteers (49). Although this detection of VRE may have represented new colonization from a contaminated food supply or unidentified occupational risk (e.g., employment in a health-care setting), it appears just as probable that exposure to glycopeptides selectively increased the number of VRE already in the large intestine to a level where colonization became detectable.

Conclusion

Questions remain regarding the human and animal origins of VRE. It is clear, however, that the use of glycopeptides in either animal or human VRE-colonized populations can promote colonization through increased selective pressure and changes in bowel flora. Genetically related VRE isolates have been found in livestock, animal carcasses, foods, outpatients, and hospitalized patients, strongly suggesting, if not proving, that interspecies transmission can occur and may contribute to colonization and infection in humans. The recovery of genetically similar isolates at various links along the food chain suggests that ingestion of the organism is a plausible mode of such interspecies transmission. The additional finding that *E. faecium* (the most common species of VRE) may better tolerate exposure to higher temperatures than *E. faecalis* makes survival in undercooked foods appear more plausible (50). After ingestion, factors such as a high organism load, reduced gastric acidity, or recent antimicrobial exposure could allow the organism to more readily establish persistent colonization in the human large intestine. After a VRE-colonized person is admitted to a health-care facility, additional antimicrobial exposure may enable small numbers of VRE in the large intestine to selectively increase to a point where interpatient transmission is promoted, colonization is more readily detected, and clinical infection is more likely to occur (Figure).

If livestock are an important source of VRE for humans, reducing the number of colonized

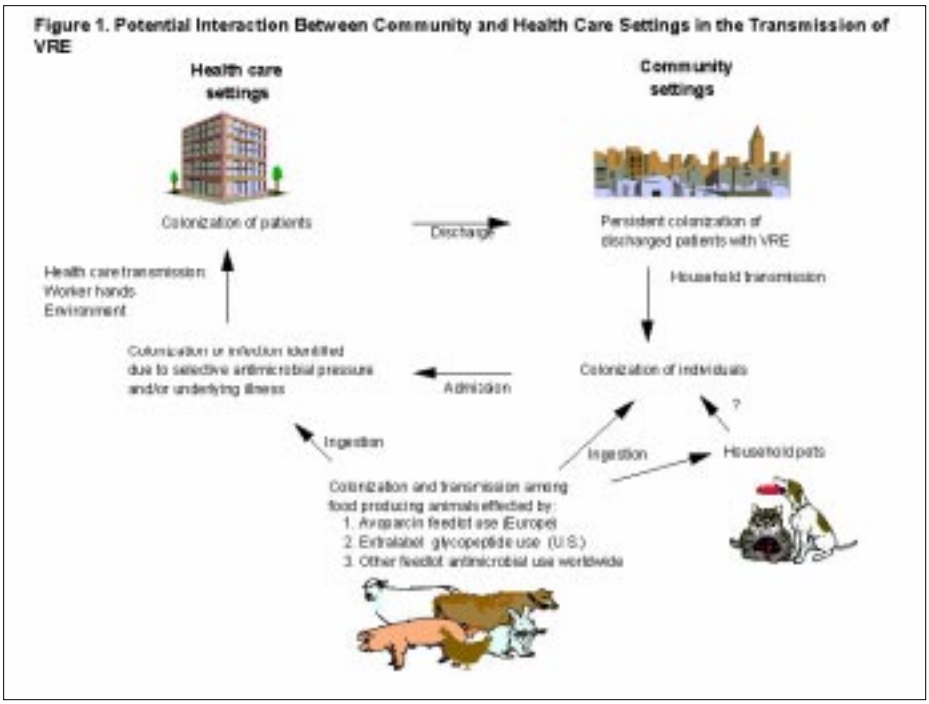


Figure. Potential interaction between community and health-care settings in the transmission of VRE.

livestock would be an effective control measure for limiting VRE infections. Since no therapy exists to eliminate VRE colonization, a rational approach would be to reduce selective pressure for the organism by limiting the use of glycopeptides. Although no glycopeptides are approved for use at subtherapeutic doses in food animals for growth promotion in the United States, vancomycin has been used therapeutically in veterinary medicine. However, a prohibition of extralabel therapeutic use of glycopeptides in food-producing animals has been announced (51). Such a ban would appear a reasonable precautionary measure against transmission of VRE within and between various animal populations and would be consistent with existing control efforts recommended by the Hospital Infection Control Practices Advisory Committee, which emphasizes prudent vancomycin use in humans (52).

The role of community transmission of VRE both within and between animal and human populations in Europe and the United States requires further study. Culture surveys for VRE among healthy human volunteers who have had no recent contact with health-care settings should be performed with sensitive culture methods. Such surveys should include family members of recently discharged patients known to be colonized or infected with VRE. Additional surveys among animal populations used for food production in the United States should also be performed. Finally, additional laboratory investigations using molecular epidemiologic methods will be required to confirm or refute present evidence for transmission of VRE between animal and human populations. If transmission of VRE from unrecognized sources can be identified and controlled, colonization of hospitalized patients may be reduced, leading to lower rates of nosocomial infections due to VRE.

Acknowledgment

We thank Frederick J. Angulo, Centers for Disease Control and Prevention, for his careful and thoughtful review of the manuscript.

Dr. McDonald is an Epidemic Intelligence Service Officer with the National Center for Infectious Diseases, CDC. His research interests include the epidemiology of nosocomial infections and antimicrobial resistance as well as diagnosis and control of these problems.

References

1. Uttley AHC, Collins CH, Naidoo J, George RC. Vancomycin-resistant enterococci. Lancet 1988;1:57-8.
2. Centers for Disease Control and Prevention. Nosocomial enterococci resistant to vancomycin—United States, 1989-1993. MMWR Morb Mortal Wkly Rep 1993;42:597-9.
3. Shay DK, Maloney SA, Monteclavo M, Banerjee S, Wormser GP, Arduino MJ, et al. Epidemiology and mortality risk of vancomycin-resistant enterococcal bloodstream infections. J Infect Dis 1995;172:993-1000.
4. Linden PK, Pasculle AW, Manez R, Kramer DJ, Fung JJ, Pinna AD, et al. Differences in outcomes for patients with bacteremia due to vancomycin-resistant *Enterococcus faecium* or vancomycin-susceptible *Enterococcus faecium*. Clin Infect Dis 1996;22:663-70.
5. Handwerger S, Raucher B, Altarac D, Monka J, Marchione S, Singh KV, et al. Nosocomial outbreak due to *E. faecium* highly resisitant to vancomycin, penicillin, and gentamicin. Clin Inf Dis 1993;16:750-5.
6. Montecalvo MA, Horowitz H, Gedris C, Carbonaro C, Tenover FC, Issah A, et al. Outbreak of vancomycin, ampicillin and aminoglycoside-resistant *Enterococcus faecium* bacteremia in an adult oncology unit. Antimicrob Agents Chemother 1994;38:1363-7.
7. Edmond MB, Ober JF, Weinbaum JL, Pfaller MA, Hwang T, Sanford MD, et al. Vancomycin-resistant *Enterococcus faecium* bacteremia: risk factors for infection. Clin Inf Dis 1995;20:1126-33.
8. Chow JW, Kuritza A, Shlaes DM, Green M, Sahm DF, Zervos MJ. Clonal spread of vancomycin-resistant *Enterococcus faecium* between patients in three hospitals in two states. 1993;31:1609-11.
9. Sader HS, Pfaller MA, Tenover FC, Hollis RJ, Jones RN. Evaluation and characterization of multiresistant *Enterococcus faecium* from 12 U.S. medical centers. J Clin Microbiol 1994;32:2840-2.
10. Morris JG, Shay DK, Hebden JN, McCarter RJ, Perdue BE, Jarvis W, et al. Enterococci resistant to multiple antimicrobial agents, including vancomycin. Establishment of endemicity in a university medical center. Ann Intern Med 1995;123:250-9.
11. Jordens JZ, Bates J, Griffiths DT. Faecal carriage and nosocomial spread of vancomycin-resistant *Enterococcus faecium*. J Antimicrob Chemother 1994;34:515-28.
12. Donnelly JP, Voss A, Witte W, Murray B. Does the use in animals of antimicrobial agents, including glycopeptide antibiotics, influence the efficacy of antimicrobial therapy in humans? [letter]. Antimicrob Chemother 1996;37:389-90.
13. Gordts B, Claeys K, Jannes H, Van Landuyt HW. Are vancomycin resistant enterococci (VRE) normal inhabitants of the GI tract of hospitalized patients? [abstract]. In: Program and Abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando. Washington (DC): American Society for Microbiology; 1994. p. 145.

14. Endtz HA, Belkum N, Braak, N, Duin J, Kluijtmans J, Koeleman J, et al. Prevalence of vancomycin-resistant enterococci in hospital and community based patients in the Netherlands [abstract]. In: Program and Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans. Washington (DC): American Society for Microbiology; 1996. p. 37.
15. Bogaard A, London N, Driessen C, Stobberingh E. Prevalence of resistant fecal bacteria in turkeys, turkey farmers and turkey slaughterers [abstract]. In: Program and Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans. Washington (DC): American Society for Microbiology; 1996. p. 86.
16. Bates J, Jordens JZ, Selkon JB. Evidence for an animal origin of vancomycin-resistant enterococci [letter]. Lancet 1993;342:490-1.
17. Klare I, Heier H, Claus H, Witte, W. Environmental strains of *Enterococcus faecium* with inducible high-level resistance to glycopeptides. FEMS Microbiol Lett 1993;106:23-30.
18. Bates J, Jordens JZ, Griffiths DT. Farm animals as a putative reservoir for vancomycin-resistant enterococci infection in man. J Antimicrob Chemother 1994;34:507-16.
19. Klare I, Heier H, Claus H, Witte W. VanA-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol Lett 1995;125:165-72.
20. Aarestrup FM. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. Microb Drug Resist 1995;1:255-7.
21. Aarestrup FM, Ahrens P, Madsen M, Pallesen LV, Poulsen RL, Westh H. Glycopeptide susceptibility among Danish *Enterococcus faecium* and *Enterococcus faecalis* isolates of animal and human origin. Antimicrob Agents Chemother 1996;40:1938-40.
22. Kruse H, Rorvik LM. The use of avoparcin as a growth promoter and the occurrence of vancomycin resistant *Enterococcus* spp. in poultry production [abstract]. In: Program and Abstracts of the 96th General Meeting of the American Society for Microbiology, New Orleans. Washington (DC): American Society for Microbiology; 1996. p. 36.
23. Klare I, Heier H, Claus H, Böhme G, Marin S, Seltmann G, et al. *Enterococcus faecium* strains with *van*A-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community. Microb Drug Resist 1995;1:265-73.
24. Arthur M, Molinas C, Depardieu F, Courvalin P. Characterization of Tn*1546*, Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J Bacteriol 1993;175:117-27.
25. Handwerger S, Skoble J. Identification of a chromosomal mobile element conferring high-level vancomycin resistance in *Enterococcus faecium*. Antimicrob Agents Chemother 1995;39:2446-53.
26. Coque TM, Tomayko JF, Ricke SC, Okhyusen PC, Murray BE, et al. Vancomycin-resistant enterococci from nosocomial, community, and animal sources in the United States. Antimicrob Agents Chemother 1996;40:2605-9.
27. Bais RK, Freundlich LF, Currie BP. Outpatient prevalence of vancomycin-resistant enterococcal (VRE) enteric colonization in the catchment area of a hospital hyperendemic for VRE [abstract]. Infect Control Hosp Epidemiol 1996;17:20.
28. Landman D, Quale JM, Oydna E, Willey B, Ditore V, Zaman M, et al. Comparison of five selective media for identifying fecal carriage of vancomycin-resistant enterococci. J Clin Microbiol 1996;34:751-2.
29. Mato R, Delencastre H, Roberts RB, Tomasz A. Multiplicity of genetic backgrounds among vancomycin-resistant *Enterococcus faecium* isolates recovered from an outbreak in a New York city hospital. Microb Drug Resist 1996;2:309-17.
30. Boyle JF, Soumakis SA, Rendo A, Herrington JA, Gianarkis DG, Thurberg BE, et al. Epidemiologic analysis and genotypic characterization of a nosocomial outbreak of vancomycin-resistant enterococci. J Clin Microbiol 1993;31:1280-5.
31. Bontin, M, Slaughter S, Hayden M, Nathan C, Van Voorhis J, Rice T, et al. Patients' endogenous flora as a source of "nosocomial" vancomycin-resistant enterococci (VRE) [abstract]. In: Program and Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans. Washington (DC): American Society for Microbiology; 1996. p. 219.
32. Coque TM, Arduino RC, Murray BE. High-level resistance to aminoglycosides: comparison of community and nosocomial fecal isolates of enterococci. Clin Infect Dis 1995;20:1048-51.
33. Thal LA, Welton LA, Perri MB, Donabedian S, McMahon J, Chow JW, et al. Antimicrobial resistance in enterococci isolated from turkeys fed virginamycin [abstract]. In Program and Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans. Washington (DC): American Society for Microbiology; 1996. p. 55.
34. Thal LA, Chow JW, Mahayni R, Bonilla H, Perri MB, Donabedian SA, et al. Characterization of antimicrobial resistance in enterococci of animal origin. Antimicrob Agents Chemother 1995;39:2112.
35. Harrison TS, Qaiyumi S, Morris JG, Bonilla H, Perri MB, Donabedian SA, et al. Incidence of multiply resistant enterococci in poultry [abstract]. In: Program and Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco. Washington (DC): American Society for Microbiology; 1995. p. 271.
36. Dunne WM, Dunne BS, Smith D. Watch out where the huskies go. American Society for Microbiology News 1996;62:283.
37. Van Belkum A, van den Braak N, Thomassen R, Verbrugh H, Endtz H. Vancomycin-resistant entero-cocci in cats and dogs [letter]. Lancet 1996;348:1038-1039.

38. Devriese LA, Ieven M, Goossens H, Vandamme P, Pot B, Hommez J, et al. Presence of vancomycin-resistant enterococci in farm and pet animals. Antimicrob Agents Chemother 1996;40:2285-87.
39. Shekar R, Chico G, Bass SN, Strozewski K, Biddle J. Household transmission of vancomycin-resistant *Enterococcus faecium*. Clin Infect Dis 1995;21:1511-2.
40. Howarth F, Poulter D. Vancomycin resistance: time to ban avoparcin? [letter]. Lancet 1996;347:1047.
41. Commission directive 97/6/EC of 30, January 1997 amending council directive 70/524/EEC concerning additives in feeding stuffs. Official Journal of the European Communities 1997;35:11-13.
42. Food and Drug Administration. Guilty verdict in veal feed case. Center for Veterinary Medicine [online] 1996; [cited 1996 Aug 29] [1 screen]. Available from: URL: http://www.cvm.fda.gov/fda/infores/updates/vitek.html
43. Hayes PW, RH Gustaeson, FK Lotgering, et al. Reply to: Does the use in animals of antimicrobial agents, including glycopeptide antibiotics, influence the efficacy of antimicrobial therapy in humans? [letter] J Antimicrob Chemother 1996;37:390-2.
44. Mudd A. Vancomycin resistance and avoparcin [letter]. Lancet 1996;347:1312.
45. Ena J, Dick RW, Jones RN, Wenzel RP. The epidemiology of intravenous vancomycin usage in a university hospital. JAMA 1993;269:598-602.
46. Swartz MN. Hospital-acquired infections: diseases with increasingly limited therapies. Proc Natl Acad Sci U S A 1994;91:2420-7.
47. Whitman MS, Pitsakis PG, DeJesus E, Osborne AJ, Levison ME, Johnson CC. Gastrointestinal tract colonization with vancomycin-resistant *Enterococcus faecium* in an animal model. Antimicrob Agents Chemother 1996;40:1526-30.
48. Suppola JP, Volin L, Valtonen VV, Vaara M. Overgrowth of *Enterococcus faecium* in the feces of patients with hematologic malignancies. Clin Infect Dis 1996;23:694-7.
49. Van der Auwera P, Pensart N, Korten V, Murray BE, Leclercq R. Influence of oral glycopeptides on the fecal flora of human volunteers: selection of highly glyco-peptide-resistant enterococci. J Infect Dis 1996;173:1129-36.
50. Panagea S, Chadwick PR. Heat tolerance of vancomycin resistant *Enterococcus faecium*. J Clin Pathol 1996;49:687-9.
51. Food and Drug Administration. Extralabel animal drug use; fluroquinolones and glycopeptides; notice of order of prohibition. Federal Register 1997;62:2744-7.
52. Centers for Disease Control and Prevention. Hospital Infection Control Practices Advisory Committee's recommendations for preventing the spread of vancomycin resistance. MMWR Morb Mortal Wkly Rep 1995;44:1-13.

Flea-borne Rickettsioses: Ecologic Considerations

Abdu F. Azad, Suzana Radulovic, James A. Higgins, B. H. Noden, and Jill M. Troyer
University of Maryland School of Medicine, Baltimore, Maryland, USA

Ecologic and economic factors, as well as changes in human behavior, have resulted in the emergence of new and the reemergence of existing but forgotten infectious diseases during the past 20 years. Flea-borne disease organisms (e.g., *Yersinia pestis, Rickettsia typhi, R. felis,* and *Bartonella henselae*) are widely distributed throughout the world in endemic-disease foci, where components of the enzootic cycle are present. However, flea-borne diseases could reemerge in epidemic form because of changes in vector-host ecology due to environmental and human behavior modification. The changing ecology of murine typhus in southern California and Texas over the past 30 years is a good example of urban and suburban expansion affecting infectious disease outbreaks. In these areas, the classic rat-flea-rat cycle of *R. typhi* has been replaced by a peridomestic animal cycle involving, e.g., free-ranging cats, dogs, and opossums and their fleas. In addition to the vector-host components of the murine typhus cycle, we have uncovered a second typhuslike rickettsia, *R. felis*. This agent was identified from the blood of a hospitalized febrile patient and from opossums and their fleas. We reviewed the ecology of *R. typhi* and *R. felis* and present recent data relevant to the vector biology, immunology, and molecular characterization and phylogeny of flea-borne rickettsioses.

A complex matrix of ecologic and economic factors and human behavior changes has resulted in the emergence of new infectious diseases during the past 20 years. Ecologic changes lead to the emergence of both known and as yet unknown pathogens circulating in the complex host and vector systems of undisturbed habitats. A drastic increase of Rocky Mountain spotted fever in the late 1970s, Lyme disease in the early 1980s, and ehrlichioses in the 1990s in the United States attests to the strong correlation of these diseases to human-made ecologic changes and further illustrates the inability of existing monitoring systems to predict outbreaks and protect at-risk populations. Unmanaged growth and expansion of the suburbs into undisturbed habitats have generated ideal ecosystems for many displaced animals. The changing ecology of murine typhus in southern California and Texas over the past 30 years is a good example of suburban expansion and environmental modifications affecting infectious disease outbreaks. In suburban areas, vector fleas are most often associated with human habitation through their natural hosts, e.g., commensal rodents and peridomestic animals, such as free-ranging cats and dogs, opossums, raccoons, and squirrels. Fleas commonly found on these animals are picked up by household pets and brought into homes. These fleas, apart from being a nuisance, may carry pathogenic organisms of concern to human health.

Of the 2,000 species and subspecies of fleas, only a handful serve as vectors of human diseases. Several bacterial pathogens of public health importance are maintained and transmitted by fleas, among them, *Yersinia pestis*, the causative agent of plague (known in history as black death). Flea-borne human pathogens are maintained in a zoonotic cycle involving mammalian hosts and fleas. They seldom cause overt disease in their natural hosts but commonly result in clinical disease, e.g., plague, murine typhus, and cat-scratch disease, in humans. The rapid spread of pathogens to human populations is due to the frequent feeding behavior and

Address for correspondence: Abdu F. Azad, Department of Microbiology and Immunology, University of Maryland School of Medicine, 655 W. Baltimore Street, Baltimore, MD 21201 USA; fax: 410-706-0282; e-mail: aazad@umabnet.ab.umd.edu.

extraordinary mobility of fleas. Flea-borne diseases are widely distributed throughout the world, in the form of endemic foci, where components of the cycle are present; however, these diseases become epidemic in human populations when infected mammalian hosts die and their fleas leave in search of a bloodmeal. Of flea-borne bacterial pathogens (e.g., *Y. pestis, Rickettsia typhi, R. felis,* and *Bartonella henselae*), the most studied and reviewed is *Y. pestis*. In this article, however, we want to examine the agents of flea-borne rickettsioses, namely *R. typhi* and the newly identified *R. felis*. Beginning with *R. typhi* ecology, we will review and discuss recent data relevant to the ecology, vector biology, immunologic and molecular characterization, and phylogeny of the flea-borne rickettsiae.

Natural History of Flea-borne Rickettsioses

"Murine typhus is a good example of a disease whose importance is not adequately appreciated—except by the patient, and, even today, in most parts of the world, he will never know what ails him because the diagnosis will not be made" (1). Murine typhus is one of the most widely distributed arthropod-borne infections endemic in many coastal areas and ports throughout the world. It occurs in epidemics or with high prevalence; is often unrecognized and substantially underreported; and, although it can be clinically mild, it can also cause severe illness and death (2,3). The severity of murine typhus infection has been associated with old age, delayed diagnosis, hepatic and renal dysfunction, central nervous system abnormalities, and pulmonary compromise. Death occurs in up to 4% of hospitalized patients (3). Thousands of human cases were reported annually in the United States (1,2). Outbreaks have been reported in Australia and recently in China, Greece, Israel, Kuwait, and Thailand (1,2,4). Recent serosurveys have demonstrated high prevalence of anti-typhus group (TG) rickettsiae in Asia and southern Europe (4). Despite the current level of reported human cases in the United States of fewer than 100 per year, murine typhus has been the subject of several recent studies (3,4). Rekindled interest in this disease is partially a result of field data and epidemiologic surveys, which have prompted a reconsideration of established components of the vector-reservoir cycle and their interaction with humans (5-7). The classic cycle of *R. typhi*, the etiologic agent of murine typhus, involves rats (*Rattus rattus* and *R. norvegicus*) and the rat flea, *Xensopsylla cheopis* (Figure 1). The flea has been considered the main vector, and the disease is transmitted by flea bites or contact with rickettsia-containing feces and tissues during or after blood feeding. Although the rat-flea-rat cycle is still the major route of human infection throughout the world, murine typhus exists in some endemic-disease foci where both rats and rat fleas are absent.

Reported cases of murine typhus in the United States are focused largely in central and southcentral Texas and the Los Angeles and Orange Counties in California; however, infected rats and their fleas are hard to document in these areas (5-7). Thirty-three cases of locally acquired murine typhus in Los Angeles County have been

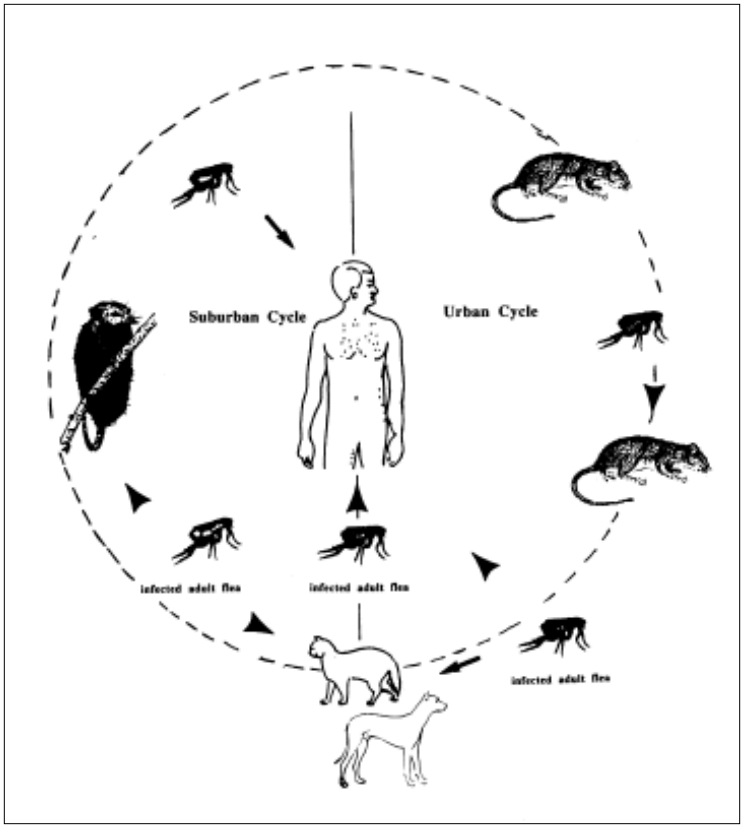


Figure 1. Urban and suburban life cycles of *Rickettsia* and mammalian hosts.

associated with seropositive domestic cats and opossums (7). More than 16 (40%) of 38 opossums and nine (90%) of 10 domestic cats collected from the case areas were seropositive for *R. typhi* antibodies. No seropositive cats (n=21) or opossums (n=36) were found in the control areas. Although flea infection was not investigated, opossums were the most heavily infested with the cat flea, *Ctencephalides felis* (104.7/animal). *C. felis* was also the most prevalent flea species (97%) collected from opossums, cats, and dogs (but not rats) in southern Texas. Surveys in other areas of the country (7,8) had similar results, which further minimizes the role of rat fleas in the maintenance of endemic typhus within the United States. The maintenance of *R. typhi* in the cat flea/opossum cycle (Figure 1) is, therefore, of potential public health importance since *C. felis* is a widespread pest that avidly bites humans (1,3,8-11).

During the past 3 years, we have investigated a new typhuslike rickettsia (initially designated as ELB and later named *R. felis*) identified in cat fleas and opossums from California and Texas murine typhus foci (8-12). Both *R. typhi* and *R. felis* were found in fleas and in opossum tissues (8-11). Additionally, a retrospective investigation of five murine typhus patients from Texas subsequently demonstrated that four patients were infected with *R. typhi* and a fifth had been infected with *R. felis* (8). Observation of human infection by *R. felis* underscores the usefulness of molecular techniques for diagnosing closely related rickettsial species. Furthermore, this documented human infection with *R. felis* and its presence in opossums and their fleas and possibly other wildlife associated with human habitation have raised concerns about the extent of *R. felis* spillover into human populations.

The Role of Household Pets and Peridomestic Animals and Their Fleas in the Maintenance of *R. typhi* and *R. felis*

Murine typhus field surveys in southern California indicated the presence of TG antibodies in opossums, striped skunks, rats, and resident cats but not in 11 other species of native mammals (5,7). The seropositive animals were associated with human cases and were heavily infested with *C. felis* fleas. Furthermore, the isolation of *R. typhi* from opossums and their fleas led these investigators to regard opossums as of epidemiologic significance. The presence of *R. felis* in cat fleas collected from opossums within the Los Angeles murine typhus focus stimulated further interest (8,10). To understand the potential role of the *R. felis* and nonrat hosts in the biology of endemic typhus, we examined the samples collected in southern Texas, a region accounting for approximately one-third of the current reported murine typhus cases in the United States. Restriction digests of polymerase chain reaction (PCR) products from 399 cat fleas collected from nine opossums had an infection rate of 3.8% for *R. felis* and 0.8% for *R. typhi*. Three of nine tested opossums were infected with *R. felis* (8,10). No *R. typhi*-infected rats (*R. norvegicus*) or rat fleas (*X. cheopis*) were found in surveyed samples. The persistence of murine typhus in both geographic foci appears to be better accounted for by infected cat fleas, opossums, and other nonrat hosts found near human populations (Figure 1, Table 1). The presence of both *R. typhi* and *R. felis* by PCR/restriction fragment length polymorphism (RFLP) in opossums from murine typhus foci in southern California and Texas thus confirms the possible role of this marsupial in the maintenance of murine typhus infection. However, our attempt to isolate either *R. felis* or *R. typhi* from PCR-positive opossum blood or spleen collected in North Carolina, Texas, and California proved unsuccessful. Although isolating rickettsiae from blood samples has proved difficult, nevertheless, our data, as well as published information, support the role of opossums in maintaining these rickettsiae and infecting fleas.

We will now focus on the role of household pets, primarily cats, in the transmission of *R. typhi*/*R. felis*. Experimental infection of cats with *R. typhi* produces a subclinical infection with rapid recovery and seroconversion (1). Cats that were used to maintain commercial cat flea colonies displayed high antibody titers to TG rickettsiae. In addition, the report by Sorvillo et al. (7) that 90% of resident cats had demonstrable anti-TG immunofluorescence antibody assay (IFA) titers prompted us to extend our seroprevalence studies to household pets to determine the role of resident and feral cats in the urban and suburban foci of murine typhus. We accomplished this through collaboration with several veterinary schools and private practice veterinarians. For the northeast U.S. cohort, 143 cat serum samples were assayed. The sources of the samples were private practice veterinarians in the northeast United States (Michigan, New York, Ohio, Pennsylvania, Tennessee, Texas, Virginia, Wisconsin),

Table 1. Results of PCR/RFLP testing of fleas for typhus group rickettsiae

Species	Host/Location (Date)	*Rickettsia typhi* / *R. felis*[a]
Ctenocephalides felis	Opossum/Corpus Christi,TX(1991)	*R. felis*
C. felis	Opossum/Corpus Christi,TX(1991)	*R. typhi*
C. felis	Opossum/Los Angeles, CA(1991)	*R. felis*
C. felis	Opossum/Los Angeles, CA(1991)	*R. typhi*
Polygenis gwyni	Opossum/Statesboro, GA (1994)	-/-
P. gwyni	Opossum/Statesboro, GA (1996)	-/-
Pulex simulans	Opossum/Statesboro, GA (1993)	-/-
Pulex irritans	Dog/Statesboro, GA (1996)	*R. felis*
C. felis	Opossum/Nashville, TN (1987)	*R. felis*
C. felis	Opossum/Statesboro, GA (1993)	*R. felis*
C. felis	Opossum/Statesboro, GA (1994)	*R. felis*
C. felis	Opossum/Statesboro, GA (1996)	*R. felis*
C. felis	Dog/Statesboro, GA (1996)	*R. felis*
C. felis	Bobcat/Statesboro, GA (1994)	*R. felis*

[a]The procedure for detecting *R. typhi* and *R. felis* in fleas followed described protocols (6,11).

who had submitted them to the Cornell University College of Veterinary Medicine, Clinical Microbiology laboratory, for testing for common feline pathogens (e.g., feline infectious peritonitis virus and feline leukemia virus). For the North Carolina cohort, 513 serum samples were tested. These were banked samples, originally collected from native cats, for use in a study of *B. henselae* seroprevalence (E.B. Breitschwerdt et al., unpub. data). IFA was used to screen cat sera for reactivity against *R. typhi* (Wilmington strain) at a 1/64 dilution. Because of the serologic cross-reactivity between *R. typhi* and *R. felis*, we have performed the definitive epitope blocking enzyme-linked immunosorbent assay (DEB-ELISA) using monoclonal anti-*R. typhi* 120 kDa surface protein antigen to rule out seropositivity to *R. typhi*. DEB-ELISA was performed on samples with an IFA titer of 1/64 (n=31 for northeast U.S. cats, n=49 for North Carolina cats) (13). For the northeast U.S. cohort, 143 samples were tested by IFA and 31 (21%) were seropositive. When these 31 positive samples were subjected to DEB-ELISA, 8 (25%) were positive, i.e., reactivity was not directed against *R. typhi*. Of 513 North Carolina cohort serum samples tested by IFA, 93 (18.1%) were seropositive at 1:64, and 49 were immunoreactive against an organism(s) other than *R. typhi*. Lack of *R. felis* monoclonal antibodies limited the usefulness of the DEB-ELISA in determining the source of infection in cats with high antibody titer against TG rickettsiae. Similar problems have been encountered in attempting to serologically identify the rickettsial species contributing to an unexplained febrile illness of dogs in the southern United States (14). Because of the antigenic cross-reactivity among the TG rickettsiae (*R. prowazekii*, *R. typhi*, and *R. canada*), and between the TG rickettsiae, *R. felis*, and *R. bellii*, serologic tests should be complemented by PCR/RFLP and/or direct isolation of rickettsiae by tissue culture.

A combination of IFA and DEB-ELISA used in our serosurvey represents the first attempt to define seroreactivity to TG rickettsiae among resident and feral cats. The positivity rate for these samples was unexpectedly high (18% to 21%), even though the sources of the samples were sick, febrile cats. The public health implications of these data are unclear. It is estimated that approximately one third of U.S. households have a pet cat; this translates into 57 million animals. If we conservatively estimate that only 5% of these animals have a patent rickettsial infection, 285,000 cats are possibly infected with a life-threatening zoonosis. Despite the serologic evidence presented above, the question of whether cats can serve as a reservoir host for *R. typhi* and *R. felis* and a source of infection for fleas remains to be elucidated.

R. typhi and *R. felis* Infection in Fleas

Cat fleas from eight commercial colonies in various regions of the United States were infected with *R. felis* (11). The infection rates as determined by selective PCR amplification and subsequent restriction digest analysis and Southern hybridization of PCR products were 43% to 93%. These flea colonies were initiated either with fleas from one supplier, in which *R. felis* was first identified (15), or with fleas from stray cats and dogs (11). In light of the latter study (11), we recently received alcohol-preserved flea samples from L. Durden (Georgia Southern University), collected from opossums, dogs, and a bobcat in Statesboro, Georgia, and Nashville, Tennessee, between 1986 and 1996. After PCR/RFLP and partial sequencing of selected PCR products, we found that more than 50% of the cat fleas

obtained from the vertebrate hosts were infected with *R. felis*. However, none of the other flea species collected from the same hosts were infected (Table 1). Although *R. felis* has been detected in cat fleas from nine states (California, Florida, Georgia, Louisiana, New York, North Carolina, Oklahoma, Tennessee, and Texas), it is probably more widespread than current data indicate. These findings demonstrate the wide distribution of *R. felis* in suburban habitats where opossums enter human habitations and share fleas with domestic dogs and cats.

Experimental infections of various laboratory colonies of fleas, e.g., *X. cheopis*, *Leptopsylla segnis*, and *C. felis*, have demonstrated a similarity in the acquisition, propagation, dissemination, and transmission of *R. typhi* (2,16). Infection in the flea is initiated when the rickettsiae, ingested in a bloodmeal, enter the midgut epithelial cells. Three to four days after infection, rickettsiae can be detected in only a small group of midgut epithelial cells (Figure 2A). Over the next 3 to 5 days, rickettsial numbers increase exponentially and spread from the initial sites of infection to the entire midgut epithelial linings. Ten days must elapse before the infected fleas can transmit *R. typhi* to susceptible hosts through infectious feces. The dynamics of *R. felis* infection have not been studied in detail experimentally, but earlier electron microscopy using infected fleas demonstrates the presence of this rickettsia in gut epithelial linings, tracheal matrix, muscle, ovaries, and epithelial sheath of the testes (15). The high rates of infection in laboratory colonies of fleas and the presence of *R. felis* in their eggs and newly emerged nonblood-fed specimens indicate that the maintenance of this rickettsiosis occurs by transovarial transmission. However, fleas may acquire both *R. typhi* and *R. felis* from rickettsemic hosts and then pass on the infection to their progeny by transovarial transmission. Neither *R. typhi* nor *R. felis* infection is lethal to fleas. There is no evidence that massive infection of flea midgut (Figure 2B) affects the feeding behavior and survival of the infected fleas. In contrast, *X. cheopis* fleas infected with *Y. pestis* starve to death while the human body lice infected with *R. prowazekii* die of the infection within 2 weeks (17). Neither *Y. pestis* nor *R. prowazekii* is maintained transovarially, and in contrast to rickettsia-infected fleas, persistence of these organisms requires constant host turnover to allow the infection cycle to perpetuate in nature. The rickettsial relationship with their arthropod hosts is considered symbiotic, yet in other instances, they act as true parasites; e.g., members of the *Rickettsia* and *Wolbachia* alter reproduction and manipulate cellular processes in their hosts (18).

Calculations based on the quantity of rickettsiae in the blood of a rickettsemic rat and the minute volume of blood ingested by fleas indicate that the invasion of flea midgut epithelium by *R. typhi* is extremely efficient, requiring only a few rickettsial organisms to result in infection (19). During the rickettsemic period in the

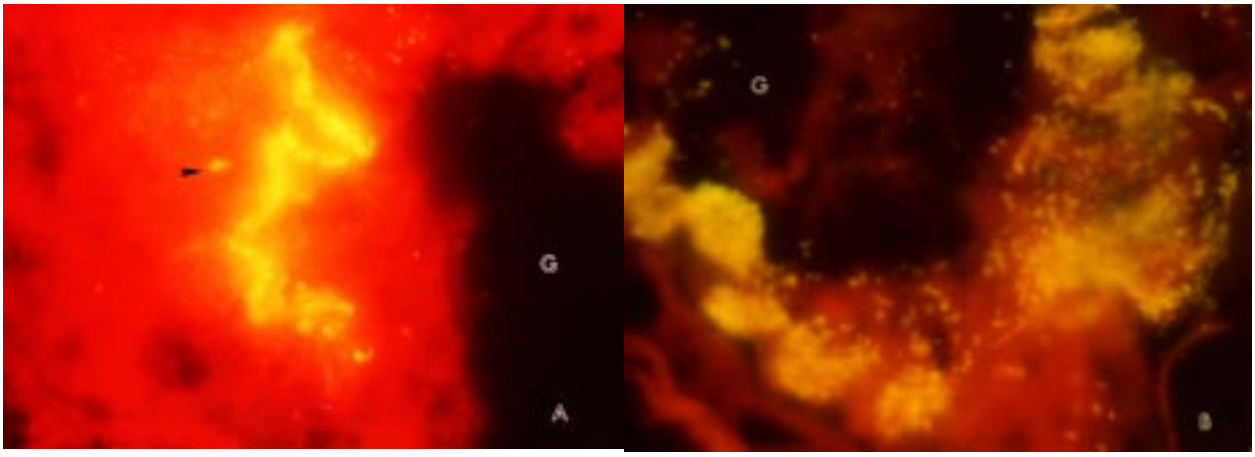


Figure 2. Direct fluorescent staining of the frozen sections of midguts of *X. cheopis* fleas showing *R. typhi*-infected epithelial cells at 3 (A) and 10 days (B) postinfectious feeding. Fleas were embedded individually in OCT compound (Miles Laboratories, Naperville, IL), sectioned (4-6 m)(16), and stained with fluorescein isothiocyanate (FITC)-labeled guinea pig anti-*R. typhi* IgG. G: gut lumen.

vertebrate host, *R. typhi* is found only in the cellular portion of the blood, particularly leukocytes. These blood cells are destroyed very rapidly (ca. 6 hours) within the flea gut (19). Experimental studies demonstrate that the rickettsial infection of flea midgut epithelium is facilitated by the flea's digestive processes. Presumably, the rapid liquefaction of the blood liberates intracellular rickettsiae from within infected blood cells. Since no peritrophic membrane is formed around the bloodmeal, there is no physical barrier to prevent freed rickettsiae from contacting and entering flea midgut epithelium. Therefore, the rapid breakdown in the cellular integrity of blood components within the flea gut lumen is probably an essential feature of the rickettsial infection of fleas. However, the biologic aspects of rickettsia-vector interactions has lagged behind other vector-borne disease studies, and only a few of the underlying phenomena involved in rickettsia-vector interactions have been partially elucidated.

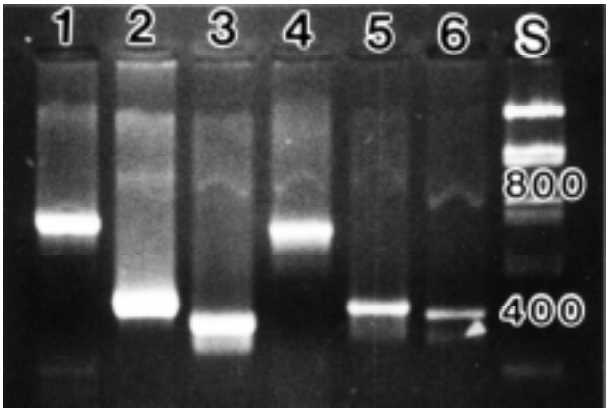


Figure 3. Amplicons for various flea-borne bacterial pathogens. Lane 1: *R. typhi* 16SrRNA (600bp); Lane 2: *R. felis* 17 kDa (434bp); Lane 3: *R. typhi* citrate synthase(384bp); Lane 4: *R. typhi* 120 kDa (612bp); Lane 5: *B. henselae* 60 kDa (414bp); Lane 6: flea 12SrRNA (414bp); and Lane S: DNA standards, with 400 and 800bp sizes indicated.

Antigenic Characterization and Phylogeny of *R. felis*

The isolation of *R. felis* from cat flea homogenates after sequential passage from infected rat spleens through embryonated chicken eggs (20) allowed its further characterization. The identity of *R. felis* in tissue samples and fleas was achieved by a combination of PCR/RFLP (with primers based on the 17 and 120 kDa antigens, citrate synthase, and 16S rRNA gene sequences) (Figure 3), the lack of rOmpA, and seroreactivity with monospecific sera and monoclonal antibodies (Table 2,3). Furthermore, *R. felis* grown in culture more strongly resembles TG than spotted fever group rickettsiae in its morphology by light microscopy, growth pattern in Vero cells, and delayed formation of small plaques (20). Immunologic characterization of *R. felis* by a battery of monoclonal and polyclonal antibodies showed various degrees of reactivity with TG group and in particular *R. typhi* (Table 2). The T65-1 monoclonal antibody that recognizes 120 kDa surface protein of *R. typhi*, but not *R. prowazekii* or *R. canada*, reacts weakly with *R. felis*. Spotted fever group polyclonal or monoclonal antibodies also exhibited lower reactivity with *R. felis*. The SDS-PAGE profile obtained for *R. felis* here clearly resembled that of *R. typhi* and *R. akari*, lacking the prominent larger rOmpA protein found in spotted fever group rickettsiae and *R. canada* (Figure 4; 21). Immunoblot studies (20) using anti-*R. felis* polyclonal antibodies further demonstrate a strong cross-reactivity with *R. typhi* lipopolysaccharides but not spotted fever group rickettsiae.

The initial *R. felis* characterization of the sequence of a portion of the 17 kDa gene and its reactivity with anti-*R. typhi* polyclonal and monoclonal antibodies suggested resemblance to typhus rickettsiae (Table 3; 20). However, its 16S rRNA sequence more closely resembled those of *R. akari* and *R. australis*, a distinct clade of the spotted fever group (22,23). PCR amplification with rOmpA primers Rr.190.70p and Rr.190.602n

Table 2. Reactivity of *Rickettsia felis*, *R. typhi*, and *R. akari* with rat and mouse typing sera and species-specific monoclonal antibodies

		Reciprocal IFA titer		
MAbs/Antisera	Antigen	*R. felis*	*R. typhi*	*R. akari*
T65-1 (IgG2a)[a]	120kDa	64	8,192	16
77-244-20 b	120-135kDa	16	16	4,096
7911-A2-H9 (IgG2a)[c]	190kDa	16	16	16
1F3-G2 (IgG2a)[d]	120-135kDa	64	128	16
Rat anti-*R. felis*		4,096	2,048	256
Rat anti-*R.typhi*		1,024	8,192	16
Mouse anti-*R. akari*		512	16	4,096

[a]MAb against *R. typhi* 120kDa (also referred to rOmpB).
[b]MAb against *R. akari*.
[c]MAb against *R. rickettsii* 190kDa (= rOmpA).
[d]MAb against *R. rickettsii* 120-135kDa (= rOmpB).

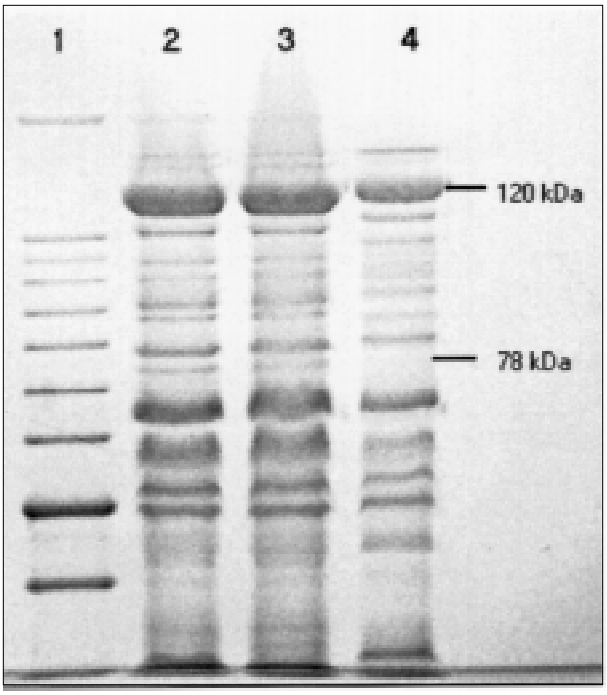


Figure 4. Coomassie blue stained polypeptide profiles of the *R. felis*, *R. typhi*, and *R. akari* plaque-purified seeds separated by SDS-PAGE (7.5%). Lane 1: 10kDa molecular mass marker, with 78 and 120kDa sizes indicated; Lane 2: *R. felis*; Lane 3: *R. typhi* (Wilmington); and Lane 4: *R. akari* (Kaplan).

was unsuccessful with *R. felis* (21). This result is perhaps not surprising since these primers also do not amplify DNA from typhus rickettsiae, *R. akari*, or *R. australis* (22,23). Recently, Andersson et al (24) reported the presence of rOmpA gene sequences in *R. prowazekii* genome, even though there is no evidence that the 190kDa protein is expressed in TG rickettsiae.

For phylogenetic studies, nucleotide sequences of all known rickettsial Citrate synthases, 17kDa surface antigen genes, and 16S r RNA sequences were obtained from Genbank and initially aligned by PILEUP (Genetics Computer Group, Madison WI) according to the progressive alignment algorithm of Feng and Doolittle (25). Nonoverlapping sequences at the trailing ends of the resulting multiple sequence file were removed and the sequences were realigned by PILEUP to give the final multiple sequence file. A distance matrix for all sequences was calculated by DNADIST, with differences between transition and transversion rates corrected according to Kimura's method (26). Evolutionary trees were computed by DNAPARS using parsimony, with the number of changes of base needed on a given tree calculated according to Fitch (27). Bootstrap analysis was performed on a 100x resampled set using SEQBOOT, with a 100x randomized input order, and the consensus tree topology was calculated by CONSENSE. The resulting cladograms for 16S rRNA and citrate synthase positioned *R. felis* within the same clade as *R. akari*, *R. australis*, and *R. helvetica*, and identified it as belonging to the spotted fever group of rickettsiae (22,23). The consensus tree for 16S rRNA is illustrated in Figure 5. The branching order within the clade comprising *R. felis*, *R. helvetica*, *R. australis*, and *R. akari* altered between different parsimony trees; however, the overall association was relatively stable. The sequence information from 17 kDa protein antigen, rOmpA, 16S rRNA, and citrate synthase genes places *R. felis* intermediate to the typhus and spotted fever group rickettsiae. The typhus and spotted fever group dichotomy, however, does not adequately reflect the evolutionary history of *R. akari*, *R. felis*, or *R. australis*.

Future Perspectives of Typhus Infections

Traditionally TG rickettsiae have been defined by antigenic characteristics of their

Table 3. Comparison of *Rickettsia felis* with other vector-borne Rickettsiae

Species	Vector	190 kDa[a]	120/ 135kDa[b]	17 kDa[c]	Hemolysis/ plaque[d]	Phylogeny[e]
R. prowazekii	louse	-	+	+	+/+	TG
R. typhi	flea	-	+	+	+/+	TG
R. canada	tick	-	+	+	-/-	TG
R. felis	flea	-	+	+	+/+	SFG
R. akari	mite	+	+	+	-/+	SFG
R. australis	tick	-	+	+	-/+	SFG
R. rickettsii	tick	+	+	+	-/+	SFG
R. conorii	tick	+	+	+	-/+	SFG

[a]rOmpA (rickettsial outer membrane protein A). Although the presence of rOmpA gene sequences or gene product have not been shown in TG rickettsiae recently, DNA sequences corresponding to rOmpA were shown in the genome of *R. prowazekii* (Ref 24).

[b]Also referred to rOmpB.

[c]Also referred to as rickettsial inner membrane protein A.

[d]Hemolysis of sheep red cells/plaque formation.

[e]Typhus group/spotted fever group rickettsiae.

+ indicates expression of gene products/growth characteristic.

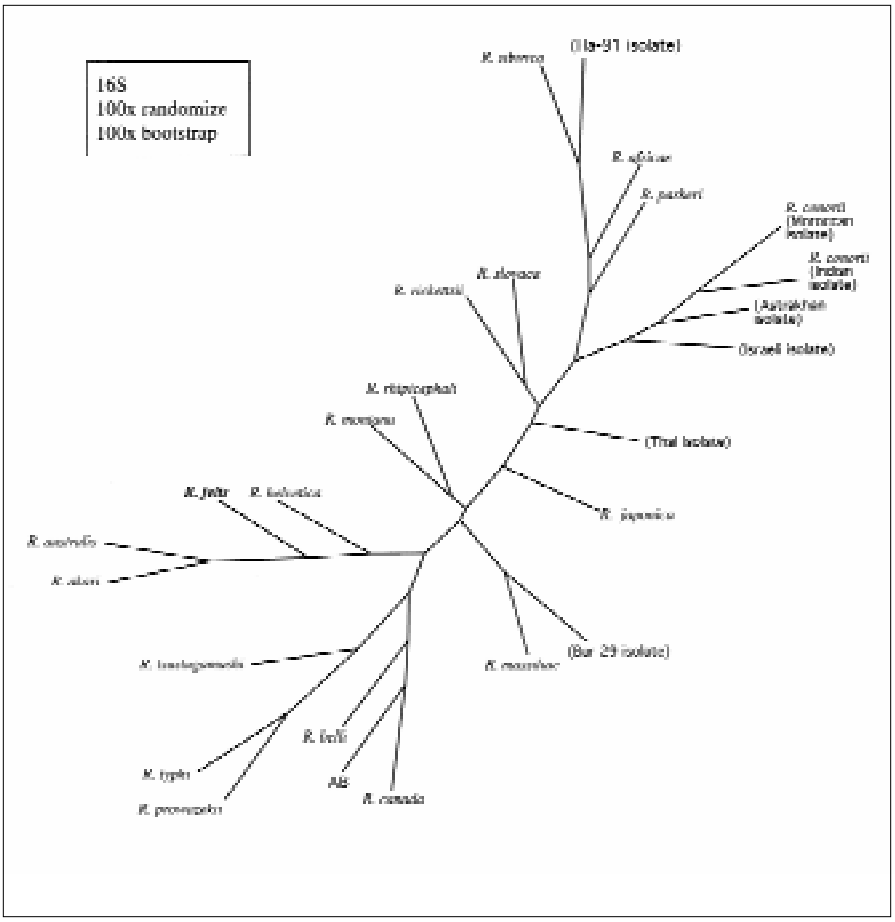


Figure 5. Consensus parsimony cladogram of rickettsial 16S ribosomal RNA sequences shows that *R. felis* (in bold) is a member of the spotted fever group.

lipopolysaccharides (4), but no fine line separates them from spotted fever group rickettsiae. Similarly the classification of rickettsiae on the basis of molecular and immunologic characterizations is also problematic because of high sequence homology and serologic crossreactivity within and between the members of TG and spotted fever group. Distinct biologic differences, however, occur between TG and SFG rickettsiae with regard to arthropod vectors, in vitro growth, antigenic repertoire, pathologic features, and clinical manifestations. Although the diagnosis can be made serologically and confirmed clinically for most of the pathogenic rickettsiae, it is unlikely to serologically distinguish *R. felis* from *R. typhi* infections. The extent of human infections with *R. felis* is unknown at this time, and the disease needs to be studied clinically. The detection and identification of *R. felis* in a single human case has been carried out by PCR/RFLP and Southern hybridization (8). The detection of both *R. typhi* and *R. felis* presents a difficult diagnostic challenge since prompt diagnosis of murine typhus or infections with *R. felis* can be established only when rickettsiae or rickettsia-PCR products from blood samples are directly isolated. DEB-ELISA using monoclonal antibodies specific to either *R. typhi* or *R. felis* would be a useful tool to differentiate between these rickettsiae and closely related species.

Reported cases of murine typhus in the United States are largely focused in central and southcentral Texas and Los Angeles and Orange Counties in California. However, murine typhus-infected rats and rat fleas are hard to document within these foci, which suggests the maintenance of *R. typhi* in the cat flea/opossum cycle. Destruction and reduction of natural habitats displace many animals and force them to move into the hospitable environments of the suburbs and cities and subsequently increase the potential for "old and new pathogens" to reemerge and generate new outbreaks. The current distribution of the opossum in more than 40 states in the United States and the invasion of urban and suburban habitats by this opportunistic marsupial have also been aided by human activities. Opossums, freeranging cats, and rats in urban and suburban habitats, where food and hospitable environments are plentiful, may live their entire lives in the same backyards. Therefore, their potential role in the murine typhus cycle as hosts to both *R. typhi*, *R. felis*, and their fleas warrants further investigation.

Acknowledgments

We thank Drs. G. A. Dasch, D. H. Walker, and J. A. Olson for the generous gift of monoclonal antibodies; Drs. E.

B. Breistchwerdt and Richard Jacobson for providing the cat serum samples; Dr. Colin Hunter for the phylogenetic analyses; and Mr. Kashif Firozvi for his technical assistance with the cat serology. This research was supported by a grant from the National Institutes of Health (AI 17828).

Dr. Azad is professor of microbiology and immunology, University of Maryland School of Medicine, Baltimore. His laboratory research focuses on host parasite interactions with particular interest in the molecular aspects of rickettsial pathogenesis.

References

1. Traub R, Wisseman CL, Jr., Azad AF. The ecology of murine typhus: a critical review. Trop Dis Bull 1978;75:237-317.
2. Azad AF. Epidemiology of murine typhus. Annu Rev Entomol 1990;35:553-69.
3. Dumler JS, Taylor JP, Walker DH. Clinical and laboratory features of murine typhus in Texas, 1980 through 1987. JAMA 1991;266:1365-70.
4. Walker DH. Advances in understanding of typhus group rickettsial infections. In: Kazar J, Toman R, editors. Ricke ttsiae and rickettsial diseases. Bratislava, Slovak Republic: VEDA Press 1996;16-25.
5. Adams WH, Emmons RW, Brooks JE. The changing ecology of murine (endemic) typhus in southern California. Am J Trop Med Hyg 1970;19:311-8.
6. Williams SG, Sacci JB Jr, Schriefer ME, Anderson EM, Fujioka KK, Sorvilo FJ. Typhus and typhus-like rickettsiae associated with opossums and their fleas in Los Angeles county, California. J Clin Microbiol 1992;30:1758-62.
7. Sorvillo FJ, Gondo B, Emmons R, Ryan P, Waterman SH, Tilzer A, et al. A suburban focus of endemic typhus in Los Angeles County: association with seropositive domestic cats and opossums. Am JTrop Med Hyg 1993;48:269-73.
8. Schriefer ME, Sacci JB Jr, Dumler JS, Bullen MG, Azad AF. Identification of a novel rickettsial infection in a patient diagnosed with murine typhus. J Clin Microbiol 1994;32:949-54.
9. Azad AF, Sacci JB Jr, Nelson WM, Dasch GA, Schmidtman ET, Carl M. Genetic characterization and transovarial transmission of a novel typhus-like Rickettsia found in cat fleas. Proc Natl Acad Sci USA 1992;89:43-6.
10. Schriefer ME, Sacci JB Jr, Higgins JA, Taylor JP, Azad AF. Murine typhus: updated role of multiple urban components and a second typhus-like rickettsiae. J Med Entomol 1994;31:681-5.
11. Higgins JA, Sacci JB Jr, Schriefer ME, Endris RG, Azad AF. Molecular identification of rickettsia-like microorganisms associated with colonized cat fleas (*Ctenocephalides felis).* Insect Mol Biol 1994;3:27-33.
12. Higgins JA, Radulovic S, Schriefer ME, Azad AF. *Rickettsia felis*: a new species of pathogenic rickettsia isolated from cat fleas. J Clin Microbiol 1996;34:671-4.
13. Radulovic S, Speed R, Feng HM, Taylor C, Walker DH. EIA with species-specific monoclonal antibodies: a novel seroepidemiologic tool for determination of the etiologic agent of spotted fever rickettsiosis. J Infect Dis 1993;168:1292-5.
14. Breitschwerdt EB, Hegarty BC, Davidson MG, Szabados NS. Evaluation of the pathogenic potential of *Rickettsia canada* and *Rickettsia prowazekii* organisms in dogs. JAVMA 1995;207:58-63.
15. Adams JR, Schmidtmann ET, Azad AF. Infection of colonized cat fleas, *Ctenocephalides felis* with a rickettsia-like microorganism. Am J Trop Med Hyg 1990;43:400-9.
16. Azad AF, Traub R, Sofi M, Wisseman CL Jr. Experimental murine typhus infection in the cat flea, *Ctenocephalides felis* (Siphonaptera:Pulicidae). J Med Entomol 1984;21:675-80.
17. Azad AF. Relationship to vector biology and epidemiology of louse and flea-borne rickettsioses. In: Walker DH, editor. Biology of rickettsial diseases. Boca Raton (FL): CRC Press; 1988. p.52-62.
18. Werren JH, Zhang W, Guo W. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. Proc R Soc Lond B Biol Sci 1995;B261:55-71.
19. Vaughan JA, Azad AF. Acquisition of murine typhus rickettsiae by fleas. Ann NY Acad Sci 1990;590:70-5.
20. Radulovic S, Higgins JA, Jaworski DC, Dasch GA, Azad AF. Isolation, cultivation and partial characterization of the ELB agent associated with cat fleas. Inf Immun 1995;63:4826-9.
21. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol 1991;173:1576-89.
22. Roux V, Raoult D. Phylogenetic analysis of the genus Rickettsia by 16S rDNA sequencing. Res Microbiol 1995;146:385-96.
23. Stothard D, Fuerst PA. Evolutionary analysis of the spotted fever and typhus group of *Rickettsia* using 16S rRNA gene sequences. Systematic Applied Microbiology 1995;18:52-61.
24. Andersson S, Eriksson A-S, Naslund AK, Andersen MS, Kurland CG. The *Rickettsia prowazekii* genome: a random sequence analysis. Microbial and Comparative Genomics 1996;1:293-315.
25. Feng DF, Doolittle RF. Progressive sequence alignment as a prerequisite to correct phylogenetic trees. J Mol Evol 1987;25:351-60.
26. Kimura M. A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16:111-20.
27. Fitch WM. Toward defining the course of evolution: minimum change for a specified tree topology. Systematic Zoology 1971;20:406-616.

Aedes albopictus in the United States: Ten-Year Presence and Public Health Implications

Chester G. Moore and Carl J. Mitchell
Centers for Disease Control and Prevention,
Fort Collins, Colorado, USA

Since its discovery in Houston, Texas, in 1987, the Asian "tiger mosquito" *Aedes albopictus* has spread to 678 counties in 25 states. This species, which readily colonizes container habitats in the peridomestic environment, was probably introduced into the continental United States in shipments of scrap tires from northern Asia. The early pattern of dispersal followed the interstate highway system, which suggests further dispersal by human activities. The Public Health Service Act of 1988 requires shipments of used tires from countries with *Ae. albopictus* to be treated to prevent further importations. Given the extensive spread of the mosquito in the United States, it is questionable whether such a requirement is still justified. *Ae. albopictus*, a major biting pest throughout much of its range, is a competent laboratory vector of at least 22 arboviruses, including many viruses of public health importance. Cache Valley and eastern equine encephalomyelitis viruses are the only human pathogens isolated from U.S. populations of *Ae. albopictus.* There is no evidence that this mosquito is the vector of human disease in the United States.

Established populations of *Aedes albopictus*, the Asian "tiger mosquito," (1) were first discovered in the continental United States in Harris County, Texas, in August 1985 (2). (*Ae. albopictus* was introduced into Hawaii sometime before 1902 (3). This mosquito may have become established in the region even earlier since an adult female was collected in Memphis, Tennessee, in 1983 (4). *Ae. albopictus* probably entered the United States in shipments of used tires from northern Asia, where the species is widely distributed (5-7). Beginning January 1, 1988, the U.S. Public Health Service required that all used tires arriving at U.S. ports from areas known to be infested with *Ae. albopictus* be dry, clean, and fumigated or otherwise "disinsected" (8). However, by the time the disinsection requirement was put in place, existing populations had become established in 15 states.

Ae. albopictus is both a nuisance and a potential disease vector. Anecdotal reports from local mosquito control agencies suggest that it has become a major pest mosquito problem in many communities in the southeastern United States. Laboratory studies show that this species is susceptible to and can transmit many arboviruses of public health importance (9-12). In this article, we summarize the reported distribution and dispersal of *Ae. albopictus* in the past 10 years and review surveillance for infection and transmission of arboviruses.

Address for correspondence: Chester G. Moore, Ph.D., Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, CDC, P.O. Box 2087, Fort Collins, CO 80522-2087, USA; fax: 970-221-6476; e-mail: cgm2@cdc.gov.

Distribution and Dispersal of *Aedes albopictus*

A national database of the distribution of *Aedes albopictus* is maintained as a passive surveillance system (13); the system is periodically stimulated by letters and telephone calls to mosquito and vector control professionals throughout the United States, as well as by articles in professional journals and newsletters and presentations at professional meetings. Data obtained from a standardized reporting form sent to potential collaborators to ensure standardization of the data are entered into a computerized database written in EpiInfo (14). Summary data are extracted from the database for reports or for transfer to a desktop mapping program.

Temporal Patterns

Figure 1 shows the changing distribution of *Ae. albopictus* over time. The mosquito is widely distributed in the southeastern United States. Established infestations are less common northward and westward, presumably because of less hospitable environments. The absence of reports from some states may reflect lack of surveillance rather than absence of the mosquito. This species may have been present in some areas for many years before discovery (particularly true in areas without active mosquito surveillance or control programs). In certain states, such as South Carolina and Kentucky, the abrupt discovery of *Ae. albopictus* in a large number of counties was the result of university graduate students' research.

Possible Dispersal Routes

During the early period of dispersal, the presence of *Ae. albopictus* appeared to be related to the proximity of a county to interstate highways (Figure 2). In December 1987, 92 counties in 15 states were infested with this mosquito. Of the 1,511 counties in states where *Ae. albopictus* was present, 582 (38.5%) had interstate highways passing through them. Were the spread of *Ae. albopictus* not related to the interstate system, only 35 (38%) of the 92 mosquito-infested counties would be expected to lie on an interstate highway. In fact, 64 of the 92 infested counties were on an interstate ($X^2 = 25.29$, df = 3, $p < 0.001$).

The postulated relationship between dispersal and major transportation routes would be expected for a species transported largely by human activities such as the commercial movement of scrap tires for retreading, recycling, or other purposes. Several of the 28 mosquito-infested sites not located on the interstate system were major tire retreading companies, other businesses that deal with large numbers of used or scrap tires, or illegal tire dumps.

Once populations of the mosquito become established, local transport and active migration should disperse the mosquito throughout the surrounding area. As would be expected if the original infestation were in Texas, the proportion of *Ae. albopictus*-infested counties on the interstate system in Texas had fallen from 1 (100%) of 1 in 1985, to 13 (65%) of 20 in 1986, and to 23 (58%) of 39 in 1987.

This distribution pattern can be explained in other ways. At least early on, searches might have been limited to *Ae. albopictus* in the major cities. Since most major cities are connected by interstates, it is impossible to separate these two possibilities. Moreover, most of the active vector control programs and other activities that would involve surveys for this mosquito are probably located in larger cities, which are connected to the interstate highway system.

Ae. albopictus as a Disease Vector

Vector Competence Studies

Reviews of many vector competence studies involving *Ae. albopictus* (9-12) provide information for 23 arboviruses and for Nodamura virus (probably not an arbovirus). In addition, *Ae. albopictus* has been recently found to be a competent experimental vector of Sindbis virus (15). A list of viruses included in vector competence experiments involving *Ae. albopictus* is shown in Table 1.

Ae. albopictus is a competent experimental vector of seven Alphaviruses: Chikungunya, eastern equine encephalitis (EEE), Mayaro, Ross River, western equine encephalitis, Venezuelan equine encephalitis, and Sindbis viruses. Only EEE virus has been isolated from *Ae. albopictus* collected in nature.

Ae. albopictus is also a competent experimental vector of the following Flaviviruses: dengue (DEN) serotypes 1, 2, 3, and 4, Japanese encephalitis, West Nile, and yellow fever viruses. In the case of an additional Flavivirus, St. Louis encephalitis virus, the amount of circulating virus in naturally infected avian hosts is generally insufficient to infect the mosquito (16).

DEN and Japanese encephalitis viruses have been isolated from specimens of *Ae. albopictus* collected outside the United States, and these viruses can be transmitted vertically under experimental conditions (9). Recently, isolation of DEN-1 virus from *Ae. albopictus* larvae in Brazil has been reported (17). *Ae. albopictus* has been involved in the transmission of DEN viruses in southeast Asia, southern China, Japan, and the Seychelles (18). If DEN viruses were introduced into areas of the United States with dense populations of *Ae. albopictus*, this mosquito could conceivably act as a vector. However, the classic epidemic vector of DEN viruses, *Ae. aegypti*, is also present in many of the southeastern states; in areas where *Ae. aegypti* is abundant, this species might be expected to play a far more important role in DEN transmission than *Ae. albopictus*.

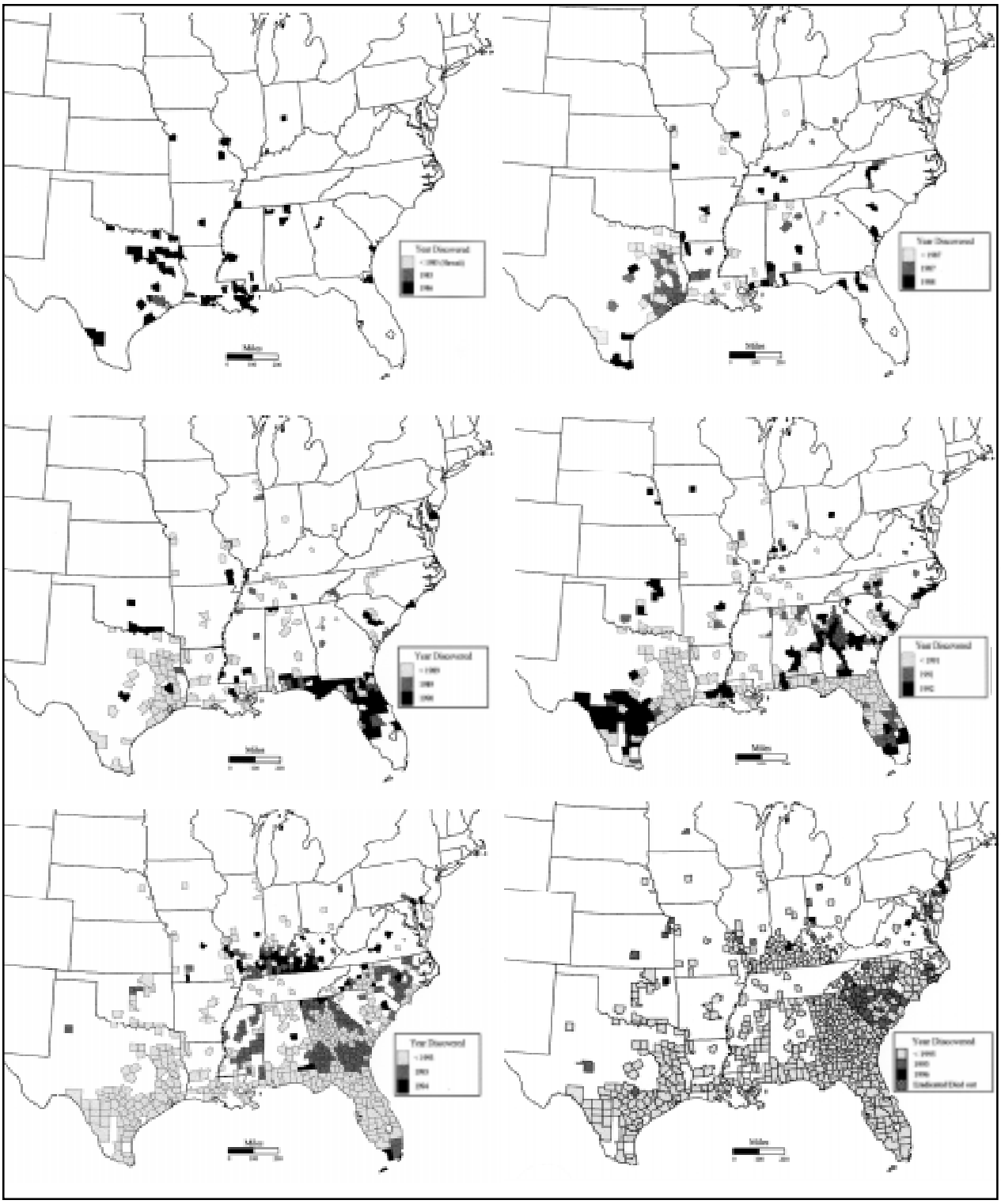


Figure 1. Reported distribution of *Aedes albopictus*, the Asian "tiger mosquito," in the continental United States, 1985-1996. Maps were generated by merging the EpiInfo database into the Atlas geographic information system.

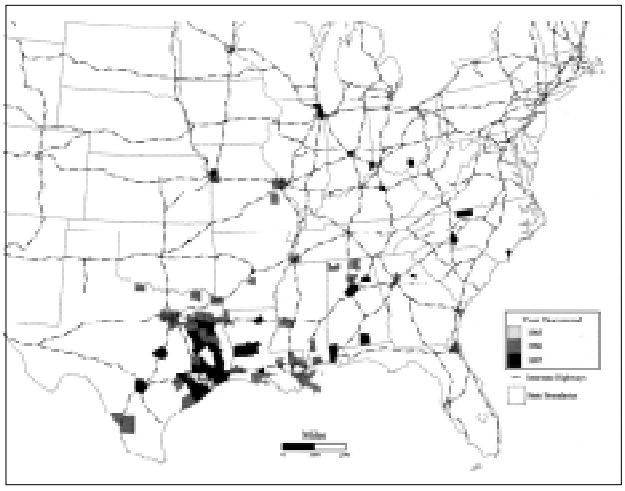


Figure 2. Apparent relationship between the early dispersal of *Aedes albopictus* and the U.S. interstate highway system, 1985-1987. Map generated by merging EpiInfo database into the Atlas geographic information system.

Vector competence tests show that eight Bunyaviridae (Jamestown Canyon, Keystone [KEY], LaCrosse, Oropouche, Potosi, Rift Valley fever, San Angelo, and trivittatus viruses) infect *Ae. albopictus* by the oral route. Only the KEY, Oropouche, and trivittatus viruses are not transmitted efficiently by bite. The KEY, LaCrosse, and San Angelo viruses can be transmitted vertically under experimental conditions.

Field Investigations

Since the discovery of *Ae. albopictus* in the United States, field-collected *Ae. albopictus* from several areas have been tested for arboviruses. From 1987 to 1995, 122,879 specimens were tested from 12 states (Table 2). Four viruses have been isolated: Potosi (19-22), EEE (23), KEY (23, 24 and R. Nasci, unpub. data), and Cache Valley (CV) virus (CDC, unpub. data). Tensaw virus was isolated by the Texas State Department of Health (23). The geographic and temporal distributions of these virus isolations are shown in Table 3.

Aside from EEE and CV viruses, the viruses isolated from *Ae. albopictus* in the United States are not of public health importance. The association of *Ae. albopictus* with EEE virus in nature has been restricted to a single incident in Polk County, Florida, in 1991. The presence of a large tire dump (ca. 1.5 million used tires) within a known enzootic focus of EEE virus (the Green Swamp) may have led to an unusual virus-vector association (23). Follow-up studies at the dump site in 1992, after the tires had been shredded, yielded fewer than 1,000 *Ae. albopictus* (none infected) (Mitchell and Niebylski, pers. comm.), and EEE virus has not been isolated from field-collected specimens of this species since the original episode. CV virus was isolated from a pool of *Ae. albopictus* collected in Jasper County, Illinois, in 1995 (CDC, unpub. data). During the same year, a case of human disease with diverse clinical manifestations due to CV virus was reported in a patient who presumably contracted the infection while deer hunting in Anson County, North Carolina (25). However, there is little reason to suspect that *Ae. albopictus* was involved in this incident. CV virus was isolated repeatedly from several other genera and species of mosquitoes before *Ae. albopictus* was present in the continental United States and, thus far, CV virus has been isolated from *Ae. albopictus* from only a single pool of specimens in Illinois.

Table 1. Susceptibility of *Aedes albopictus* to oral infection with arboviruses and ability to transmit by bite*

	Ae. albopictus strains			
	Hawaii and areas outside W. Hemisphere		North and South America	
Viruses	Infect.	Trans.	Infect.	Trans.
Chikungunya	+	+	+	+
Dengue 1, 2, 3, 4	+	+	+	+
Eastern equine encephalitis	+	+	+	+
Jamestown Canyon			+	+
Japanese encephalitis	+	+		
Keystone			+	-
La Crosse			+	+
Mayaro			+	+
Nodamura	+	?		
Oropouche			+	-
Orungo	+	+		
Potosi			+	+
Rift Valley fever			+	+
Ross River	+	+	+	+
San Angelo	+	+		
Sindbis			+	+
St. Louis encephalitis	+	+		
Trivittatus			+	-
West Nile	+	+		
Western equine encephalitis	+	+	+	+
Venezuelan equine encephalitis			+	+
Yellow fever	+	+	+	+

* Modified from Mitchell (1991)(10)

Table 2. Field-collected *Aedes albopictus* tested for virus, 1987-1995*

State of origin	Number tested
Alabama	64
Arkansas	1,234
Florida	18,862
Illinois	10,921
Indiana	516
Louisiana	47,320
Mississippi	128
Missouri	35,797
North Carolina	4,590
Ohio	1,604
South Carolina	72
Tennessee	1,771
TOTAL	122,879

*Tests were conducted by the Centers for Disease Control and Prevention

Table 3. Arboviruses isolated from *Aedes albopictus* in the United States, 1987-1996*

Virus	State	County or parish	Year
Potosi	Missouri	Washington	1989
	North Carolina	Anson	1994
	Illinois	Jasper	1994
Eastern equine encephalitis	Florida	Polk	1991
Keystone	Florida	Polk	1991
	Florida	Orange	1993
	Louisiana	Calcasieu	1995
Tensaw	Texas	Montgomery	1991
Cache Valley	Illinois	Jasper	1995

*All viruses except Tensaw were isolated in the CDC laboratory in Fort Collins, Colorado; Tensaw virus was isolated by the Texas State Health Department, Austin, Texas.

Conclusions

Ae. albopictus is firmly established in the United States. In the 10 years since its discovery, this species has spread throughout much of the East. The species occurs in all counties in at least four states—Delaware, Florida (26), Georgia (27), and South Carolina. It probably occurs in all or most counties in Alabama, Mississippi, and Louisiana, but surveys from those states are incomplete. *Ae. albopictus* seems to be approaching the northern limit predicted by Nawrocki and Hawley (28). The westward dispersal of this mosquito has been very slow, perhaps because the drier environment of the Great Plains region inhibits westward movement of this species. Data suggest that the current practice of requiring the disinsection of used tires entering the United States from other countries with *Ae. albopictus* does not influence the dynamics and spread of this species within this country. If disinsection is to remain in force, other justification will be needed.

Observations on early dispersal of *Ae. albopictus* are consistent with the hypothesis of dispersal by human activities, probably movement of scrap tires through the interstate highway system. This information might be useful in designing monitoring programs for possible future introductions of mosquitoes.

Collectively, the above information indicates that *Ae. albopictus* is a competent vector for a wide variety of arboviruses under experimental conditions, has been found to be naturally infected with DEN, Japanese encephalitis, Potosi, KEY, Tensaw, CV, and EEE viruses, and can serve as an epidemic vector of DEN viruses. The capacity of *Ae. albopictus* to vertically transmit certain arboviruses may also enhance the possibility of establishing new enzootic and endemic foci of some viruses. *Ae. albopictus* is a major biting pest throughout much of its range and is of justifiable concern to mosquito control and public health agencies for this reason alone. Nonetheless, in terms of its role as an arbovirus vector, evidence is lacking to incriminate *Ae. albopictus* as the vector of even a single case of human disease in the United States.

Acknowledgments

The *Aedes albopictus* distribution database is the result of the effort of nearly 100 colleagues in state and local health and vector control agencies, universities, and military agencies. We acknowledge K.W. Blank and G.M. Beavers, University of Kentucky, and D. Berge, University of South Carolina, for sharing unpublished data for the distribution maps.

References

1. Robertson RC, Hu SMK. The tiger mosquito in Shanghai. The China Journal 1935;23:299-306.
2. Usinger, RL. Entomological phases of the recent dengue epidemic in Honolulu. Public Health Rep 1944;59:423-30.
3. Sprenger D, Wuithiranyagool T. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. J Am Mosq Control Assoc 1986;2:217-9.
4. Reiter P, Darsie RF Jr. *Aedes albopictus* in Memphis, Tennessee (U.S.A.): an achievement of modern transportation? Mosquito News 1984;44:396-9.
5. Hawley WA, Reiter P, Copeland RW, Pumpuni CB, Craig, Jr GB. *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. Science 1987;236:1114-6.

6. Reiter P, Sprenger D. The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitoes. J Am Mosq Control Assoc 1987;3:494-501.
7. Craven RB, Eliason DA, Francy DB, Reiter P, Campos EG, Jakob WL, et al. Importation of *Aedes albopictus* and other exotic mosquito species into the United States in used tires from Asia. J Am Mosq Control Assoc 1988;4:138-42.
8. Centers for Disease Control. Requirement of certification of used tire casings from Asia prior to entry into the United States. Federal Register 1987;52(224):44646.
9. Shroyer DA. *Aedes albopictus* and arboviruses: a concise review of the literature. J Am Mosq Control Assoc 1986;2:424-8.
10. Mitchell CJ. Vector competence of North and South American strains of *Aedes albopictus* for certain arboviruses. J Am Mosq Control Assoc 1991;7:446-51.
11. Mitchell CJ. Geographic spread of *Aedes albopictus* and potential for involvement in arbovirus cycles in the Mediterranean basin. Journal of Vector Ecology 1995;20:44-58.
12. Mitchell CJ. The role of *Aedes albopictus* as an arbovirus vector. Proceedings of the Workshop on the Geographic Spread of *Aedes albopictus* in Europe and the Concern among Public Health Authorities; 1990 Dec 19-20; Rome, Italy. Parassitologia; 1995;37:109-13.
13. Teutsch SM. 1994. Considerations in planning a surveillance system. In: Teutsch SM, Churchill RE, editors. Principles and practice of public health surveillance. New York: Oxford University Press; 1994. p. 18-30.
14. 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 epidemiology on microcomputers. Atlanta (GA): Centers for Disease Control and Prevention: 1994.
15. Dohm DJ, Logan TM, Barth JF, Turell MJ. Laboratory transmission of Sindbis virus by *Aedes albopictus*, *Ae. aegypti*, and *Culex pipiens* (Diptera: Culicidae). J Med Entomol 1995;32:818-21.
16. Savage HM, Smith GC, Mitchell CJ, McLean RG, Meisch MV. Vector competence of *Aedes albopictus* from Pine Bluff, Arkansas, for a St. Louis encephalitis virus strain isolated during the 1991 epidemic. J Amer Mosq Control Assoc 1994;10:501-6.
17. Serufo JC, Montes de Oca H, Tavares VA, Souza AM, Rosa RV, Jamal MC, et al. Isolation of dengue virus type 1 from larvae of *Aedes albopictus* in Campos Altos City, State of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz 1993;88:503-4.
18. Hawley WA. 1988. The biology of *Aedes albopictus*. J Am Mosq Control Assoc 1988;4(Suppl. 1):1-40.
19. Francy DB, Karabatsos N, Wesson DM, Moore Jr CG, Lazuick JS, Niebylski ML, et al. A new arbovirus from *Aedes albopictus*, an Asian mosquito established in the United States. Science 1990;250:1738-40.
20. Mitchell CJ, Smith GC, Miller BR. Vector competence of *Aedes albopictus* for a newly recognized *Bunyavirus* from mosquitoes collected in Potosi, Missouri. J Am Mosq Control Assoc 1990;6:523-7.
21. Mitchell CJ, Smith GC, Karabatdsos N, Moore CG, Francy DB, Nasci RS. Isolations of Potosi virus from mosquitoes collected in the United States, 1989-1994. J Am Mosq Control Assoc 1996;12:1-7.
22. Harrison BA, Mitchell CJ, Apperson CS, Smith GC, Karabatsos N, Engber BR, et al. Isolation of Potosi virus from *Aedes albopictus* in North Carolina. J Am Mosq Control Assoc 1995;11:225-9.
23. Mitchell CJ, Niebylski ML, Smith GC, Karabatsos N, Martin D, Mutebi J-P, et al. Isolation of eastern equine encephalitis from *Aedes albopictus* in Florida. Science 1992;257:526-7.
24. Mitchell CJ, Morris CD, Smith GC, Karabatsos N, Vanlandingham D, Cody E. Arboviruses associated with mosquitoes from nine Florida counties during 1993. J Am Mosq Control Assoc 1996;12:255-62.
25. Sexton DJ, Rollin PE, Breitschwerdt EB, Corey GR, Myers SA, Hegarty BC, et al. Brief report: severe multiorgan failure following Cache Valley virus infection. N Engl J Med 1997;336:547.
26. O'Meara GF, Evans Jr LF, Gettman AD, Cuda JP. Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera: Culicidae) in Florida. J Med Entomol 1995;32:554-62.
27. Womack ML, Thuma TS, Evans BR. Distribution of *Aedes albopictus* in Georgia, USA. J Am Mosq Control Assoc 1995;11:237.
28. Nawrocki SJ, Hawley WA. Estimation of the northern limits of distribution of *Aedes albopictus* in North America. J Am Mosq Control Assoc 1987;3:314-7.

Using a Mathematical Model to Evaluate the Efficacy of TB Control Measures

Laura Gammaitoni and Maria Clara Nucci
University of Perugia, Perugia, Italy

We evaluated the efficacy of recommended tuberculosis (TB) infection control measures by using a deterministic mathematical model for airborne contagion. We examined the percentage of purified protein derivative conversions under various exposure conditions, environmental control strategies, and respiratory protective devices. We conclude that environmental control cannot eliminate the risk for TB transmission during high-risk procedures; respiratory protective devices, and particularly high-efficiency particulate air masks, may provide nearly complete protection if used with air filtration or ultraviolet irradiation. Nevertheless, the efficiency of these control measures decreases as the infectivity of the source case increases. Therefore, administrative control measures (e.g., indentifying and isolating patients with infectious TB) are the most effective because they substantially reduce the rate of infection.

After decades of steady decline and a subsequent relaxation of infection control practices in health-care facilities, the number of tuberculosis (TB) cases has been increasing dramatically in the United States (1) and Western Europe (2) since 1985; the increase is due to human immunodeficiency virus (HIV) infection, immigration, poverty, homelessness, and drug addiction.

Epidemiologic studies have shown that primary pulmonary TB is caused by inhaling the tubercle bacillus in a droplet nucleus form (3). Airborne contagion is crucial in the indoor transmission of all respiratory infections from person to person (4,5). Infective persons may contaminate the air by coughing, sneezing, and spitting (4,5), which generate a large number of small respiratory droplets that evaporate almost instantly into small droplet nuclei, disperse into the environment, and implant themselves in the lung when inhaled (4). Droplet nuclei have a leading role in airborne contagion (3-5).

Outbreaks of TB have been reported from prisons, nursing homes, residential centers for HIV-infected persons, urban homeless shelters, aircraft, schools, and bars. All outbreaks occurred under crowded living conditions with prolonged close exposure to an infectious person. Nosocomial TB transmission is also associated with cough-generating procedures (6), bronchoscopy (7), endotracheal intubation and suctioning (8), open abscess irrigation (9), and autopsy (10). Workers involved in such procedures are at high and increasing risk for TB (11) because of the resurgence of the disease, the emergence of multidrug-resistant (MDR) strains causing outbreaks in hospitals among patients and health-care workers, and compromised TB control due to decreased funding of health-care agencies responsible for TB control.

After studying hospital outbreaks that resulted in TB transmission to health-care workers, various authorities have recommended measures to prevent nosocomial TB transmission (12-15). The implementation of a TB infection control program requires risk assessment and development of a TB infection control plan including early identification, treatment, and isolation of infectious TB patients; effective engineering controls (environmental controls such as general ventilation, high-efficiency particulate air [HEPA] filters, or ultraviolet germicidal irradiation [UVGI]); the adoption of appropriate respiratory protection (surgical masks and particulate respirators such as HEPA masks); health-care worker TB training, education, counseling, and screening; and evaluation of the program's effectiveness (13). Several reviews of environmental control measures and respiratory protective

Address for correspondence: Maria Clara Nucci, Dipartimento di Matematica, Università di Perugia, 06123 Perugia, Italy; fax: 011-39-75-585-5024; e-mail: nucci@unipg.it.

devices are available (16-20). Implementing recommended measures would require massive expenditures in all hospitals that admit TB patients. At several hospitals where MDR-TB outbreaks have occurred, measures similar to those recommended in TB prevention guidelines have been implemented. Such implementation halted and prevented MDR-TB transmission to health-care workers and patients (21-23), but which of the many implemented control measures played a key role in reducing risk is not clear. However, data suggest that implementing administrative control measures, in particular identifying and isolating patients with infectious TB, substantially reduces the risk for transmission (22).

In this article, we review the most important TB infection control measures and evaluate their efficacy in various settings by a deterministic mathematical model for airborne contagion (24). The model describes how a person can become infected by staying in a room where a source of airborne infection and an air disinfection device are present.

Two existing mathematical models describe the propagation of airborne contagion indoors. The first model was developed in 1955 by William F. Wells (4), who introduced the so-called quantum theory. Wells defined a quantum to be the number of infectious droplet nuclei required to infect 1 - 1/*e* of susceptible persons. His ninth postulate states, "The response to inhaled droplet nuclei contagium is quantal; the Poisson equation expresses reasonably well the relation between dosage and initial response, a quantum infecting 63.2% of homogeneously exposed hosts by definition." Wells explains, "When on the average one animal breathes one quantum, (omissis) 36.8% of the animals will survive, since this is the fraction whose negative natural logarithm is 1. Thus 1 quantum of contagium has been breathed per animal when 63.2% of the animals become infected (4, p. 124)." In 1978, Edward R. Riley, G. Murphy, and Richard L. Riley elaborated another model (25), which deals with the probability of a susceptible person becoming infected by inhaling a quantum of infection. They used the Reed Frost modification of the Soper equation for airborne transmission and Poisson's law of small chances. This model contains Wells' model as a particular case.

Our model is based on Wells' experiments and postulates and includes the Riley/Murphy/Riley model as a particular case (24). In this article, we describe some of the most important TB infection control measures, such as environmental controls and respiratory protective devices, and review recommendations made by various agencies and organizations. Then, we consider four recent TB outbreaks during which health-care workers became infected while performing bronchoscopy, jet irrigation of a thigh abscess, autopsy, and intubation. Using our deterministic mathematical model, we simulate the adoption of different environmental control strategies and respiratory protective devices, and we give the number of purified protein derivative (PPD) conversions predicted to occur under various exposure conditions. Moreover, we use our mathematical model to evaluate the efficacy of administrative control measures by analyzing data related to PPD conversions found on an HIV ward before and after implementing such control measures.

Environmental Control Strategies and Respiratory Protective Devices

A TB infection control program should be based on control measures that address the most important factors involved in TB transmission: the concentration of droplet nuclei in the air and the rate at which droplet nuclei are inhaled by susceptible persons. The concentration of infectious droplet nuclei in the air can be reduced by environmental controls. These controls include diluting and removing contaminated air by general ventilation and air cleaning by air filtration (e.g., through HEPA filters) or UVGI.

In general ventilation systems, uncontaminated supply air mixes with contaminated room air (dilution), which is subsequently removed from the room by the exhaust system (removal). Two types of general ventilation systems can be used for dilution and removal of contaminated air: single-pass systems and recirculating systems. In single-pass systems the supply air is uncontaminated fresh outside air, and after it passes through the ventilated area, 100% of that air is exhausted to the outside. In a recirculating system, a small portion of the exhaust air is discharged to the outside and is replaced with fresh outside air, which mixes with the portion of exhaust air that was not discharged to the outside. The resulting mixture, which can contain a large proportion of contaminated air, is then recirculated. The rate at which airborne particles are removed from an enclosed space (e.g., a room) by ventilation is usually expressed

as the number of air changes per hour (ACH), which is the ratio between the volume of air entering the room per hour and the room volume. A minimum of 6 ACH is recommended for TB isolation rooms and treatment rooms (26-28). Where feasible, this airflow rate should be increased to 12 ACH or more, and in areas where the nature of work is exceptionally hazardous, such as autopsy rooms, airflow rates of 15-25 ACH have been recommended (29,30).

HEPA filtration units or UVGI can be used as a supplement to ventilation control measures in settings where adequate airflow cannot be provided with the general ventilation system alone (13). HEPA filters have a demonstrated and documented minimum removal efficiency of 99.97% of particles whose diameter is ≥ 0.3 µm (droplet nuclei are an estimated 1 to 5 µm in size [13], but they can have an aerodynamic diameter of less than 1 µm [31]). They can be used in fixed or portable HEPA recirculation systems that can achieve 12 ACH or more (13). UVGI can kill or inactivate tubercle bacilli under experimental conditions (32), especially where air mixing is accomplished mainly by convection (13). The effect of UVGI in a room without supplemental ventilation (33) and in another with a ventilation rate of 6 ACH (34) is estimated at 10 and 39 ACH, respectively. Greater rates of ventilation may decrease the killing of bacteria (34), but the optimal relationship between ventilation and UVGI is not known. However, general ventilation plus UVGI has a disinfection rate of 45 ACH if properly installed (34).

Personal protective devices are recommended when engineering controls are not likely to protect against inhaling infectious airborne droplet nuclei (e.g., in TB isolation rooms, treatment rooms in which cough-inducing or aerosol-generating procedures are performed, and ambulances during the transport of infectious TB patients) (13). The respiratory protective devices used in these settings should have a filter efficiency ≥ 95% with an obtainable face-seal leakage not greater than 10% (13). The efficiency of standard cup-shaped surgical masks in preventing the inhalation of droplet nuclei with a diameter of 1 to 5 µm is less than 50% (11) with a face-seal leakage of 0% to 20% (35). Since 1990, the use of particulate respirators including HEPA masks tested to filter 99.97% of 0.3 µm particles with a face-seal leakage less than 2% has been recommended (13).

Because of the very high costs of HEPA masks, the breathing difficulties they may cause, and the odd appearance of people wearing them, which may have a psychological impact on patients, the recommendation for personal respiratory devices is the most controversial aspect of existing TB guidelines. Reported cases of TB and skin-test conversions among health-care workers have led many to conclude that HEPA respirators are justified. On the other hand, many believe that less stringent personal protective devices, such as dust/mist respirators, may provide sufficient protection, with less discomfort and lower cost (31). New certification rules (42 CFR 84) have been recently approved that allow the use of dust/mist respirators, in addition to HEPA respirators, in TB control settings (36).

The Model

In the case of an airborne infection outbreak in a single room (e.g., a hospital room) of volume V, where a certain number $I_0 \geq 1$ of infective persons and S_0 of susceptible persons are present at initial time t = 0, the number of susceptible persons can be expressed as a function of the time interval from the beginning of the exposure (the time of exposure t, measured in minutes), the rate at which quanta are produced by the infective persons (the infection rate q), and the number of effective or equivalent air changes (AC) in the unit time (the disinfection rate C) (24).

The following assumptions (4,24,25) are taken into consideration: differences in susceptibility are neglected; the rate at which quanta of infection are added to the air by infectious persons is considered constant; the latent period of the disease is longer than the time scale of the model, and the number of infective persons in the room is constant; droplet nuclei are instantaneously and evenly distributed in the room; droplet nuclei are assumed to be removed by fresh air ventilation of the room and ultraviolet irradiation at a constant rate; fresh air is at the same temperature and pressure as the air already present in the room; and the number of infected persons is proportional to the number of encounters between susceptible persons and quanta of infection, (and the encounter rate is proportional to both the number of susceptible persons and quanta of infection in the room [law of mass action]).

If the constant of proportionality between the number of infected persons and the number of

susceptible persons times the number of quanta of infection Q is the ratio between the pulmonary ventilation p (we assume p = 0.01 m^3/min) and the volume V of the room, the following equations can be derived:

1. $$\frac{dS}{dt} = -\frac{p}{V}QS,$$
$$\frac{dQ}{dt} = -CQ + q, \quad t \geq 0,$$

with the initial conditions

2. $$S(0) = S_0 > 0,$$
$$Q(0) = Q_0 \geq 0.$$

The solution of equation 1 - equation 2 is

3. $$S(t,q,C) = S_0 \exp\left\{-\frac{pq}{V}\frac{Ct + e^{-Ct} - 1}{C^2}\right\},$$

4. $$Q(t,q,C) = \left(Q_0 - \frac{q}{C}\right)e^{-Ct} + \frac{q}{C}.$$

The percentage of PPD conversion among health-care workers is the following:

5. $$I_w(t,q,C) = \left[1 - \frac{S(t,q,C)}{S_0}\right] \times 100.$$

The rate of production of quanta of infection during each outbreak is not known a priori. Therefore, we do not know the real value of q during a specific epidemic. However, it can be derived a posteriori when the number $\overline{S}$ of susceptible persons who were not infected is known. In fact, solving equation $S(t,q,C) = \overline{S}$ with respect to q, with S given by formula 3 yields

6. $$q = \frac{V}{p}\frac{C^2}{Ct + e^{-Ct} - 1}\log\frac{S_0}{\overline{S}}$$

By substituting the given values of p, V, S_0, $\overline{S}$, t, and C in the right-hand side of formula 6, we obtain the value for q during each epidemic.

In this article, we simulate the adoption of various environmental control strategies (e.g., HEPA filtration devices or UVGI combined with ventilation) by giving suitable values to the parameter C. Also, we simulate the adoption of personal protective devices (e.g., surgical masks, dust/mist respirators, and HEPA masks) by scaling the rate at which quanta of infection are breathed (pulmonary ventilation p), which depends both on the mask's filter efficiency and face-seal leakage. If the masks adopted have a filter efficiency of X% and a face-seal leakage of Y%, then we can describe the effective filter efficiency of the mask as Z%, with Z = (X-XY/100). The scaling factor for p, therefore, is (100-Z)%.

For example, dust/mist respirators should have a filter efficiency X% equal to 95% and a face-seal leakage Y% not greater than 10%; then, their effective filter efficiency is Z = X-XY/100 = 85.5%, and the scaling factor for p is (100-Z)% = 14.5%. We derive a scaling factor of 60% and 3% for surgical and HEPA masks, respectively (13). Moreover, we assume C = 6 ACH (0.1 AC/min) for general ventilation alone, and C = 18 ACH (0.3 AC/min) or C = 45 ACH (0.75 AC/min) when HEPA filtration or UVGI is adopted in combination with ventilation (34).

Model Application

We consider two types of nosocomial exposure to TB infection: exposure during high-risk procedures and exposure during normal working conditions on a ward where a source of TB infection is present. Using data from published TB outbreaks among health-care workers and assuming the use of different environmental control strategies (general ventilation, HEPA filtration, UVGI) and respiratory protective devices (surgical masks, dust/mist respirators, HEPA masks), we derive the percentage of PPD conversions that can occur.

High-Risk Procedures

We use data from four published TB outbreaks during which health-care workers were infected while performing bronchoscopy (7), jet irrigation of a thigh abscess (9), autopsy (10), and intubation (8) (Table 1). The values for q vary from six (7) to 514 (8) quanta per minute (qpm), indicating a high level of infectiveness of the index case in each outbreak. The patient with the highest rate of quanta production was considered a dangerous disseminator of TB (8).

Table 2 shows the percentage of PPD conversions that can occur during four procedures (bronchoscopy, abscess irrigation, autopsy, intubation) if

Table 1. Nosocomial TB outbreaks involving health-care workers who performed high-risk procedures

	Pulmonary ventilation $p(m^3/min)$	Room volume $V(m^3)$	No. of susceptible persons S_0	No. of uninfected persons $\bar{S}$	Exposure time t (min)	Infection rate q (qpm)	Disinfection rate C (ACH)
Bronchoscopy	10^{-2}	200	13	3	150	6	1.26
Abscess irrigation	6×10^{-3}[a]	200[b]	5	1	150[b]	38	6[b]
Autopsy	10^{-2}	200[b]	4	0[c]	150[b]	94	11
Intubation	6×10^{-3}[a]	3000	3	1	66	514	2.4[d]

[a]Surgical masks were adopted.
[b]Estimated value; actual data are not available
[c]We assumed $S=0.001 \cong 0$.
[d]In the emergency department where this outbreak developed, 60% of the air was recirculated without filtration; we assumed an airflow rate of 6 ACH, obtaining an effective disinfection rate equal to 40% of 6 ACH.

different environmental control strategies were adopted, with and without the adoption of surgical masks, dust/mist respirators, or HEPA masks.

The percentage of PPD conversions that can occur as a function of the time of exposure t during the least risky procedure—bronchoscopy—if different environmental control strategies were adopted, no personal protective devices were used, and the time of exposure varied from 50 to 400 minutes is shown in Figure 1.

Normal Working Conditions

We consider an MDR-TB outbreak that occurred on an HIV ward (21). During the initial period, the percentage of PPD conversions among health-care workers was 28%. Inadequate TB control programs or facilities (delays in TB diagnosis and in determining drug susceptibility and inadequate patient isolation precautions) facilitated the transmission. Administrative measures similar to those subsequently recommended (37) were implemented on the HIV ward, and early follow-up period showed a decrease in PPD conversions among healthcare workers (18%), who were required to wear surgical masks.

The actual time of exposure during the initial period is not available. Assuming that the ratio between the time of exposure t_{in} and the time span T_{in}=150 days of the initial period (January–May 1990) is equal to the ratio between the time of exposure t_{ef}=135 days (average between 139 and 129 days) and the time span T_{ef}=270 days of the early follow-up period (June 1990–February 1991), we assume that $t_{in}=T_{in} \times t_{ef}/T_{ef} \cong 75$ days. The remaining data are summarized in Table 3.

We calculate the rate of infection in the initial period to be q = 0.006; and after administrative control measures were implemented, it decreased to q = 0.003 in the early follow-up period. Administrative control measures decreased the rate of infection by 50%, thus confirming their key role in preventing TB transmission.

Figure 2 shows the percentage of PPD conversions predicted to occur during the initial period with the use of different environmental control strategies and respiratory devices.

Table 2. Percentage of purified protein derivative conversions predicted to occur during various procedures, by using various environmental control strategies and respiratory protective devices

Control measures[a]	Bronchoscopy q=6, t=150 min[b]	Abscess irrigation q=38, t=150 min	Autopsy q=90, t=150 min	Intubation q=514, t=66 min
GV	34.3	93	99.9	61.7
GV+HF	13.6	60.5	90	30.1
GV+UVGI	5.8	31.4	60.5	13.7
GV+SM	22.3	79.7	98.1	43.8
GV+HF+SM	8.4	42.7	74.8	19.3
GV+UVGI+SM	3.5	20.2	42.8	8.5
GV+DMR	5.9	32	61.5	13
GV+HF+DMR	2.1	12.6	28.3	5.1
GV+UVGI+DMR	0.9	5.3	12.6	2.1
GV+HM	1.3	7.7	17.9	2.8
GV+HF+HM	0.4	2.7	6.7	1.1
GV+UVGI+HM	0.2	1.1	2.8	0.4

[a]GV=general ventilation; HF=HEPA filtration; SM=surgical masks; DMR=dust/mist respirators; HM=HEPA masks
[b]q=infection rate; t=exposure time.

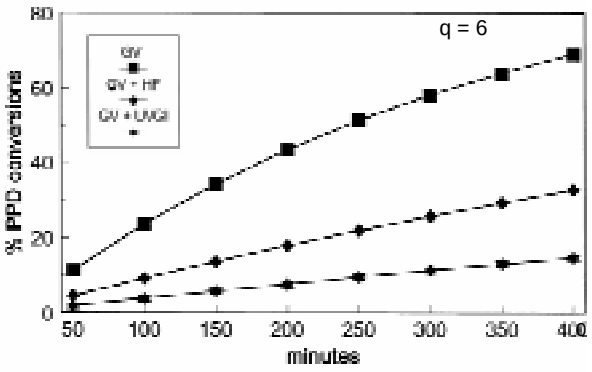


Figure 1. Percentage of PPD conversions predicted to occur as a function of time of exposure t during bronchoscopy, if different environmental control strategies were adopted, no personal protective devices were used, and the time of exposure varied from 50 to 400 minutes.

Conclusions

Our mathematical model indicates that a certain number of persons exposed to a source of TB infection will be infected in spite of all precautions. Nevertheless, if the infection rate is known, this number can be kept low by increasing the disinfection rate and decreasing the time of exposure.

We have evaluated the probability of acquiring TB infection under various exposure conditions and found that the efficacy of environmental control strategies depends on the duration of exposure and the infection rate. In particular, if the recommended levels of airflow are the only means of reducing the concentration of droplet nuclei in a room, they can neither eliminate TB contagion nor provide tolerable value for the risk of contagion. If HEPA filtration or UVGI is combined with general ventilation, protection against TB transmission is ensured at low infection rates, especially for brief exposures. As the infection rate increases, higher disinfection rates appear less and less effective in reducing the risk for transmission, and in situations of very intensive exposure, not even HEPA filtration or UVGI combined with general ventilation can keep the risk for transmission low enough. In these circumstances, respiratory protective devices may be used to protect health-care workers from inhaling droplet nuclei. The adoption of HEPA

q = 0.006, t = 75 days

Figure 2. Percentage of PPD conversions predicted to occur on the HIV ward during the initial period, if different environmental control strategies were adopted and with and without the adoption of surgical masks (SM), dust/mist respirators (DMR), or HEPA masks (HM).

Table 3. Nosocomial TB outbreaks involving health-care workers under normal working conditions

	Pulmonary ventilation p (m^3/min)	Room volume V (m^3)	No. of susceptible persons S_o	No. of uninfected persons S	Exposure time t (days)	Infection rate q (qpm)	Disinfection rate C (ACH)
Initial period	10^{-2}	200[a]	25	18	75	0.006	6
Early follow-up period	6×10^{-3}[b]	200[a]	17	14	135	0.003	6

[a]Estimated value; actual data are not available.
[b]Surgical masks were adopted.

masks would provide nearly complete protection, even for long exposures, if used together with HEPA filtration or UVGI. Neverthless, the efficacy of these control mea-sures decreases as the infectivity of the source case increases; therefore, the only control measures that significantly reduce the infection rate are administrative.

Dr. Gammaitoni is a postgraduate fellow at the University of Perugia and a computer specialist for ALENIA-Italy. Her research interests include mathematical models applied to environmental health problems.

Dr. Nucci is a researcher at the University of Perugia. Her recent interests include application of mathematical modeling techniques to epidemiology. Her work is supported in part by Ministero Università Ricerca Scientifica Tecnologica: Fondi (60% and 40%).

References

1. Centers for Disease Control and Prevention. Tuberculosis morbidity—United States, 1992. MMWR Morb Mortal Wkly Rep 1993;42:696-704.
2. World Health Organization. Tuberculosis control program. Secular trends of tuberculosis in Western Europe. Geneva: World Health Organization Technical Bulletin; 1992.
3. Nardell EA. Dodging droplet nuclei: reducing the probability of nosocomial tuberculosis transmission in the AIDS era. American Review of Respiratory Diseases 1990;142:501-3.
4. Wells WF. Airborne contagion and air hygiene: an ecological study of droplet infections. Cambridge (MA): Harvard University Press; 1955.
5. Riley RL. Airborne infection. Am J Med 1974;57:466-75.
6. Malasky C., Potulski F, Jordan T, Reichman LB. Occupational tuberculous infections among pulmonary physicians in training. American Review of Respiratory Diseases 1990;142:505-7.
7. Catanzaro A. Nosocomial tuberculosis. American Review of Respiratory Diseases 1982;125:559-62.
8. Haley CE, McDonald RC, Rossi L, Jones WD Jr, Haley RW, Luby JP. Tuberculosis epidemic among hospital personnel. Infect Control Hosp Epidemiol 1989;10:204-10.
9. Hutton MD, Stead WW, Cauthen GM, Bloch AB, Ewing WM. Nosocomial transmission of tuberculosis associated with a draining abscess. J Infect Dis 1990:161:286-95.
10. Kantor HS, Poblete R, Pusateri SL. Nosocomial transmission of tuberculosis from unsuspected disease. Am J Med 1998;84:833-8.
11. Menzies D, Fanning A, Yuan L, Fitzgerald M. Tuberculosis among health care workers. N Engl J Med 1995;332:92-8.
12. American Thoracic Society. Control of tuberculosis in the United States. American Review of Respiratory Diseases 1992;146:1623-33.
13. Centers for Disease Control and Prevention. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care facilities. MMWR Morb Mortal Wkly Rep 1994;43/RR-13.
14. Occupational safety and health standards, personal protective equipment, respiratory protection, C.F.R. No. 1910.134 (1996).
15. National Institute for Occupational Safety and Health. Protect yourself against tuberculosis—a guide for health care workers. Cincinnati (OH): U.S. Department of Health and Human Services, Public Health Service 1996; DHHS (NIOSH) Publication No. 96-102.
16. Nardell EA, Keegan J, Cheney SA, Etkind SC. Airborne infection: theoretical limits of protection achievable by building ventilation. American Review of Respiratory Diseases 1991;144:302-6.
17. Riley RL. Ultraviolet air disinfection: rationale for whole building irradiation. Infect Control Hosp Epidemiol 1994;15:324-5.
18. Segal-Maurer S, Kalkut GE. Environmental control of tuberculosis: continuing controversy. Clin Infect Dis 1994;19:299-308.
19. Winters RE. Guidelines for preventing the transmission of tuberculosis: a better solution? Clin Infect Dis 1994;19:309-10.
20. Rutala WA, Weber DJ. Environmental interventions to control nosocomial infections. Infect Control Hosp Epidemiol 1995;16:442-3.
21. Wenger PN, Otten J, Breeden A, Orfas D, Beck-Sague CM, Jarvis WR. Control of nosocomial transmission of multidrug-resistant *Mycobacterium tuberculosis* among healthcare workers and HIV-infected patients. Lancet 1995;345:235-40.
22. Jarvis WR. Nosocomial transmission of multidrug-resistant *Mycobacterium tuberculosis*. Am J Infect Control 1995;23:146-51.
23. Williams J, Schneider N, Gilligan ME. Implementing a tuberculosis control program. Am J Infect Control 1995;23:152-5.
24. Gammaitoni L, Nucci MC. Using Maple to analyze a model for airborne contagion. Maple Tech 1997. In press.
25. Riley ER, Murphy G, Riley RL. Airborne spread of measles in a suburban elementary school. Am J Epidemiol 1978;107:421-32.
26. American Society of Heating, Refrigerating and Air-Conditioning Engineers. Chapter 7: Health Care Facilities. In: 1995 ASHRAE Handbook. HVAC Applications. Atlanta (GA): American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc.; 1995. p. 7.1-7.13.
27. The American Institute of Architects. Chapter 7: General Hospital. In: 1996-97 Guidelines for Design and Construction of Hospital and Health Care Facilities. Washington (DC): The American Institute of Architects Press; 1996. p. 11-61.
28. Health Resources and Services Administration. Guidelines for Construction and Equipment of Hospital and Medical Facilities. Rockville (MD): U.S. Department of Health and Human Services, Public Health Service; 1984. Publication No. (HRSA)84-14500.
29. McCracken RC. Facility engineering, planning and construction letter. Department of Medicine and Surgery, Veterans Administration, Heating, Ventilation, Air Conditioning Criteria; 1982. Publication No. IL

10A4-82-23.
30. U.S. Department of Health, Education and Welfare. Minimum requirements of construction and equipment for hospital and medical facilities. Washington (DC): U.S. Government Printing Office; 1979 Department of Health, Education and Welfare Publication No. 79-14500.
31. Vesley DL. Respiratory protection devices. Am J Infect Control 1995;23:165-8.
32. Riley RL, Nardell EA. Clearing the air: the theory and application of ultraviolet air disinfection. American Review of Respiratory Diseases 1989;139:1286-94.
33. Riley RL, Knight M, Middlebrook G. Ultraviolet susceptibility of BCG and virulent tubercle bacilli. American Review of Respiratory Diseases 1976;113:413-8.
34. Kethley CW, Branch K. 1972. Ultraviolet lamps for room air disinfection: effect of sampling location and particle size of bacterial aerosol. Arch Environ Health 1972;25:205-14.
35. National Institute for Occupational Safety and Health. Guide to industrial respiratory protection. Cincinnati (OH): U.S. Department of Health and Human Services, Public Health Service; 1987 DHHS (NIOSH) Publication No. 87-116.
36. National Institute for Occupational Safety and Health. Guide to selection and use of particulate respirators certified under 42 CFR 84. Cincinnati (OH): U.S. Department of Health and Human Services, Public Health Service; 1996 DHHS (NIOSH) Publication No. 96-101.
37. Centers for Disease Control and Prevention. Guidelines for preventing the transmission of tuberculosis in health-care settings, with special focus on HIV-related issues. MMWR Morb Mortal Wkly Rep 1990;39/RR-17:1-29.

Borna Disease Virus Infection in Animals and Humans

Jürgen A. Richt,* Isolde Pfeuffer,* Matthias Christ,* Knut Frese,† Karl Bechter,‡ and Sibylle Herzog*
*Institut für Virologie, Giessen, Germany; †Institut für Veterinär-Pathologie, Giessen, Germany; and ‡Universität Ulm, Günzburg, Germany

The geographic distribution and host range of Borna disease (BD), a fatal neurologic disease of horses and sheep, are larger than previously thought. The etiologic agent, Borna disease virus (BDV), has been identified as an enveloped nonsegmented negative-strand RNA virus with unique properties of replication. Data indicate a high degree of genetic stability of BDV in its natural host, the horse. Studies in the Lewis rat have shown that BDV replication does not directly influence vital functions; rather, the disease is caused by a virus-induced T-cell–mediated immune reaction. Because antibodies reactive with BDV have been found in the sera of patients with neuropsychiatric disorders, this review examines the possible link between BDV and such disorders. Seroepidemiologic and cerebrospinal fluid investigations of psychiatric patients suggest a causal role of BDV infection in human psychiatric disorders. In diagnostically unselected psychiatric patients, the distribution of psychiatric disorders was found to be similar in BDV seropositive and seronegative patients. In addition, BDV-seropositive neurologic patients became ill with lymphocytic meningoencephalitis. In contrast to others, we found no evidence is reported for BDV RNA, BDV antigens, or infectious BDV in peripheral blood cells of psychiatric patients.

Borna disease (BD), first described more than 200 years ago in southern Germany as a fatal neurologic disease of horses and sheep, owes its name to the town Borna in Saxony, Germany, where a large number of horses died during an epidemic in 1885. The virus etiology of BD was proven in the early 1900s when Zwick and coworkers (1) in Giessen, Germany, successfully transmitted brain homogenates from infected horses to experimental animals. Other milestones in BD-related research were the demonstration of virus growth in cell cultures (2-4); the finding that the pathogenesis of BD is caused by a T-cell–dependent immune mechanism (5-8); and most recently, the molecular characterization of the etiologic agent of BD, the highly neurotropic Borna disease virus (BDV) (9-17).

BD is characterized by a disseminated nonpurulent meningoencephalomyelitis with infiltration of mononuclear cells (1,8,18,19) and a predilection for the gray matter of the cerebral hemispheres and the brain stem (8,19). In neurons, sometimes in glia cells, acidophilic intranuclear inclusion bodies, called Joest-Degen inclusion bodies, are occasionally found. BD occurs sporadically in Germany and Switzerland; its presence in other countries has not yet been substantiated. Natural infections in other Equidae, ruminants, rabbits, cats, and ostriches have also been described (19-21).

This review discusses the etiology of Borna disease, the natural and experimental infection in various animal species, the pathogenesis of the disease in the experimental rat model, the genetic stability of BDV, and the possible link between BDV or a similar agent and human neuropsychiatric disorders.

Etiology

The etiologic agent of BD, BDV, has been recently characterized as an enveloped, nonsegmented, negative-stranded RNA virus with a genomic size of approximately 9 kb and a nuclear site for replication and transcription (14-17). The

Address for correspondence: Jürgen A. Richt, Institut für Virologie, Frankfurterstrasse 107, D-35392 Giessen, Germany; fax: 49-641-99-38359; e-mail: juergen.a.richt@vetmed.uni-giessen.de.

genomic organization is similar to that of members of the *Mononegavirales* order; therefore, BDV is the prototype of the new family *Bornaviridae* within this order. The *Mononegavirales* also include *Filoviridae* (e.g., Marburg and Ebola viruses), *Paramyxoviridae* (e.g., mumps, measles virus), and *Rhabdoviridae* (e.g., rabies, vesicular stomatitis virus). Six major open reading frames (ORFs)(I,II,III, IV,V,x1) are predicted in the genome sequence (16,17). Only five ORFs correspond to previously identified proteins with molecular weights of 10 (ORF x1: p10 BDV gene; 22), 18 (ORF III: gp18 BDV gene; 23), 24 (ORF II: p24 BDV gene; 24), 38/39 (ORF I: p38 BDV gene; 25), and 94 (ORF IV: p57 BDV gene; 26) or 84 (ORF IV: p57 BDV gene; 27, Richt et al., unpub. obs.) kDa.

Natural and Experimental Infection

Natural Infection

Extensive epizootiologic studies in horses have shown that BD is rare but occurs all over Germany, extending beyond the classic disease-endemic regions (28-30). Furthermore, BDV-specific antibodies were detected in horses in several European countries, Israel (28,29), Japan (31), Iran (32), and the United States (33). Since BDV-specific antibodies are frequently found in clinically healthy horses (20,28,29), natural infections in horses seem to remain subclinical in most cases. Unknown exogenous and endogenous factors might influence the genesis of the disease (20).

In addition to its predominant natural host, the horse, other Equidae, sheep, cattle, rabbits, goats, deer, alpacas, llamas, cats, pigmy hippopotamus, sloth, vari monkeys (*memur variegatus*), and ostriches have become naturally infected with BDV (8,19,20,34,35). In sheep flocks, clinical BD can affect large numbers of animals; however, in horse stables, usually only a few animals show clinical signs. The virus is assumed to be transmitted through salival, nasal, or conjunctival secretions because BDV-specific RNA has been found in these secretions (20,28,29,36). Animals become infected by direct contact with these secretions or by exposure to contaminated food or water. A minimum incubation period of 4 weeks is estimated for horses and sheep with nonspecific signs such as hyperthermia, anorexia, colic, and constipation in the initial phase of the disease. During the acute phase of disease, neurologic signs such as ataxia, depression, circular movement, standing in awkward positions, collapsing, running into obstacles, and paralysis, result from nonpurulent meningoencephalomyelitis. Clinical illness usually lasts 1 to 3 weeks, and death rates for diseased horses are 80% to 100% (18-20,37). In surviving animals, recurrent episodes are possible, especially after stress (18). Clinical manifestations, however, may vary among individual animals and various species.

BD tends to occur in spring and early summer and is more frequent in some years than in others; therefore, arthropods have been discussed as a potential vector. BDV, however, has never been isolated from insects in Europe. In the Near East, ticks have been associated with transmission of an equine encephalomyelitis similar to BD (38). A definite virus reservoir for BDV has not been found; various rodents most likely represent such a reservoir. In addition, since many seropositive horses with subclinical infections have infectious virus or BDV-specific RNA in various secretions, they can be potential sources of infection for other animals and humans (29,36).

Experimental Infection

BDV can be experimentally transmitted to a wide variety of animal species, from chickens to nonhuman primates (1,8,19,20,37). Incubation periods, clinical signs, and severity of the disease depend on the animal species and the virus variants. Rabbits, Lewis rats, and guinea pigs are highly susceptible, whereas chickens, monkeys, cattle, and tree shrews (Tupaia glis;39) are less susceptible (8,19). Some animals (hamsters, black-hooded rats, mice, ferrets, pigeons, and dogs), however, do not develop disease despite being persistently infected with BDV (1,8,18,19). The pathohistologic picture after experimental infections resembles that of natural infections. Most experimentally infected animals develop perivascular and parenchymal central nervous system (CNS) infiltrations and have infectious virus in the brain tissue. Frequently, the clinical picture in experimentally infected animals does not differ from that of spontaneously infected natural hosts.

The result of BDV infection in rats depends on the inbred rat strain and the virus isolate used for infection. Black-hooded rats do not show clinical signs after BDV infection with BDV isolates despite persistent virus infection and mononuclear infiltration of the CNS (40). Lewis rats, however, are highly susceptible and are

therefore used extensively for studies of BD pathogenesis (see below; 5,8,19). When infected with various BDV variants, Lewis rats exhibited clinical manifestations such as behavioral disorders, paralytic disease, or obesity, in addition to fertility problems (8,19), which indicates that BDV can form virus variants with different biologic properties.

Pathogenesis

BDV is a highly neurotropic agent that gains access to the CNS, probably by intraaxonal migration through the olfactory nerve or nerve endings in the oropharyngeal and intestinal regions (18,41). Virus spreads throughout the CNS by intraaxonal transport and centrifugally into the peripheral nerves. Antibody titers in naturally infected animals, such as in horses with clinical BD, are relatively low and are found in sera and cerebrospinal fluid (CSF) (19,20,28,29). Whether BDV-specific antibodies are neutralizing is not certain (5,42); the coexistence of BDV-specific antibodies and infectious virus in the CSF contradicts this assumption (43).

Extensive studies in the Lewis rat showed that the immune response to viral antigens after BDV infection does not elicit protective immunity but rather an immunopathologic reaction in which T cells play an important role (5,44). After adult rats are infected intra-cerebrally or intranasally, productive virus replication is found in the entire CNS. Once introduced into the rat's CNS, BDV usually causes a persistent infection with continuous productive replication in the brain and spinal cord (5,8). In immunocompetent animals, no infectivity was found in extraneural tissues at any stage of infection (5,45). In newborn animals, in contrast, the virus spreads throughout the whole organism; BDV-specific antigen was found in parenchymal cells of numerous organs, and infectious virus was found in excretions (45). The age of rats at the time of infection affects the capacity of host cells to support virus replication and, therefore, to control the spread of BDV.

The reason for the variation in virus replication is still unclear, and differences in the host's immunocompetence cannot be the only factor, since in adult immunosuppressed rats the virus is strictly neurotropic (45). All immunocompetent animals develop antibodies to BDV-specific antigens, and these antibodies coexist with infectious virus in the CNS (5). The outcome of BDV infections in rats depends on the rat strain and the passage history of the virus (see above; 19,40).

Despite productive virus replication in the CNS, immunocompromised animals (immunologically incompetent newborn and athymic rats) and immunosuppressed (by cyclophosphamide or cyclosporine A) animals do not become ill with BD or encephalitis, as do immunocompetent animals (5-8,29,44,46,47). The hypothesis of a virus-induced cell-mediated immunopathologic basis of BD was confirmed in adoptive transfer experiments in which spleen cells from infected rats were transferred into immunosuppressed virus carriers (5). Recipient rats developed BDV-specific antibodies, encephalitis and disease (5). The critical role of T cells in the pathogenesis of BD was demonstrated in adoptive transfer experiments: transfer of BDV-specific major histocompatibility complex class II-restricted T_H1 cell lines into drug-tolerized virus carriers resulted in acute meningoencephalitis and disease (Table 1; 6-8,29,47).

Consequently, conventional immunization with inactivated virus preparations or purified virus-specific antigens did not confer protective immunity against clinical BD, despite detectable levels of virus-specific antibodies after immunization (25; Richt, unpub. data). However, when viable BDV-specific T_H1 cells were passively transferred into naive rats before virus infection, animals were protected against clinical BD after challenge (Table 1; 29,47,48, Schmeel et al., unpub. obs.). In contrast, injection of the same viable BDV-specific T_H1 cells after BDV-infection induced clinical disease faster in BDV-uninfected animals than in BDV-infected control animals (29,47,48, Schmeel et al., unpub. obs.). Thus, in

Table 1. Adoptive transfer of Borna disease virus (BDV) T_H1 cells before and after BDV infection

Group	CY treatment	Transfer of T_H1-cells	Clinical BD
Control	yes	day +10	no
BDV	yes	no	no
BDV	no	day +10	yes
BDV	yes	day +3	yes
BDV	yes	day +10	yes
BDV	no	day -25 and -5	no

Four-week-old Lewis rats were inoculated intracerebrally with BDV; some rats were treated with cyclophosphamide (CY) (150 mg/kg) for immunosuppression 24 hours later. Animals were intravenously injected with $>2x10^6$ BDV T_H1 cells on the indicated days, either before (-) or after (+) infection with BDV.

BDV infections of the CNS with immunopathologic lesions, the same virus-specific immune cells that cause disease can also prevent disease, depending on the dynamics of virus replication at the time of the T-cell response.

Several reports of the pathogenesis of BD in BDV-infected animals indicate that the pathogenesis of BD in naturally and experimentally infected animals is also immunopathologic since the pathohistologic picture, including CNS-infiltrating cells in naturally BDV-infected animals, resembles that of experimentally infected rats (19); and immunosuppression or splenectomy of experimentally BDV-infected rabbits (49) and splenectomy of BDV-infected monkeys (Macaca mulatta; 50) affected the severity of clinical signs or prevented disease.

BDV Isolates from Naturally Infected Horses

To study the genetic variability of BDV, virus isolates were derived from three clinically ill horses in Germany (Lower Saxony, Rhineland-Palatina, Bavaria), euthanized between 1989 and 1995. BDV-specific antibodies were detected in the serum, and infectious virus, viral proteins, and viral RNA were detected in the CNS of all three horses (Pfeuffer et al., unpub. obs., 51). Molecular analysis of five different ORFs was performed. The coding regions of ORFs I, II, III, IV, and x1 were amplified by the reverse transcriptase-polymerase chain reaction (RT-PCR) technique with total RNA isolated from the CNS (36); the amplified cDNA was cloned and sequenced. The sequences of the respective ORFs were aligned and compared with viral sequences derived from a cell-culture–adapted virus strain, BDV strain V (14), which was isolated in 1929 from a horse with clinical BD. In the analyzed clones, two deletions resembling post-transcriptionally spliced mRNA species were identified (Pfeuffer et al., unpub. obs., 51). Similarly, two introns were recently identified when cell-culture–adapted BDV isolates were molecularly analyzed (16,17).

The degree of homology between the analyzed BDV isolates and the reference BDV strain V isolated more than 60 years ago (14) for the p24 BDV gene is summarized in Table 2. There is a maximal divergence of 2.0% at the nucleotide level and 2.5% at the amino acid level (Table 2). These data agree with recently published observations showing a high degree of homology within the p24 BDV gene of wild-type and cell-adapted viruses (52,53). When the other four ORFs were similarly analyzed, a homology of >95% at the nucleotide level and >97% at the amino acid level was found (Pfeuffer et al., unpub. obs., 51), indicating that in addition to the previously analyzed p24 (ORF II; 52,53) and p38 (ORF I; 53) genes, the other BDV-ORFs (ORFs III, IV, x1) also exhibit high genetic stability within one host species, the horse (Pfeuffer et al., unpub. obs., 51).

Nevertheless, antigenic variations of BDV wild-type isolates were found when brain material from approximately 50 horses with BDV infections were screened by immunocytochemistry and immunoblot assays with BDV-specific monoclonal and monospecific antibodies (51). Two isolates (horse #215, horse #2,300) showed a remarkable difference in antigenicity (Figure 1A,B). These isolates were not recognized by the p38 specific monoclonal antibody (mAb) Bo18 (25,51; Figure 1A). Polyclonal reconvalescent (51) and monospecific p38 antiserum or monospecific p24 antiserum (Figure 1B), however, reacted with BDV-specific proteins from both isolates in immunocytochemistry assays (Figure 1B) and immunoblots (51), indicating that the p38 and p24 BDV-proteins were synthesized. The recognition site for the mAb Bo18 within the p38 BDV-protein, however, was altered. To obtain detailed information on the location of these changes, we cloned and analyzed the p38 BDV gene of two horse isolates. Sequence analysis showed several nucleotide mutations within the respective p38 BDV genes; only one, however, led to an amino acid replacement (51). The

Table 2. Percentage of p24 Borna disease virus gene sequence homology

	Strain V	Horse #215	Horse #640	Horse #2300
Strain V		99.0	97.5	99.0
Horse#215	98.7		98.5	100.0
Horse#640	98.0	98.2		98.5
Horse#2300	98.8	98.7	98.2	

Numbers above diagonal are %homology at the amino acid level; numbers below diagonal represent the %homology among nucleotides. Strain V: isolated 1929 from a horse with clinical BD from Lower Saxony. Horse #215: isolated 1989 from a horse with clinical BD from Rhineland Palatine. Horse #640: isolated 1993 from a horse with clinical BD from Lower Saxony. Horse #2300: isolated 1995 from a horse with clinical BD from

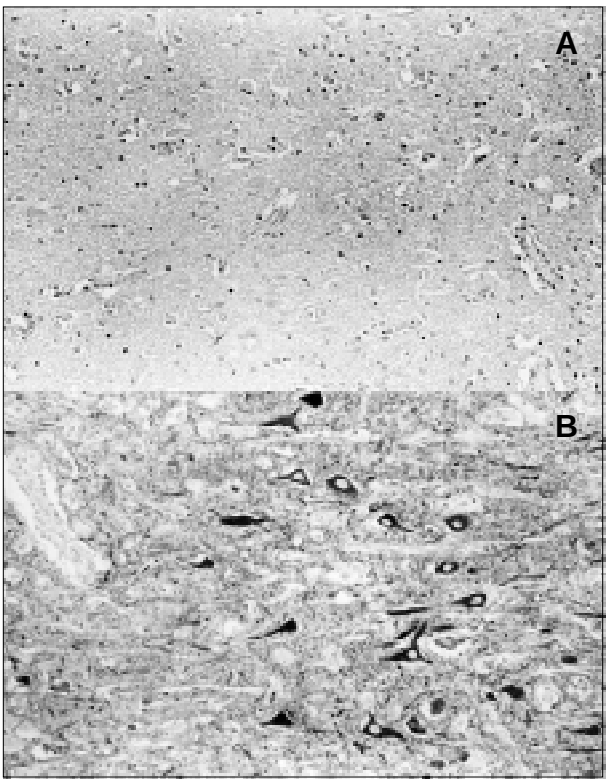


Figure 1. Immunohistologic analysis of consecutive brain sections from the cerebral cortex of horse #215 with A) the monoclonal antibody Bo18, specific for the p38/p39 BDV-protein and B) a rabbit monospecific serum, specific for the p24 BDV-protein. Immunoreactive neurons are only detected with the p24-specific antiserum. Papanicolaou-counterstain, x130.

amino acid replacements were adjacent to each other within the N-terminal part of the p38 protein, indicating that they are within the epitope of mAb Bo18 (51).

BDV and Human Neuropsychiatric Disorders

BDV's wide host range and its association with behavioral abnormalities in many species such as rhesus macaques (1,50), tree shrews (*Tupaia glis*; 39), and rats (5,8) suggest that BDV may be involved in human neuropsychiatric illnesses. Seroepidemiologic studies have demonstrated BDV-specific antibodies in sera of psychiatric and neurologic patients in Germany, Japan, and the United States (13,51,54-71). Recent studies with many patients showed a significantly higher prevalence of BDV serum antibodies among hospitalized psychiatric patients and a moderately higher seroprevalence among neurologic patients than among controls (13,56,60,68). Investigations of CSFs of BDV-seropositive patients acutely ill with psychiatric disorders (mainly schizophrenia and affective psychoses) showed intrathecally synthesized BDV-specific immunoglobulin (Ig) G in 25% of the patients (59).

A slightly higher level of seroprevalence was found among psychiatric patients in an area with high incidence of natural BD in horses (the BD-endemic area around Günzburg in Southern Germany) than among psychiatric patients in an area without endemic BD in the United States (Philadelphia area) (6.8% vs. 4.5%). The incidence in healthy controls was considerably lower in the United States (0% to 1%) than in the Günzburg area (3% to 3.5%; 55). These studies indicated a high number of clinically inconspicuous BDV-seropositive persons in the BD-endemic area. Therefore, as is the case in horses, inapparent BDV infections may be very common in humans (28,29,55).

An epidemiologic investigation of BDV seroprevalence in a large number (n = 4,673) of surgical (control), neurologic, and psychiatric patients showed continuous age-dependent increase in sero-prevalence in controls and neurologic patients (Figure 2; 55); the dynamics of seropositivity were different in psychiatric patients. A twofold higher seroprevalence in young neurologic patients paralleled a higher prevalence in patients with acute or chronic meningo-encephalitis and psychiatric disorders (56,71). Seroprevalence among the youngest quartile (aged 17-30 years) of psychiatric patients was 600% higher than among surgical patients (Figure 2;55), which suggests a role of BDV infections in the pathogenesis of psychiatric disorders (psychiatric disorders frequently begin in younger persons).

Initial investigations indicated a correlation between BDV-specific antibodies and affective psychoses (54,67). In a random group of psychiatric patients, however, we found a similar distribution of psychiatric disorders BDV-seropositive and BDV-seronegative patients (psychiatric disorders and brain atrophy were higher among the more severely ill BDV-seropositive patients [71]).

If a causal relationship exists between BDV infections in naturally infected animals and BDV seropositivity in humans, identifying patients

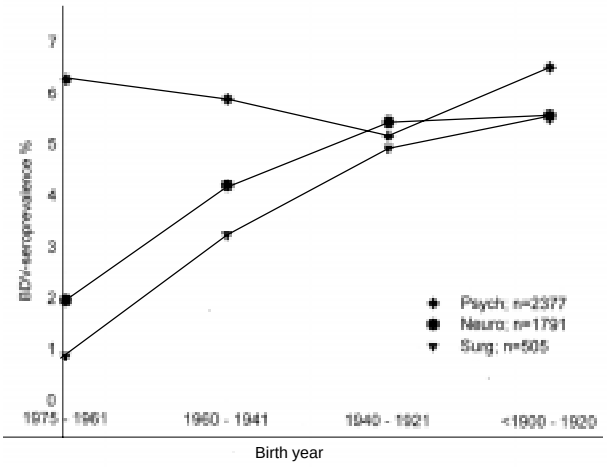


Figure 2. Borna disease virus seroprevalence among different age groups.
Psych=psychiatric patients; Neuro=neurologic patients; Surg=surgical (control) patients. Reproduced with permission from Habilitationsschrift der Fakultät für Klinische Medizin der Universität Ulm (55).

with symptoms and disorder progression similar to those of BDV-infected animals (paresis, weakness of legs, excitability, or depression) would be important. Recently, we identified a BDV-seropositive neurologic patient who exhibited abnormal body postures, depressive and apathetic behavior, and paresis (57). The patient worked at his father's farm, where one of four horses and four of 10 sheep had antibodies specific for BDV (57). The patient initially had a cerebral seizure and later neurologic and psychiatric symptoms resembling those of BD in animals and not typical of other known human encephalitic disorders, possibly implicating an acute, mild BDV encephalitis. Although isolation and molecular analysis of the respective viruses did not show an infectious chain, BDV transmission from animals to humans seems possible.

In addition, to study possible horizontal transmission of BDV within families, we reviewed the medical history of approximately 200 BDV-seropositive neuropsychiatric patients for coincident onset of mental or neurologic disorders during 1 year within each family. Of four families meeting the criteria, two were studied in detail; both had neuropsychiatric illness and BDV seropositivity in the primary patient and also in a second family member in the household (58). In two additional families, only the primary patients, not the other psychiatrically ill family members, showed BDV seropositivity (58). In summary, humans can be infected by BDV or a similar agent; a correlation between BDV infection in animals and humans exists; and BDV infections seem to contribute to or initiate various psychiatric syndromes.

To verify the etiologic role of BDV in human neuropsychiatric disorders, we tested specimens from the CSF of more than 40 BDV-seropositive neuropsychiatric patients for isolates of BDV or a related agent. The CSFs were spread onto cell cultures or injected intracerebrally into rabbits. BDV or a related agent was detected in the CSF of three neuropsychiatric patients (two patients with acute lymphocytic meningoencephalitis, one patient with acute schizophrenic psychosis; 67). The CSF of the schizophrenic patient showed high amounts of BDV-specific IgG, which became normal 5 years later when the CSF of this patient was again analyzed during myelography before lumbar surgery; at that time the patient was mentally healthy (60). Injection of the respective CSFs onto cell cultures showed expression of BDV-specific antigens (by BDV-specific staining pattern in an indirect immunofluorescence assay). Expression of BDV antigen, however, was lost, when these cells were passaged (13,67). In addition, all three cerebrospinal fluids induced BDV-specific antibodies in inoculated animals; however, neither BDV replication nor BDV-specific antigen was detected in the CNS of any of these animals (13,67).

Most recently, de la Torre and co-workers (72) detected both BDV-specific antigen and BDV-RNA in four autopsied human brains with hippocampal sclerosis and astrocytosis, which confirms previous studies that BDV can infect human brain tissue; whether BDV infection contributes to the pathophysiology of human neuropsychiatric disorders, however, warrants further investigation.

Before this study, BDV-specific proteins (73), BDV-specific RNA (63,64,69,74,75), and infectious BDV (76) were found in peripheral blood mononuclear cells of psychiatric patients. In addition, the presence of BDV-RNA in these cells in 4% to 5% of Japanese blood donors was also reported (63). Since these results have important implications for BD pathogenesis, the *intra vitam* diagnosis, and the safety of blood and blood products, we conducted a double-blind study of BDV-specific markers in the blood of psychiatric patients. Samples were aliquoted before distribution to two different laboratories and

tested independently. Forty-two seropositive psychiatric patients and four seronegative controls from a BD-endemic area in Germany were analyzed. Consecutive samples obtained over a 9-month period were included in the study. The nested RT-PCR method was used with primers specific for the p24 BDV-gene, the internal primers identical to the one used by Kishi and co-workers (63,64). Positive results for BDV-specific RNA were obtained only in the laboratory where BDV is routinely studied. Simultaneous analysis of the samples in another laboratory yielded negative results (62). In addition, cocultivation of peripheral blood mononuclear cells from 47 samples of seropositive patients over two passages and from eight samples over 8 to 10 passages with rabbit embryonic brain cells did not provide evidence of BDV or BDV-specific antigens (62). We thus concluded that contami-nation can have a serious influence on the out-come of studies using nested RT-PCR methods to probe for BDV-specific sequences in the peripheral blood mononuclear cells of psychiatric patients.

Viral infections of the CNS are often difficult to diagnose because conventional viral and serologic laboratory methods are still unsatisfactory. Existing standard techniques are either insensitive and slow (virus culture) or highly invasive and rare (brain biopsy) and, therefore, are unsatisfactory as a measure for the accuracy of a new technique such as PCR or RT-PCR (77). PCR techniques (PCR, RT-PCR, nested PCR), with their speed and high molecular sensitivity possess great potential for the diagnosis of viral infections of the CNS.

To overcome the discrepancies of recent RT-PCR studies about whether BDV or BDV-RNA can be detected in the peripheral blood of humans and animals, a multicenter study needs to be conducted with an independent center responsible for collecting and distributing samples to prevent potential contamination by BDV-RNA. These laboratories need to use similar RNA isolation and RT-PCR techniques. Furthermore, the methods used by the laboratories conducting sera or CSF analysis for BDV-specific antibodies in animals and humans urgently need further standardization.

Conclusion

Studies of the epizootiology and epidemiology of BDV infections in the last decade underscore the importance of BD as an emerging zoonosis. Data clearly indicate that BDV or an analogous virus can also infect humans. In addition to the seroepidemiologic results, data from preliminary studies on the isolation of BDV from the CSF and the detection of BDV-specific antigen and BDV-RNA in human brain confirm this conclusion. Therefore, humans belong to the wide spectrum of animal species, from birds to mammals, that are susceptible to BDV infections.

The question of whether BDV infections in humans contribute to human neuropsychiatric disorders remains unsolved. A convincing argument might be the significantly increased BDV-seroprevalence in psychiatric patients compared with healthy controls. That some mentally or neurologically healthy persons are BDV seropositive does not contradict this hypothesis, since the natural infection in horses remains subclinical in most cases and experimental intracerebral application of BDV into highly susceptible animals does not always result in clinical disease (Rott, pers. comm.). In addition, epizootiologic studies suggest that BDV is not highly contagious since only individual horses within a stable succumb to clinical disease. Similarly, analysis of a possible horizontal transmission of BDV within family members and spouses of approximately 200 BDV-seropositive neuropsychiatric patients showed only few cases of coincident onset of mental or neurologic disorders within 1 year. Whether BDV or BDV-RNA is present in human peripheral blood mononuclear cells remains questionable. Depending on the virus strain and the host, BDV can induce in animals a variety of clinical manifestations, from inapparent infections, behavioral abnormalities, obesity, fertility problems to fatal neurologic disease. Animals frequently show behavioral abnormalities during the course of the disease. This was very obvious when tree shrews (*Tupaia glis*), classified phylogenetically at the root of primates, were infected with BDV. Here, mainly disturbances in social behavior and severe apathy were observed. In contrast, an unequivocal classification of a definite psychiatric disease with BDV infections in humans is at present not possible.

To establish the etiologic role of BDV infections in human psychiatric symptoms, the following findings must be considered: symptomatology of BD in animals is highly variable depending upon many factors such as age, immune status, and strain of the animal used for infection, in addition to the genetic make-up of the virus; psychiatric symptomatology is nonspecific (78) even for known genetic causes, which has represented a major problem for research investigating known neuropsychiatric disorders caused by infectious agents. Despite the similarities in BDV infections of animals and humans, additional future molecular, seroepidemiologic, and clinical studies will be required to substantiate the possible contribution of BDV to the pathophysiology of neuropsychiatric disorders.

This paper is dedicated to Prof. Hermann Becht on the occasion of his 65th birthday.

Acknowledgments

The work was supported by the German Research Foundation, SFB 272 (I.P and J.A.R.), the Gemeinnützige Hertie-Stiftung (M.C. and J.A.R.), and the Bundesministerium für Gesundheit (S.H. and J.A.R.). We thank Prof. R. Rott for his continuous support and Dr. B. Boschek for critically reading the manuscript.

Dr. Richt is a research scientist and group leader at the Institute of Virology, Justus-Liebig-Universität Giessen, Germany. His current research topic is molecular pathogenesis of Borna disease virus infections. His interests include the epidemiology/epizootiology and immunopathology of Borna disease virus infections and the replication strategy of Borna disease virus.

References

1. Zwick W. Bornasche Krankheit und Encephalomyelitis der Tiere. In: Gildenmeister E, Haagen E, Waldmann O, editors. Handbuch der Viruserkrankungen.Gustav Fischer Verlag, Jena; 1939. p. 252-354.
2. Mayr A, Danner K. Production of Borna virus in tissue culture. Proc Soc Exp Biol Med 1972;140:511-5.
3. Ludwig L, Becht H, Groh L. Borna disease (BD), a slow virus infection—biological properties of the virus. Med Microbiol Immunol (Berl) 1973;158:275-89.
4. Herzog S, Rott R. Replication of Borna disease virus in cell cultures. Med Microbiol Immunol (Berl) 1980;173:153-8.
5. Narayan O, Herzog S, Frese K, Scheefers H, Rott R. Behavioral disease in rats caused by immunopathological responses to persistent Borna virus in the brain. Science 1983;220:1401-3.
6. Richt JA, Stitz L, Wekerle H, Rott R. Borna disease, a progressive encephalomyelitis as a model for CD4+ T cell-mediated immunopathology in the brain. J Exp Med 1989;170:1045-50.
7. Richt JA, Stitz L, Deschl U, Frese K, Rott R. Borna disease virus-induced meningoencephalomyelitis caused by a virus-specific CD4+ T cell-mediated immune reaction. J Gen Virol 1990;71:2565-73.
8. Richt JA, VandeWoude S, Zink MC, Clements JE, Herzog S, Stitz L, et al. Infection with Borna disease virus: molecular and immunobiological characerization of the agent. Clin Inf Dis 1992;14:1240-50.
9. Lipkin WI, Travis GH, Carbone KM, Wilson MC. Isolation and characterization of Borna disease agent cDNA clones. Proc Natl Acad Sci U S A 1990;87:4184-8.
10. VandeWoude S, Richt JA, Zink MC, Rott R, Narayan O, Clements JE. A Borna virus cDNA encoding a protein recognized by antibodies in humans with behavioral diseases. Science 1990;250:1278-81.
11. Richt JA, VandeWoude S, Zink MC, Narayan O, Clements, JE. Analysis of Borna disease virus-specific RNAs in infected cells and tissues. J Gen Virol 1990;72:2251-5.
12. Richt JA, Clements JE, Herzog S, Pyper J, Wahn K, Becht H. Analysis of virus-specific RNA species and proteins in Freon-113 preparations of the Borna disease virus. Med Microbiol Immunol (Berl) 1993;182:271-80.
13. Richt JA, Herzog S, Pyper JM, Clements JE, Narayan O, Bechter K, Rott R. Borna disease virus: nature of the etiologic agent and significance of infection in man. Arch Virol 1993;7 Suppl:101-9.
14. Briese T, Schneemann A, Lewis AJ, Park YS, Kim S, Ludwig H, Lipkin WI. Genomic organization of Borna disease virus. Proc Natl Acad Sci U S A 1994;91:4362-6.
15. Cubitt B, Oldstone C, de la Torre JC. Sequence and genome organization of Borna disease virus. J Virol 1994;68:1382-96.
16. de la Torre, JC. Molecular biology of Borna disease virus: prototype of a new group of animal viruses. J Virol 1994;68:7669-75.
17. Schneemann A, Schneider PA, Lamb RA, Lipkin WI. The remarkable coding strategy of Borna disease virus: a new member of the nonsegmented negative strand RNA viruses. Virology 1995;210:1-8.
18. Heinig A. Die Bornasche Krankheit der Pferde und Schafe. In: Roehrer H, editor. Handbuch der Virusinfektionen bei Tieren. VEB Fischer Verlag: Jena 1969;4:83-148.
19. Rott R, Becht H. Natural and experimental Borna disease in animals. Borna disease. Curr Top Microbiol Immunol 1995;190:17-30.
20. Becht H, Richt JA. Borna disease. In: Studdert MJ, editor. Virus diseases of equines. Amsterdam: Elsevier Science Publishers BV; 1996. p. 235-44.
21. Malkinson M, Weisman Y, Perl S, Asash E. A Borna-like disease of ostriches in Israel. Borna Disease. Curr Top Microbiol Immunol 1995;190:31-8.
22. Wehner T, Ruppert A, Frese K, Herden C, Becht B, Richt JA. Detection of a novel Borna disease virus encoded protein of 10 kilodalton in infected cells and tissues. J Gen Virol 1997;78. In press.
23. Kliche S, Briese T, Henschen AH, Stitz L, Lipkin WI. Characterization of a Borna disease virus glycoprotein, gp18. J Virol 1994;68:6918-23.
24. Thiedemann N, Presek P, Rott R, Stitz L. Antigenic relationship and further characterization of two major Borna disease virus proteins. J Gen Virol 1992;73:1057-64.

25. Haas B, Becht H, Rott R. Purification and properties of an intranuclear virus-specific antigen from tissues infected with Borna disease virus. J Gen Virol 1986;67:235-41.
26. Schneider PA, Hatalski CG, Lewis AJ, Lipkin WI. Biochemical and functional analysis of the borna disease virus G protein. J Virol 1997;71:331-6.
27. Gonzalez-Dunia D, Cubitt B, Grässer FA, de la Torre JC. Characterization of Borna disease virus p56 protein, a surface glycoprotein involved in virus entry. J Virol 1997;71:3208-18.
28. Herzog S, Frese K, Richt JA, Rott R. Ein Beitrag zur Epizootiologie der Bornaschen Krankheit des Pferdes. Wien Tierärztl Wschr 1994;81:374-9.
29. Richt JA, Herzog S, Schmeel A, Frese K, Rott R. Current knowledge about Borna disease. In: Nakajima H, Plowright W, editors. Equine infectious diseases VII. Newmarket: R & W Publications; 1994. p. 55-60.
30. Dürrwald R. Die natürliche Borna-Virus-Infektion der Einhufer und Schafe: Untersuchungen zur Epidemiologie, zu neueren diagnostischen Methoden (ELISA, PCR) und zur Antikörperkinetik bei Pferden nach Vakzination mit Lebendimpfstoff. Inaugural-Dissertation, Freie Universität Berlin; 1993.
31. Nakamura Y, Kishi M, Nakaya T, Asahi S, Tanaka H, Sentsui H, et al. Demonstration of Borna disease virus RNA in peripheral blood mononuclear cells from healthy horses in Japan. Vaccine 1995;13:1076-9.
32. Bahmani MK, Nowrouzian I, Nakaya Y, Nakamure Y, Hagiwara K, Takahashi H, et al. Varied prevalence of Borna disease virus infection in Arabic, thoroughbred and their cross-bred horses in Iran. Virus Res 1996;45:1-13.
33. Kao M, Hamir AN, Rupprecht CE, Fu ZF, Shankar V, Koprowski H, Dietzschold B. Detection of antibodies against Borna disease virus in sera and CSF of horses in the USA. Vet Rec 1993;132:241-4.
34. Schüppel KF, Kinne J, Reinacher M. Bornavirus-Antigennachweis bei Alpakas (Lama pacos) sowie bei einem Faultier (Choleopus didiactylus) und einem Zwergflu§pferd. Verh Erkr Zootiere 1994;36:189-93.
35. Schüppel KF, Kinne J, Lebelt J, Reinacher M. Zwei Fälle von Bornascher Krankheit bei Varis (Memur variegatus). 38. Tagung der Fachguppe "Pathologie der DVG", Hamburg; 1995.
36. Richt JA, Herzog S, Haberzettl K, Rott R. Demonstration of Borna disease virus-specific RNA in secretions of naturally infected horses by the polymerase chain reaction. Med Microbiol Immunol (Berl) 1993;182:293-304.
37. Ludwig H, Kraft W, Kao M, Gosztonyi G, Dahme E, Krey HF. Die Borna-Krankheit bei natürlich und experimentell infizierten Tieren: Ihre Bedeutung für Forschung und Praxis. Tierärztl Praxis 1985;13:421-53.
38. Daubney R, Mahlau EA. Viral encephalomyelitis of equines and domestic ruminants in the Near East. Res Vet Sci 1967;8 (Pt. 1):375-97.
39. Sprankel H, Richarz K, Ludwig H, Rott R. Behavior alterations in tree shrews (*Tupaia glis*, Diard 1820) induced by Borna disease virus. Med Microbiol Immunol (Berl) 1978;165:1-18.
40. Herzog S, Frese K, Rott R. Studies on the genetic control of resistance of black hooded rats to Borna disease. J Gen Virol 1991;72:535-40.
41. Morales JA, Herzog S, Kompter C, Frese K, Rott R. Axonal transport of Borna disease virus along olfactory pathways in spontaneously and experimentally infected rats. Med Microbiol Immunol (Berl) 1988;12:938-46.
42. Ludwig H, Furuya K, Bode L, Klein N, Dürrwald R, Lee DS. Biology and neurobiology of Borna disease viruses (BDV), defined by antibodies, neutralizability and their pathogenic potential. Arch Virol 1993;7 Suppl:111-33.
43. Ludwig H, Koester V, Pauli G, Rott R. The cerebrospinal fluid of rabbits infected with Borna disease virus. Arch Virol 1977;55:209-23.
44. Stitz L, Dietzschold B, Carbone KM. Immunopathogenesis of Borna disease. Curr Top Microbiol Immunol 1995;190:75-92.
45. Herzog S, Kompter C, Frese K, Rott R. Replication of Borna disease virus in rats: age-dependent differences in tissue distribution. Med Microbiol Immunol (Berl) 1984;173:171-7.
46. Stitz L, Soeder D, Deschl U, Frese K, Rott R. Inhibition of immune-mediated meningoencephalitis in persistently Borna disease virus-infected rats by Cyclosporine A. J Immunol 1989;143:4250-6.
47. Richt JA, Schmeel A, Frese K, Carbone KM, Narayan O, Rott R. Borna diseae virus-specific T cells protect against or cause immunopathological Borna disease. J Exp Med 1994;179:1467-73.
48. Schmeel A, Frese K, Richt JA. Immunopathogenesis of Borna disease virus infections in rats: role of BDV-specific TH1 cells in induction and prevention of Borna disease. In: Schwyzer M, Ackermann M, Bertoni G, Kocherhans R, McCullough K, Engels M, Wittek R, Zanoni R, editors. Immunobiology of viral infections. Proceedings of the 3rd Congress ESVV Interlaken 1995 Sep; 1995. p. 182-7.
49. Gierend M, Ludwig H. Influence of immunosuppressive treatment on Borna disease in rabbits. Arch Virol 1981; 67:217-28.
50. Stitz L, Krey HF, Ludwig H. Borna disease in rhesus monkeys as a model for uveo-cerebral symptoms. J Med Virol 1980; 6:333-40.
51. Herzog S, Pfeuffer I, Haberzettl K, Feldmann H, Frese K, Bechter K, Richt JA. Molecular characterization of Borna disease virus from naturally infected animals and possible links to human disorders. Arch Virol 1997;13 Suppl.
52. Binz T, Lebelt J, Niemann H, Hagenau K. Sequence analysis of the p24 gene of Borna disease virus in naturally infected horse, donkey and sheep. Virus Res 1994;34:281-9.
53. Schneider PA, Briese T, Zimmermann W, Ludwig H, Lipkin WI. Sequence conservation in field and experimental isolates of Borna disease virus. J Virol 1994;68:63-8.
54. Amsterdam JD, Winokur A, Dyson W, Herzog S, Gonzalez F, Rott R, Koprowski H. Borna disease virus: a possible etiological factor in human affective disorders? Arch Gen Psychiatry 1985;42:1093-6.
55. Bechter K. Borna disease virus—mögliche Ursache neurologischer und psychiatrischer Störungen des Menschen. Habilitationsschrift der Fakultät für Klinische Medizin der Universität Ulm. 1995.

56. Bechter K, Herzog S, Schüttler R .Possible significance of Borna disease for humans. Neurology Psychiatry Brain Research 1992; 1:23-9.
57. Bechter K, Herzog S, Schüttler R. Case of neurological and behavioral abnormalities: due to Borna disease virus encephalitis? Psychiatry Res 1992;42:193-6.
58. Bechter K, Herzog S, Schüttler R. Borna disease virus: possible causal agent in psychiatric and neurological disorders in two families. Psychiatry Res 1992;42:291-4.
59. Bechter K, Herzog S, Behr W, Schüttler R. Investigations of cerebrospinal fluid in Borna disease virus seropositive psychiatric patients. European Psychiatry 1995;10:250-8.
60. Bechter K, Herzog S, Schüttler R. Borna disease-possible cause of human neuropsychiatric disorders. Neurology Psychiatry Brain Research 1996;4:45-52.
61. Bode L, Riegel S, Lange W, Ludwig H. Human infections with Borna disease virus: seroprevalence in patients with chronic diseases and healthy indviduals. J Med Virol 1992;36:309-15.
62. Richt JA, Alexander RC, Herzog S, Hooper DC, Kean R, Spitsin S, et al. Failure to associate human psychiatric disorders with Borna disease virus infection. J Neurovirol 1997;3:174-8.
63. Kishi M, Nakaya T, Nakamura Y, Kakinuma M, Takahashi TA, Sekiguchi S, et al. Prevalence of Borna disease virus RNA in periopheral blood mononuclear cells from blood donors. Med Microbiol Immunol (Berl) 1995;184:135-8.
64. Kishi M, Nakaya T, Nakamura Y, Zhong Q, Ikeda K, Senjo M, et al. Demonstration of human Borna disease virus RNA in human peripheral blood mononuclear cells. FEBS Lett 1995; 3645:293-7.
65. Kitze B, Herzog S, Rieckmann P, Poser S, Richt JA. No evidence of Borna disease virus-specific antibodies in multiple sclerosis patients in Germany. J Neurol 1996;234:660-1.
66. Kubo K, Fujijoshi T, Yokoyama MM, Kamei K, Richt JA, Kitze B, et al. Lack of association of Borna disease virus and human T-cell leukemia virus type I infections with psychiatric disorders among Japanes patients. Clin Diagn Lab Immunol 1997;4:189-94.
67. Rott R, Herzog S, Fleischer B, Winokur A, Amsterdam J, Dyson W. Detection of serum antibodies to Borna disease virus in patients with psychiatric disorders. Science 1985;228:755-6.
68. Rott R, Herzog S, Bechter K, Frese K. Borna disease, a possible hazard for man? Arch Virol 1991;118:143-9.
69. Sauder C, Müller A, Cubitt B, Mayer J, Steinmetz J, Trabert W, et al. Detection of Borna disease virus (BDV) antibodies and BDV RNA in psychiatric patients: evidence for high sequence conservation and human blood-derived BDV RNA. J Virol 1997;70:7713-24.
70. Waltrip RW II, Buchanan RW, Summerfelt A, Breier A, Carpenter WT, Bryant NL, et al. Borna disease virus and schizophrenia. Psychiatry Res 1995;56:33-44.
71. Bechter K, Herzog S, Estler HC, Schüttler R. Increased psychiatric comorbidity in Borna disease virus seropositive psychiatric patients. Acta Psychiatr Belg. In press 1997.
72. de la Torre JC, Gonzalez-Dunia D, Cubitt B, Mallory M, Mueller-Lantzsch N, Grässer FA, et al. Detection of Borna disease virus antigen and RNA in human autopsy brain samples from neuropsychiatric patients. Virology 1996;223:272-82.
73. Bode L, Steinbach F, Ludwig H. A novel marker for Borna disease virus infection. Lancet 1994;343:297-8.
74. Bode L, Zimmermann W, Ferszt R, Steinbach F, Ludwig H. Borna disease virus genome transcribed and expressed in psychiatric patients. Nat Med 1995;1:232-6.
75. Bode L. Human infections with Borna disease virus and potential pathogenic implications. Borna Disease. Curr Top Microbiol Immunol 1995;190:103-30.
76. Bode L, Dürrwald R, Rantam FA, Ferszt R, Ludwig H. First isolates of infectious human Borna disease virus from patients with mood disorders. Molecular Psychiatry 1996;1:200-12.
77. Jefferey KJM, Read SJ, Peto TEA, Mayon-White RT, Bangham CRM. Diagnosis of viral infections of the central nervous system: clinical interpretation of PCR results. Lancet 1997;349:313-7.
78. Buchsbaum MS, Rieder R. Biologic heterogeneity and psychiatric research. Arch Gen Psychiatry 1979;36:1163-9.

Deer Ticks (*Ixodes scapularis*) and the Agents of Lyme Disease and Human Granulocytic Ehrlichiosis in a New York City Park

Rodent trapping and drag sampling in Van Cortlandt Park, New York City, yielded all stages of *Ixodes scapularis*, the deer tick vector of Lyme disease and human granulocytic ehrlichiosis (HGE). Polymerase chain reaction analyses of the ticks showed *Borrelia burgdorferi* and the *Ehrlichia* sp. that causes HGE.

Lyme disease, a tick-borne spirochetosis transmitted by the deer tick (*Ixodes scapularis* Say), was reported from 46 states in the United States in 1996; for the past 6 years, an average of 20% of those cases have been from Westchester and Suffolk Counties, New York (1). While spread of the deer tick population in New York State has been documented north and west of Westchester County (2), movement of ticks southward toward New York City has been largely ignored, despite rising Lyme disease case numbers in southern Westchester and a relatively high incidence of human parasitism by vector ticks. The discovery in Westchester County of human granulocytic ehrlichiosis (HGE), a second, potentially fatal, tick-borne disease (3), and of the causative *Ehrlichia equi*-like rickettsial agent in *I. scapularis* (4) highlights the significance of defining the geographic range of the deer tick. This is particularly important in urban areas, where residents may not be familiar with tick-borne diseases common in nearby suburban and rural areas. Foci of Lyme disease can occur in forested urban areas, as well as in rural sites, if ticks and their hosts are present (5).

Because of the proximity of Van Cortlandt Park to areas of southern Westchester where *I. scapularis* have been collected (Falco, unpub. data), the park's relatively large wooded area (approximately 60% of 468 ha), and the wide range of vertebrate hosts on which this tick feeds, we examined rodents live-trapped in the park to determine if deer ticks were present.

For one night in August 1995, during the period of larval *I. scapularis* activity, trapping was conducted on five study grids, each 50 m by 50 m. Fifty Sherman mouse traps (H.B. Sherman, Tallahassee, FL) and nine Tomahawk traps (Tomahawk Live Traps, Tomahawk, WI) for larger mammals were baited and placed on each grid. Mean distance between neighboring grids was 400 m, and all were located in the northern half of the park, where woodland is concentrated. Captured animals were lightly anesthetized with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IN); they were examined, and their age, sex, and weight were determined. All ectoparasites were collected, identified, and counted. Animals were released at the capture site after recovery from anesthesia.

The presence of this tick species in the park might lead to a Lyme disease or HGE focus and, therefore, the need for additional surveillance efforts. To further evaluate the risk for park visitors from infected ticks, host-seeking ticks were sampled in the summer (July 1996), when nymphal *I. scapularis* were active. Drag sampling, in which a 1 m^2 panel of white corduroy cloth is pulled along the ground and over vegetation to collect host-seeking ticks, was conducted. Any ticks found on the drag cloth or on researchers were removed with forceps, placed in a glass vial, and held live until identification. Specimens were stored in 70% ethanol until testing.

Polymerase chain reaction (PCR) analysis was conducted on all ticks collected in 1995 and 1996 (6). For nymphal and adult ticks, each specimen was dissected with sterile needles, and DNA was extracted by the Isoquick DNA extraction kit (ORCA Research, Bothell, WA), according to manufacturer's directions. Final DNA pellets were suspended in 50 µl of sterile water. Each tick extract was tested for *B. burgdorferi* and the HGE agent by PCR amplification of a 10 µl aliquot.

Even though neither *B. burgdorferi* nor other ehrlichiae are efficiently transovarially

transmitted (7,8), white-footed mice are competent reservoirs of both agents (9). Given the likelihood that transovarial transmission of the *Ehrlichia* sp. causing HGE is extremely low, larval *I. scapularis* collected from mice might have acquired either agent while feeding. Seven of the nine larvae removed from hosts were tested in pools of two (n = 2) or three (n = 1) specimens; the remaining two larvae were tested individually. Larvae were pooled only with ticks that had been removed from the same host animal. Larval specimens were likewise dissected in a tube, and DNA was extracted as described above.

B. burgdorferi-specific PCR targeted the spacer region between duplicated 23S rRNA genes with primer IS1 and IS2 (10). Amplified products were electrophoresed on a 1.5% agarose gel, and DNA was transferred to nylon membranes hybridized with a *B. burgdorferi*-specific probe (P19) (10). The HGE agent was detected by amplifying a 151 bp fragment of 16S rDNA with primers GER3 and GER4 (11). PCR products were resolved by electrophoresis on 2% agarose gels and visualized by staining with ethidium bromide.

Of 33 captured mammals examined in the summer of 1995, 19 were white-footed mice (*Peromyscus leucopus*), the primary reservoir of *B. burgdorferi*. Four (21%) mice hosted *I. scapularis*; two mice each hosted a single larva, one hosted two larvae, and one mouse hosted five larvae. Mice that hosted ticks were captured on three of the five trapping grids. Examination of the nine chipmunks (*Tamias striatus*), four gray squirrels (*Sciurus carolinensis*), and one flying squirrel (*Glaucomys volans*) that also were captured did not show any *I. scapularis* or other tick species.

To evaluate the relative density of host-seeking *I. scapularis*, i.e., unattached ticks available to parasitize a passing host, 5,840 m^2 of woodland habitat was drag sampled on or adjacent to the five trapping grids. One nymphal and two adult male *I. scapularis* were collected, along with a single *I. dentatus* nymph.

Results of PCR analyses indicated that one pool of two larvae, removed from a white-footed mouse that hosted five *I. scapularis*, was positive for the *Ehrlichia* agent of HGE. Of the four host-seeking ticks examined, two male *I. scapularis* were infected with *B. burgdorferi*; the single *I. dentatus* nymph was not infected with either agent. No specimens were infected with both agents. The primers used to amplify the HGE agent DNA would also yield PCR product with the closely related *E. platys* (an agent of canine ehrlichiosis) and a recently described *Ehrlichia* species from white-tailed deer (12). However, neither of these bacteria has been reported in hosts in the northeastern United States, nor are the invertebrate vectors known (although *Amblyomma americanum* is a suspected vector of the deer *Ehrlichia*) (12,13). Since the prevalence rate of the HGE agent in *I. scapularis* collected in Westchester County, New York, is approximately 20% (14), it is reasonable to conclude that in the current study, positive PCR results reflect the presence of the HGE agent.

Although anecdotal reports of Lyme disease by New York City residents who have not traveled to disease-endemic sites have previously suggested the presence of *I. scapularis* within city limits, to our knowledge, this is the first instance in which the deer tick has been confirmed on wildlife hosts resident in the city. Examinations of tick collections at both the American Museum of Natural History, New York (L.N. Sorkin, pers. comm.), and the U.S. National Tick Collection at the Institute of Arthropodology and Parasitology, Georgia Southern University (L.A. Durden, pers. comm.), also indicate that no specimens of *I. scapularis* previously collected from wildlife in New York City have been deposited.

These findings have several implications. First, the distribution of infested hosts suggests at least three potential tick population foci within Van Cortlandt Park. From these, a growing tick population may develop. Second, the larvae collected in this study were likely derived from eggs laid by replete females in the park. Thus, it is probable that host-seeking adults had successfully found medium- to large-sized mammals on which to feed during the previous adult season. The collection of nymphs and adult ticks further supports our conclusion that a population of *I. scapularis* is established in Van Cortlandt Park, though at a low level. By comparison, average drag densities at a woodland site in central Westchester County typically are one nymph per approximately 16 m^2 during a comparable period in the nymphal activity cycle (Daniels and Falco, unpub. data). Third, the potential exists for increased exposure to the agents of Lyme disease and HGE by park visitors. Rather than creating the peridomestic exposure that marks suburban habitats, tick populations in urban areas will likely result in more focal exposure, restricted to woodland habitat "islands,"

which exist primarily as parkland. The presence of white-tailed deer (*Odocoileus virginianus*) in such parks, even if it occurs on an intermittent, seasonal basis (as appears to be the case in Van Cortlandt Park), may serve to introduce new ticks into the park from adjoining disease-endemic areas. In this case, Westchester County is the apparent source of the sporadic deer migration. In addition, the presence of deer in the park can feed host-seeking ticks active at that time and thereby help increase the tick population. Even in the absence of white-tailed deer, the preferred host of adult *I. scapularis*, small populations of deer ticks may be maintained by medium-sized mammals such as raccoons (*Procyon lotor*). Therefore, these ticks may be overlooked by physicians unaware of the potential risk to their patients, resulting in undiagnosed cases of Lyme disease and HGE.

The *I. scapularis* population in Van Cortlandt Park may have been present for many years, though at such low densities as to be unnoticed. Moreover, other wooded parks in the city, which can provide a refuge for urban wildlife and ticks, may pose a risk of encountering ticks infected with either or both of these tick-borne disease agents. Further surveillance that measures the extant tick population is needed to assess the risk for Lyme disease and ehrlichiosis in this urban area.

Acknowledgments

We are grateful to Ramona Weidel, whose study of urban forests provided the basis for this investigation, and to members of her field crew for their considerable help during the trapping session; Tony Emmerich, City Parks Foundation; Charles Nilon and Theresa Boccia for help conducting field work and ectoparasite collections; and Lance A. Durden, Louis N. Sorkin, and Dennis J. White for helpful comments and additional information related to this study. This work was partially supported by the New York State Department of Health Tick-Borne Disease Institute grant C011999 (TJD), the American Lyme Disease Foundation (RCF), and NIH grant AR41511 (IS). This is contribution number 158 to the Louis Calder Center, Fordham University.

Thomas J. Daniels,*† Richard C. Falco,*†
Ira Schwartz,† Shobha Varde,†
and Richard G. Robbins‡
*Fordham University, Armonk, New York, USA;
†New York Medical College, Valhalla, New York, USA; ‡Walter Reed Army Medical Center, Washington, D.C., USA

References

1. Centers for Disease Control and Prevention. Lyme disease surveillance summary 1997;8:2.
2. White DJ, Chang HG, Benach JL, Bosler EM, Meldrum SC, Means RG, et al. The geographic spread and temporal increase of the Lyme disease epidemic. JAMA 1991;266:1230-6.
3. Centers for Disease Control. Human granulocytic ehrlichiosis—New York, 1995. MMWR Morb Mortal Wkly Rep 1995;44:593-5.
4. Bakken JS, Dumler JS, Chen SM, Eckman MR, Van Etta LL, Walker DH. Human granulocytic ehrlichiosis in the upper midwest United States. JAMA 1994; 272:212-8.
5. Magnarelli LA, Denicola A, Stafford KC, Anderson JF. *Borrelia burgdorferi* in an urban environment: white-tailed deer with infected ticks and antibodies. J Clin Microbiol 1995;33:541-4.
6. Schwartz I, Varde S, Nadelman RB, Wormser GP, Fish D. Inhibition of efficient polymerase chain reaction amplification of *Borrelia burgdorferi* DNA in blood-fed ticks. Am J Trop Med Hyg 1997; 56:339-42.
7. Magnarelli LA, Anderson JF, Fish D. Transovarial transmission of *Borrelia burgdorferi* in *Ixodes dammini* (Acari: Ixodidae). J Infect Dis 1987;156:234-6.
8. Dumler JS, Bakken JS. Ehrlichial diseases of humans: emerging tick-borne infections. Clin Infect Dis 1995; 20:1102-10.
9. Telford III SR, Dawson JE, Katavolos P, Warner CK, Kolbert CP, Persing DH. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. Proc Natl Acad Sci U S A 1996; 93:6209-14.
10. Schwartz I, Wormser GP, Schwartz JJ, Cooper D, Weissensee P, Gazumyan A, et al. Diagnosis of early Lyme disease by polymerase chain reaction amplification and culture of skin biopsies from erythema migrans lesions. J Clin Microbiol 1992;30:3082-8.
11. Munderloh UG, Madigan JE, Dumler JS, Goodman JL, Hayes SF, Barlough JE, et al. Isolation of the equine granulocytic ehrlichiosis agent, *Ehrlichia equi*, in tick cell culture. J Clin Microbiol 1996;34:664-70.
12. Dawson JE, Warner CK, Baker V, Ewing SA, Stallknecht DE, Davidson WR, et al. *Ehrlichia*-like 16S rDNA sequence from wild white-tailed deer (*Odocoileus virginianus*). J Parasitol 1996;82:52-8.
13. Rikihisa Y. The tribe *Ehrlichiae* and ehrlichial diseases. Clin Microbiol Rev 1991;4:286-308.
14. Schwartz I, Fish D, Daniels TJ. Prevalence of the rickettsial agent of human granulocytic ehrlichiosis in ticks from a hyperendemic focus of Lyme disease. N Engl J Med 1997;337:49-50

Jail Fever (Epidemic Typhus) Outbreak in Burundi

We recently investigated a suspected outbreak of epidemic typhus in a jail in Burundi. We tested sera of nine patients by microimmunofluorescence for antibodies to *Rickettsia prowazekii* and *Rickettsia typhi*. We also amplified and sequenced from lice gene portions specific for two *R. prowazekii* proteins: the gene encoding for citrate synthase and the gene encoding for the rickettsial outer membrane protein. All patients exhibited antibodies specific for *R. prowazekii*. Specific gene sequences were amplified in two lice from one patient. The patients had typical clinical manifestations, and two died. Molecular techniques provided a convenient and reliable means of examining lice and confirming this outbreak. The jail-associated outbreak predates an extensive ongoing outbreak of louse-borne typhus in central eastern Africa after civil war and in refugee camps in Rwanda, Burundi (1), and Zaire.

Typhus group rickettsioses are distributed worldwide. Rickettsial typhus has two forms: endemic (murine) typhus and epidemic typhus. Endemic typhus is caused by *R. typhi*, usually transmitted to humans by the rat flea, *Xenopsylla cheopis*; epidemic typhus is caused by *R. prowazekii*, transmitted by the body louse, *Pediculus humanus humanus* (2). In the United States, *R. prowazekii* has also been found in a sylvatic cycle involving flying squirrels and their ectoparasites (3). Typhus group rickettsioses, especially epidemic typhus, are associated with wars and human disasters.

During World Wars I and II, typhus spread through North Africa, the Pacific Islands, and Europe, especially in German concentration camps. In North Africa, the epidemics involved mainly civilian populations. U.S. forces were protected by the first vaccine applied on a large scale, the Cox-type vaccine (4). Epidemic typhus began to decline at the end of World War II when DDT started to be used as an insecticide. Ethiopia, Rwanda, Nigeria, and Burundi were the main foci of louse-borne typhus in Africa in the 1970s. Ethiopia reported 1,931 cases in 1983 and 4,076 cases in 1984 (5), with a death rate of 3.8%(6). However, these data were difficult to interpret because serologic testing was usually performed with inadequate techniques, and epidemiologic data were unavailable.

Political instability in Eastern European and African countries has recently caused conflicts in regions where the risk for typhus outbreaks is very high. In Yugoslavia and Rwanda, older persons who contracted epidemic typhus as youths can develop recrudescent illness and become the source of resurgent epidemics if louse infestation becomes prevalent. Recently, an outbreak of typhus was suspected in a Burundi jail; we diagnosed epidemic typhus by amplifying and sequencing portions of the citrate synthase and the rOmpB genes of *R. prowazekii* in lice.

Patients

In a jail in N'Gozi, Burundi, a local nurse observed patients with abrupt fevers of up to 40°C in association with a body louse outbreak from December 1995 to January 1996. Typhus was suspected, and chloramphenicol was given orally or intravenously. Blood specimens were obtained from nine patients (all young black men), and sera were sent to Marseille for serologic testing. Two lice per patient were obtained from the clothes of Patients Four, Five, Seven, Eight, and Nine; clinical information was also obtained (Table).

Serologic Investigation

Patients' sera were tested by microimmunofluorescence. Vero cell–grown rickettsia were purified (7) and placed on slides as spots. *R. prowazekii* Breinl strain and *R. typhi* Wilmington strain were used. Both antigens were placed on either side of the same slide to compare the serum reactivity with the two antigens. Sera were diluted from 1/32 to 1/4,096. Immunoglobulin G (IgG) antibodies were detected by gamma chain specific anti-human Ig (Bio Merieux, Marcy l'Etoile, France), and IgM was detected after IgG was removed by RF adsorbent (Behring) (7). The diagnostic cutoff for microimmunofluorescence in

Table. Clinical and serologic data (by microimmunofluorescence) for nine patients with louse-borne typhus

Patient number	One	Two	Three	Four	Five	Six	Seven	Eight	Nine
Age (yrs)	28	21	33	20	27	25	28	50	27
Onset date	1/25/96	1/26/96	1/6/96	1/19/96	1/20/96	1/19/96	12/16/95	1/13/96	1/20/96
Peak fever (°C)	40	39.7	40	39.5	40	39.5	39.7	39	40
Neurologic signs	no	no	yes	yes	yes	yes	yes	yes	yes
mental confusion	-	-	yes	no	yes	yes	yes	yes	yes
seizures	-	-	no	yes	no	no	no	no	no
coma	-	-	yes	no	no	no	yes	yes	no
Rash	yes	no	yes	no	no	no	no	no	no
purpuric	no	-	yes	-	-	-	-	-	-
Pulmonary signs	yes	no	no	yes	yes	yes	yes	no	no
cough	yes	-	-	yes	yes	no	no	-	-
dyspnea	yes	-	-	no	no	yes	yes	-	-
Vomiting	yes	no	yes	yes	no	yes	yes	no	no
Splenomegaly	no	yes	yes	no	no	no	yes	no	no
Hypotension	yes	no	no	no	yes	no	yes	no	yes
Death	no	no	no	no	no	no	yes	no	yes
Anti-*R. prowazekii* IgG titer	256	1024	2048	256	256	512	4096	1024	256
Anti-*R. prowazekii* IgM titer	256	512	512	256	512	512	256	1024	512
Anti-*R. typhi* IgG titer	64	512	128	256	64	0	1024	1024	64
Anti-*R. typhi* IgM titer	256	512	512	256	512	512	256	1024	512

diagnosing rickettsiosis is 1/128 for IgG and 1/64 for IgM (7,8). All nine patients were positive for both antigens; however, titers were identical for both antigens in Patients Four and Eight. In the other patients, antibodies were higher for *R. prowazekii* than for *R. typhi* by one-to-five dilution for IgG. IgM antibodies were at the same level against both antigens in all patients (Table).

Polymerase Chain Reaction (PCR) Detection in Lice

Lice were sent to Marseille and arrived dry; they were crushed, and DNA suitable for use as a template in PCR was extracted using QIAGEN columns (QIAamp Tissue Kit, QIAGEN, Hilden, Germany). Citrate synthase PCR amplification incorporated primers 120-M59 (CCGCAGGGTTGGTAACTGC) (9) and 120-508 (CCAAGTGATAAGAGGAGCTT) selected from the *R. prowazekii ompB* sequence (10). The citrate synthase amplicons were subjected to restriction fragment length polymorphism analysis using the enzyme *AluI* and were identical to that previously described for *R. prowazekii* (11). Sequence determinations were performed by using an automated laser fluorescent DNA sequencer as previously described (12). The citrate synthase and rOmpB sequences obtained from the lice had 100% homology with that of *R. prowazekii* strain Breinl. Of the 10 tested, two lice from the same patient (four) were positive.

Cause, Symptoms, and Diagnosis

Social conditions are critical factors influencing the reemergence of disease. Louse infestation occurs when cold weather, poor hygiene, and poverty are prevalent; these conditions have been present in refugee camps in Rwanda and Zaire recently (13,14). Louse infestation has also been found in several eastern European countries and in homeless persons in western Europe (15).

Epidemic typhus was suspected in a refugee camp in Goma, Zaire, in 1994, but the outbreak was unconfirmed (14). Typhus is usually exanthematic, and a careful clinical examination will find a rash in more than half of the cases. In the present series, clinical data were recorded retrospectively mainly on the basis of a nurse's report and are probably incomplete (Table). In an outbreak, looking for exanthema is critical for typhus suspicion. The typhus exanthema is frequently purpuric and is observed even on dark skin in 33% of cases (6). The other typical symptoms are neurologic signs including seizures, coma, and mental confusion, which gave the disease its name (ORIG: from the Greek word "typhos," which means smoke, cloud, stupor arising from fever). Neurologic involvement was noted in seven of the nine cases: symptoms included coma, seizures, mental confusion, and delirium. Rash was described in only two patients (Figure); splenomegaly in three patients; cough in three patients; dyspnea in three patients; and

hypotension in four patients. Two patients died. The other patients recovered rapidly after receiving chloramphenicol. The prevalence of pulmonary symptoms is high in general as noticed here.

In situations like those observed in this jail, one can suspect either murine or epidemic typhus. Murine typhus is less severe. However, in poor hygienic conditions, rats are prevalent, and the risk exists for both diseases. Consequently,

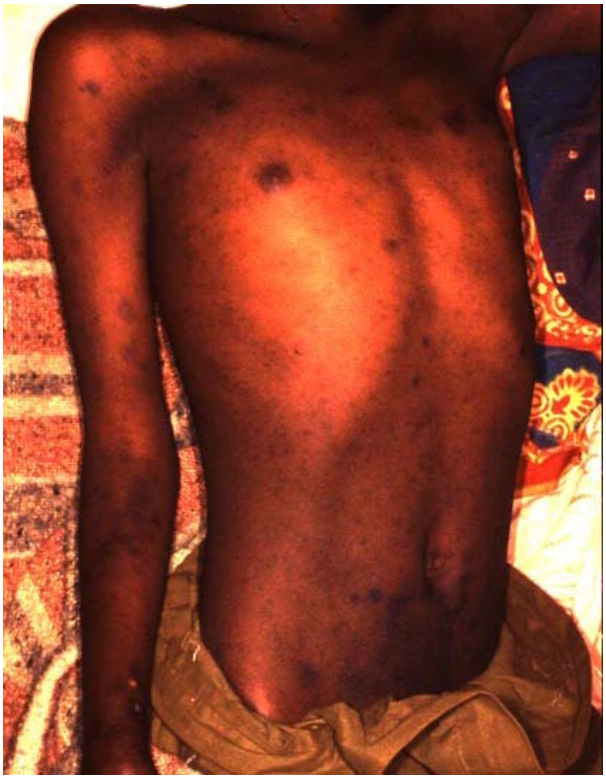


Figure. Rash in a man with epidemic typhus in Burundi.

in such situations it is critical to discriminate between *R. prowazekii* and *R. typhi* infections because they do not have the same epidemic potential. Diagnosis is usually provided by microimmunofluorescence; in some cases antibody levels are sufficient to differentiate between the two diseases. IgG titers are usually different, but IgM titers are not. When, as in Patients Four and Eight, antibodies are at the same level, cross-adsorption, a tedious and expensive procedure requiring large amounts of antigens and serum (not available in our study [16]), can differentiate between the two diseases. The use of arthropod vectors for gene amplification and disease recognition has been reported (17). In a case of laboratory-acquired human typhus, diagnosis was confirmed by PCR (18). However, in the present work, we report for the first time the use of the louse in diagnosing epidemic typhus in the field.

The confirmation of a typhus outbreak in Burundi is of concern, given the relative political instability in this area of the world. A preliminary report from the World Health Organization discusses a current large outbreak in Burundi with an estimated 24,000 cases (1). It is uncertain if the outbreak we observed is related to the current epidemic, but our present work confirms continuing cycles of louse-borne typhus in Burundi. Delousing (14) is critical to typhus prevention. The treatment for typhus is simple and inexpensive: a single dose of 200 mg of doxycycline provides cure (19).

We stress the major risk for exanthematic typhus in central eastern Africa including Burundi, Zaire, and Rwanda and the importance of sending lice to reference laboratories to identify the pathogens when a typhus outbreak is observed. Molecular identification by PCR and sequencing offers a rapid, sensitive, and specific identification method for *Rickettsia*, even when epidemiologic investigations are undertaken.

D. Raoult,* V. Roux,* J.B. Ndihokubwayo,† G. Bise,‡ D. Baudon,§ G. Martet,§ and R. Birtles*
*Université de la Méditerranée, Faculté de Médecine, Marseille, France; †CHU de Bujumbura, Laboratoire de Biologie Médicale, République du Burundi; ‡World Health Organization, CICR; Geneva, Switzerland; §Institut de Médecine Tropicale, Service de Santé des Armées, Marseille, France

References

1. World Health Organization. A large outbreak of epidemic louse-borne typhus in Burundi. Wkly Epidemiol Rec 1997;21:152-3.
2. Azad AF. Relationship of vector biology and epidemiology of louse- and flea-borne rickettsioses. In: Walker DH, editor. Biology of rickettsial diseases. Boca Raton (FL): CRC Press; 1991. p. 51-61.
3. McDade JE. Evidence of *Rickettsia prowazekii* infections in the United States. Am J Trop Med Hyg 1980;29:277-83.
4. Woodward TE. Rickettsial vaccines with emphasis on epidemic typhus. Initial report of an old vaccine trial. S Afr Med J 1986; Suppl:73-6.

5. World Health Organization. Louse-borne typhus, 1983-1984. Wkly Epidemiol Rec 1986;7:49-50.
6. Perine PL, Chandler BP, Krause DK, McCardle P, Awoke S, Habte-Gabr E, et al. A clinico-epidemiological study of epidemic typhus in Africa. Clin Infect Dis 1992;14:1149-58.
7. Eremeeva ME, Balayeva NM, Raoult D. Serological response of patients suffering from primary and recrudescent typhus: comparison of complement fixation reaction, Weil-Felix test, microimmunofluorescence, and immunoblotting. Clin Diag Lab Immunol 1994;1:318-24.
8. Ormsbee R, Peacock M, Philip R, Casper E, Plorde J, Gabre-Kidan T, Wright L. Serologic diagnosis of epidemic typhus fever. Am J Epidemiol 1977;105:261-71.
9. Wood DO, Williamson LR, Winkler HH, Krause DC. Nucleotide sequence of the *Rickettsia prowazekii* citrate synthase gene. J Bacteriol 1987;169:3564-72.
10. Regnery RL, Spruill CL, Plikaytis BD. Genomic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol 1991;173:1576-89.
11. Carl M, Dobson ME, Ching WM, Dasch GA. Characterization of the gene encoding the protective paracrystalline-surface-layer protein of *Rickettsia prowazekii:* presence of a truncated identical homolog in *Rickettsia typhi*. Proc Natl Acad Sci U S A 1990;87:8237-41.
12. Joblet C, Roux C, Drancourt M, Gouvernet J, Raoult D. Identification of *Bartonella* (*Rochalimaea*) species among fastidious Gram-negative bacteria based on the partial sequence of the citrate-synthase gene. J Clin Microbiol 1995;33:1879-83.
13. World Health Organization. Global surveillance of rickettsial diseases: memorandum from a WHO meeting. Bull World Health Organ 1993;71:293-6.
14. World Health Organization. Epidemic typhus risk in Rwandan refugee camps. Wkly Epidemiol Rec 1994;34:259
15. Stein A, Raoult D. Return of trench fever. Lancet 1995;345:450-1.
16. Raoult D, Dasch GA. Immunoblot cross-reactions among *Rickettsia*, *Proteus* spp. and *Legionella* spp. in patients with Mediterranean spotted fever. FEMS Immunol Med Microbiol 1995;11:13-8.
17. Azad AF, Sacci JB, Nelson WM, Dasch GA, Schmidtmann ET, Carl M. Genetic characterization and transovarial transmission of a typhus-like rickettsia found in cat fleas. Proc Natl Acad Sci U S A 1992;89:43-6.
18. Carl M, Tibbs CW, Dobson ME, Paparello SF, Dasch GA. Diagnosis of acute typhus infection using the polymerase chain reaction. Ann N Y Acad Sci 1990;590:439-44.
19. Perine PL, Krause DW, Awoke S, McDade JE. Single-dose doxycycline treatment of louse-borne relapsing fever and epidemic typhus. Lancet 1974;2:742-4.

Hantavirus Transmission in the United States

In 1996, investigation of a hantavirus pulmonary syndrome (HPS) outbreak in southern Argentina found evidence of person-to-person transmission of a hantavirus. The infection control ramifications of this finding led to this review of hantavirus epidemiology in the United States; the review suggests that Sin Nombre virus infection is rarely, if ever, transmitted from person to person and that existing guidelines for prevention of HPS remain appropriate for North America.

The public health significance of New World hantaviruses (genus Hantavirus, family Bunyaviridae) was first recognized in 1993 when healthy young adults in the southwestern United States contracted a mysterious pulmonary illness and died. The ensuing investigation identified a novel hantavirus, Sin Nombre virus (SNV), as the cause of a new syndrome, hantavirus pulmonary syndrome (HPS). A case-control study performed early in the outbreak showed that high density peridomestic rodent populations were a key risk factor for disease (1), a finding consistent with knowledge of Old World hantaviruses (including Puumala, Seoul, Hantaan, and Dobrava viruses), which can cause hemorrhagic fever with renal syndrome (HFRS) in humans who inhale aerosolized excreta from infected rodents (2). Thousands of episodes of HFRS occur in Eurasia each year, yet person-to-person transmission has never been reported. When person-to-person transmission of a hantavirus was suggested by the results of an HPS outbreak investigation in southern Argentina (3), we reviewed the epidemiology of HPS in the United States for evidence of nosocomial or secondary transmission.

Since 1993, a U.S. HPS registry has been maintained that includes surveillance case reports, environmental and epidemiologic case investigations, patients' medical records, and laboratory databases. This registry was used to identify the clusters of hantaviral infections shown in the Figure. A cluster was defined as the association of a patient with confirmed HPS with one or more contacts who had both laboratory evidence of SNV infection and a history of a febrile illness within 8 weeks of the onset of symptoms. Presence of serum immunoglobulin M (IgM) or IgG reacting with SNV antigen or immunohistochemical detection of SNV antigen in autopsy specimens was accepted as laboratory evidence of SNV infection. Eight weeks was selected as a conservative estimate of the incubation period on the basis of HFRS occurrence among United Nations (U.N.) military personnel within 7 days of arrival in (and as long as 46 days after departure from) a hantavirus-endemic area (4).

We identified five clusters (involving 12 patients) of hantaviral infections (Figure). The interval between cases was much shorter than 8 weeks in each instance, and contact between the linked patients from episodes 1, 3, and 4 occurred before patients were admitted to a hospital. All infected persons could have been exposed to rodent excreta in their home or work environments. Exposure risk was documented by results of rodent trapping in episodes 2, 3, and 4 (Table). In episodes 1 and 5, the men had been sleeping and working in rural environments where rodent infestation was noted, but trapping was not performed.

Viral products were amplified from the second of the two agricultural workers in episode 4 and from rodents captured at the ranch visited by the two men. The rodent and human viral sequences differed sufficiently to suggest that the second man's infection had been acquired elsewhere (S. St. Jeor, pers. comm.). Viral products could not be obtained from the other agricultural worker or from patients in the other clusters.

The U.S. case registry has on file 160 patients with clinical and laboratory evidence of HPS. Household or social contacts (n=320) of 40 of these HPS patients (including the HPS patients in the Figure) have been tested for antibodies that react with SNV antigen. Most contacts were household members or work colleagues of patients in whom HPS was diagnosed during the 1993 outbreak; testing sera from contacts of patients has not been routine in later years. Of these 320 household or social contacts, 310 had no serologic evidence of hantaviral infection,

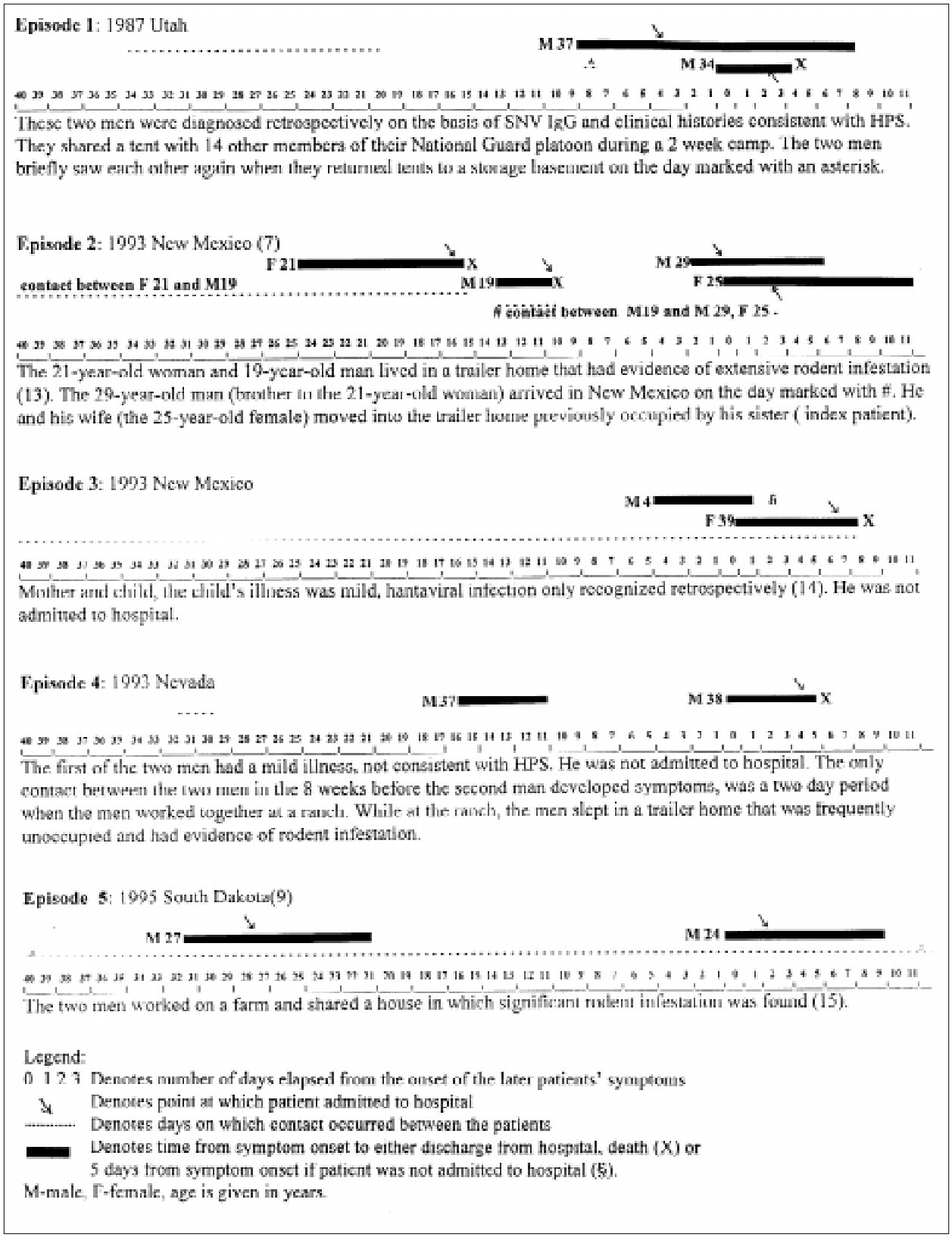


Figure. Clusters of hantaviral infections in the United States.*
*From a hantavirus pulmonary syndrome registry maintained by the Centers for Disease Control and Prevention

three had IgG that reacted with SNV antigen (SNV-IgG) but no illness, one had SNV-IgG and was diagnosed with HPS retrospectively (episode 1, Figure), and six had IgM that reacted with SNV antigen (SNV-IgM). Four of the six contacts with SNV-IgM had confirmed cases of HPS (episodes 2 and 5), and two had a clinical course not consistent with that of HPS (episodes 3 and 4) (5). While person-to-person transmission cannot be ruled out, these data may simply reflect common exposure of the persons involved to infected rodents.

Nosocomial transmission of HPS has never been documented in the United States. In 1993, a survey of 396 health-care workers from New Mexico examined evidence of nosocomial transmission of HPS (6). Although 266 of these workers recalled recent exposure to a patient with HPS, none became ill with HPS or had serologic evidence of infection with SNV. Statistically, this estimate could have been obtained (with 95% confidence intervals) if true hantaviral seroprevalence among these workers was $\leq$ 3%. During the first month of the 1993 outbreak, health-care workers inconsistently adhered to precautions to avoid respiratory or blood contact. Small numbers of needlestick exposures and mouth-to-mouth resuscitation attempts did not result in documented hantavirus infection (F. Koster, pers. comm.). The only health-care worker in the United States to have a documented SNV infection had evidence of peridomestic rodent infestation and no known contact with other HPS patients. HFRS was not seen in attendants, nurses, doctors, or research workers involved in caring for more than 3,000 U.N. military personnel who contracted HFRS in Korea during the early 1950s (2).

In contrast, one hospital receptionist and five doctors were among the 20 patients involved in the HPS outbreak in southern Argentina (3). Only two of the six recalled seeing rodents in the 6 weeks before onset of HPS; and when trapping was performed in the homes of four of these persons, no evidence of rodent infestation was found and no rodents were captured (J. Mills, pers. comm.).

While HPS in Argentinean health-care workers was unexpected, clustering of HPS patients is in keeping with rodent-borne zoonoses. Small localized clusters of HFRS were not uncommon among U.N. troops in Korea, with 1- to 3-week intervals between the onset of symptoms in the first and last patients (7). A cross-sectional study of hantavirus antibody prevalence in rural China identified localized clustering of HFRS patients, but the association between patients with HFRS was not statistically significant (8). Outbreaks of HFRS among laboratory workers also is well recognized. Epidemiologic investigation of one such outbreak found no hantaviral infections in household contacts of 113 persons who became infected after entering a building that housed a laboratory colony of infected rodents (9).

Electron microscopy and immunohistochemical studies of tissues from HPS patients suggest why these viruses may have such a low propensity to cause secondary hantaviral infections in humans. Despite immunohistochemical examination of tissues from HPS patients showing high levels of hantaviral antigens in the pulmonary microvasculature, mature virus particles are rarely visualized (10). Disease in humans appears to result from an overwhelming immunologic response after exposure to hantaviral antigens. The direct cytopathic effects of virus on host cells (as seen in some other devastating viral infections, such as that caused by Ebola virus) is not a feature of HPS. Differences in infectiousness could reflect the extent of viral replication and spread before the immune response becomes active or failure of the immune response to promptly eradicate virus.

Although infected rodents develop antibodies that react with hantaviral antigens, they do not have the immunologically mediated manifestations characteristic of HPS in humans. Rodents can continue to excrete virus in their

Table. Seropositivity of humans and rodents from episodes in which clustering of human hantaviral infections were identified in the United States

	Household contacts	Nonhousehold contacts	Site contacts[a]		Seropositive rodents
Episode	No. Positive (No. tested)	No. Positive (No. tested)	No. Positive (No. tested)	Rodents No. trapped[b]	Positive / No. tested (%)
1	0 (5)	0 (19)	0 (0)	nd	nd
2	3 (9)	0 (11)	0 (0)	40	8/27 (30)
3	1 (7)	0 (0)	0 (0)	87	15/41 (37)
4	1 (2)	0 (20)	0 (9)	80	7/80 (9)
5	1 (1)	0 (2)	0 (0)	nd	nd

[a]Persons with exposure to the home or worksite of the index patient but without exposure to the patient
[b]Trapping occurred in and around index patient's home/worksite
nd= not done

urine, saliva, and feces for prolonged periods despite the presence of serum antibodies. Hantaviruses have been isolated (albeit with difficulty) from infected rodents but not from tissues of HPS patients. Viral RNA is plentiful, however, and frequently can be amplified by reverse transcriptase and polymerase chain reaction (RT-PCR) from the blood and tissues of HPS patients. When the amino acid composition of viral proteins from HPS patients (coded for by these RT-PCR products) from southern Argentina was compared with that of other New World hantaviruses, differences varied from 13.6% to 23.9% for fragments of the G2 protein regions and from 8.5% to 12.5% for a 528 base pair fragment of the amino terminal region of the nucleocapsid protein (11). Thus, SNV (the etiologic agent responsible for most hantaviral infections in the United States) is phylogentically related to, but clearly distinct from, the Argentinean hantavirus, provisionally named Andes virus. The rodent host for the southern Argentinean virus is *Oligoryzomys longicaudatus*, a species not found in North America (12).

Milder disease was not a feature of the southern Argentinean outbreak; but interestingly, two patients with mild disease were identified in our search for HPS clusters. A small amount of mild disease resulting from SNV infection probably goes unrecognized in the United States. For example, in episodes 3 and 4, a diagnosis of febrile illness in the first patients was made only after the more severe course of HPS in the later patients led to testing for hantaviral antibodies. Hantaviral antibodies in sera from only 78 U.S. residents (in addition to the 160 confirmed patients) were identified by the Centers for Disease Control and Prevention, despite testing sera from approximately 7,000 U.S. residents as part of diagnostic work-ups, surveys of potential high-risk populations, and blinded population surveys.

Whether the secondary transmission of HPS observed in Argentina is a feature peculiar to hantaviruses in southern Argentina or a rare interaction between host and virus pertinent to all members of the genus is not known. In outbreaks associated with some highly pathogenic viruses (e.g., Ebola virus), "super-spreaders" (patients who transmit virus to many persons) have been an important factor in ongoing transmission of disease (A.S. Khan, pers. comm.). It is unlikely that such a phenomenon was solely responsible for the 1996 outbreak of HPS in southern Argentina, however, because multiple chains of transmission appear to have occurred (3).

This investigation highlights the importance of maintaining surveillance systems for emerging diseases such as HPS. Five clusters of patients in the U.S. HPS case registry were not unexpected on the basis of previous experience with hantaviral diseases. Although person-to-person transmission of SNV cannot be completely excluded, the clusters were consistent with the exposure of multiple persons to infected rodents. Thorough future investigation of clusters of SNV-infected patients will be important, but now it appears that existing recommendations have been sufficient to prevent secondary transmission of HPS in the United States.

Acknowledgments

We thank John O'Connor for his editorial comments and the following people for their assistance in clarifying the many questions raised during investigation of the five clusters: B. Tempest, F. Koster, G. Simpson, W. Frampton, J. Childs, S. St Jeor, and J. Tappero.

Rachel M. Wells, Joni Young, R. Joel Williams, Lori R. Armstrong, Kristi Busico, Ali S. Khan, Thomas G. Ksiazek, Pierre E. Rollin, Sherif R. Zaki, Stuart T. Nichol, and C.J. Peters
Centers for Disease Control and Prevention,
Atlanta, Georgia, USA

References

1. Zeitz PS, Butler JC, Cheek JE, Samuel MC, Childs JE, ShandsLA, et al. A case-control study of hantavirus pulmonary syndrome during an outbreak in the southwestern United States. J Infect Dis 1995;171:864-70.
2. Lee HW, van der Groen G. Hemorrhagic fever with renal syndrome. Prog Med Virol 1989;36:62-102.
3. Wells RM, Sosa Estani S, Yadon ZE, Enria D, Padula P, Pini N, et al. An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Emerg Infect Dis 1997;3:171-4.
4. Powell GM. Hemorrhagic fever: a study of 300 cases. Medicine (Baltimore) 1954;33:97-153.
5. Centers for Disease Control and Prevention. Case defnitions for infectious conditions under public health surveillance. MMWR Morb Mort Wkly Rep 1997;46:RR-10.
6. Vitek CR, Breiman RF, Ksiazek TG, Rollin PE, McLaughin JC, Umland ET, et al. Evidence against person-to-person transmission of hantavirus to health care workers. Clin Infect Dis 1996;22:824-6.
7. Gauld RL, Craig JP. Epidemiological pattern of localized outbreaks of epidemic hemorrhagic fever. American Journal of Hygiene 1954;59:32-8.

8. Ruo SL, Li YL, Tong Z, Ma QR, Liu ZL, Tang YW, et al. Retrospective and prospective studies of hemorrhagic fever with renal syndrome in rural China. J Infect Dis 1994;170:527-34.
9. Kulagin CM, Fedorova H, Ketiladze EC. Laboratory outbreak of hemorrhagic fever with a renal syndrome (clinico-epidemiological characteristics). Journal of Microbiology, Epidemiology and Immunology 1962;33:121-6.
10. Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foucar K, et al. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am J Pathol 1995;146:552-79.
11. Lopez N, Padula P, Rossi C, Lazaro ME, Franze-Fernandez MT. Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. Virology 1996;220:223-6.
12. Musser GG, Carleton MD. Family Muridae. In: Wilson DE, Reeder DM, editors. Mammal species of the world, a taxonomic and geographic reference. 2nd ed. Washington (DC): Smithsonian Institution 1993:501-755.
13. Duchin JS, Koster FT, Peters CJ, Simpson GL, Tempest B, Zaki S, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. N Engl J Med 1994;330:949-55.
14. Armstrong LR, Bryan RT, Sarisky J, Khan AS, Rowe T, Ettestad PJ, et al. Mild hantaviral disease caused by Sin Nombre virus in a four-year-old child. Pediatr Infect Dis J 1995;14:1108-10.
15. Centers for Disease Control and Prevention. Hantavirus pulmonary syndrome—United States, 1995 and 1996. MMWR Morb Mort Wkly Rep 1996;45:291-5.

Population Dynamics of the Deer Mouse (*Peromyscus maniculatus*) and Sin Nombre Virus, California Channel Islands

Hantavirus pulmonary syndrome, first documented in 1993, is caused by Sin Nombre virus (SNV), which is carried by the *Peromyscus* species. In 1994, high SNV antibody prevalence was identified in deer mice from two California Channel Islands. We sampled two locations on three islands to estimate mouse population density and SNV prevalence. Population flux and SNV prevalence appear to vary independently.

A new acute respiratory illness, hantavirus pulmonary syndrome (HPS), was first documented in May 1993 in New Mexico (1). The death rate was initially more than 90% (1,2) and is now approximately 50% (3). Serologic surveys indicated positive reactions with previously known hantavirus antigens but not with any agents usually associated with severe respiratory illness (2). Four distinct serotypes of hantaviruses, which are carried by rodents, were known before 1993 (2). The virus causing HPS in the Four Corners area, Sin Nombre virus (SNV), represents an unusual fifth serotype that affects the lungs and has a high death rate (1,4).

During the 1993 outbreak, rodents were trapped in and near homes with confirmed SNV cases and tested for hantavirus antibodies (1). *Peromyscus maniculatus* (deer mouse) was the most common rodent captured and had the highest antibody prevalence. Average prevalence was 30.4% (0% to 51.3% range) for 813 *P. maniculatus* captured at 21 sites (1).

P. maniculatus is the only species of mouse on four of the five islands that make up Channel Islands National Park; Santa Cruz Island also has populations of *Reithrodontomys megalotis*. Channel Islands National Park (Figure 1) has been monitoring deer mouse populations on Santa Barbara Island for 19 years (recorded data are incomplete) and on San Miguel and Anacapa Islands since 1993 (5; C. Schwemm, pers. comm.). Concern for the health of persons trapping mice and others on the islands prompted testing of mice on San Miguel, Santa Rosa, and Santa Barbara Islands for hantavirus in 1994. After SNV was identified in mice from these islands, mice from the other five Channel Islands were tested. Blood samples from technicians and

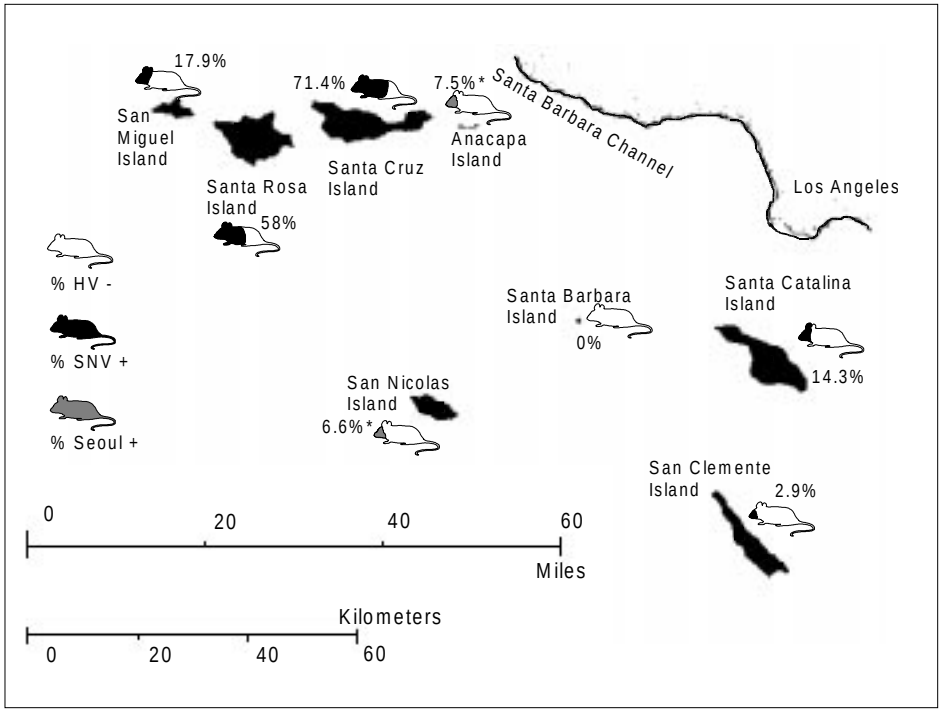


Figure 1. The California Channel Islands, with prevalence of Sin Nombre virus (SNV) and Seoul* hantaviruses on each island in 1994 (6). Channel Islands National Park comprises San Miguel, Santa Rosa, Santa Cruz, Anacapa, and Santa Barbara Islands.

others living or working on the islands in close contact with mice were also tested.

These tests indicated that the prevalence of SNV antibodies was 0% to 71% in mice tested from each island and that cross-reactivity to the Seoul hantavirus occurred on some islands (6) (Figure 1). Prevalence values for Santa Cruz (71%) and Santa Rosa (58%) Islands were higher than for any mainland population (1). No antibodies to hantaviruses were found in blood from any Channel Islands National Park or Santa Rosa ranch employees, including those living in mouse-infested cabins on islands with high hantavirus antibody prevalence in mice (K. Reilly, pers. comm.). Despite the lack of evidence of previous infection, concern for the health of visitors and employees remains.

P. maniculatus populations were sampled at only one location on each island during the 1994 survey. To understand the dynamics of the *P. maniculatus*–SNV relationship, the geographic variability of SNV on each island should be determined, requiring a more extensive sampling program. Few surveys have estimated rodent population densities in conjunction with testing for hantavirus; thus, the actual percentage of a population carrying the virus cannot be estimated, and prevalence values between sites cannot be compared (7,8). Furthermore, because mice were not trapped systematically and mouse densities were not estimated during sampling in 1994, comparisons between islands or sample times must be made with caution. Simultaneous monitoring of mouse populations and SNV prevalence in them should improve understanding of viral transmission from mouse to mouse and of the dynamics of changes in SNV antibody prevalence relative to mouse population fluctuations. The objectives of this pilot study were to determine whether prevalence differs spatially among populations on each island and between islands and whether prevalence differs temporally within each population.

We sampled mice at two locations on San Miguel, Santa Rosa, and Santa Cruz Islands. Sites were far enough apart that individual mice were unlikely to move directly from one site to the other. One site on each island was near concentrated human activity; the sites included the areas sampled in 1994 but not the exact locations. Mice were trapped in September 1995 (San Miguel and Santa Rosa Islands) and in February (San Miguel Island) 1996 and March (Santa Rosa and Santa Cruz Islands) 1996. These months generally represent the high and low population levels in the annual cycle on the islands (5). We trapped three nights at each site using 200 Sherman live traps arranged in a radial web design (9). Mouse population densities were estimated directly by the distance sampling theory (10). The number of new mice captured each day in each ring of traps is used to calculate density (number of mice per hectare) with the program DISTANCE (10).

Blood was collected from the suborbital sinuses of all mice captured except those from the San Miguel Island airstrip web in September 1995; only 34 of 247 mice captured there were sampled because of time constraints. Blood was refrigerated until transfer to the University of California, Davis. An enzyme immunosorbent assay with recombinant antigen (1) was used to determine the percentage of infected rodents and their antibody titer. Samples collected in September 1995 were analyzed by the Centers for Disease Control and Prevention; samples from February and March 1996 were analyzed at the University of California, Davis.

On the three islands, 531 mice were captured in 6,000 trap nights. Of these, 316 were tested for SNV antibodies, and 54 were positive (overall prevalence estimate 17%). Capture success was lower in 1996 on all islands as were density estimates (Table). We caught no mice at one web on Santa Rosa Island in September 1995, and only one mouse, which was seropositive, on the other web; a few days earlier on San Miguel Island we had caught 365 mice in the two webs. More mice were caught in September at the San Miguel Island airstrip web than at any other site or sample period (Table), but the program DISTANCE generated an unrealistic density estimate (> 8,000 mice per hectare). The number of mice caught near the center of the web was apparently too large to fit the models in DISTANCE (D. Anderson, pers. comm.). Therefore, we generated a naive density estimate for the San Miguel Island airstrip population in 1995 by dividing the total number of mice caught by the area covered by the web. We caught nine mice on Santa Cruz Island, two *P. maniculatus* on the Ranch web, and six *P. maniculatus* and one *R. megalotis* on the East Val web. Density at the East Val web was estimated as total mouse density using all seven mice. The density of *P. maniculatus* (Table) was calculated by multiplying total density by

Table. Numbers of *Peromyscus maniculatus* captured and tested on San Miguel (SMI), Santa Rosa (SRI), and Santa Cruz (SCI) Islands in 1995 and 1996, and results of serologic assay on blood collected from captured mice

Island (Site)	Dates	Total captures (no.)	Mice (no.)	Density no. ha^{-1} (SE)	Tested for SNV (no.)	SNV-positive (%)	Female: Male	Female: Male SNV-positive
SMI (Helipad)	3-5 Sep 95	152	118	104.3 (9.6)	116	15.5	59:57	6:12
SMI (Helipad)	15-17 Feb 96	67	49	43.3 (6.19)	49	14.3	25:24	3:4
SMI (Airstrip)	3-5 Sep 95	353	247	218[a] (NA)	34	5.9	17:17	1:1
SMI (Airstrip)	15-17 Feb 96	90	70	61.9 (7.4)	70	30	37:33	10:11
SRI (Airstrip)	7-9 Sep 95	2	1	NA	1	100	0:1	0:1
SRI (Torrey)	7-9 Sep 95	0	0	NA	0	NA	0:0	0:0
SRI (Campgr)	27-29 Mar 96	43	22	19.5 (4.15)	22	13.6	9:13	0:3
SRI (Cherry)	27-29 Mar 96	27	15	13.3 (3.42)	15	13.3	5:10	0:2
SCI (East Val)	23-25 Mar 96	10	6	5.3 (2.01)	6	0.0	2:4	0:0
SCI (Ranch)	23-25 Mar 96	5	2	NA	2	0.0	1:1	0:0

[a] Density was not estimated using DISTANCE, but directly, as the number of mice caught (247) divided by the area covered by the web (1.13 ha) .

the proportion of *P. maniculatus* (0.857) caught. The eight *P. maniculatus* and the *R. megalotis* were seronegative for SNV antibodies.

The prevalence of SNV is plotted against estimated mouse density for all island sampling sites and periods (Figure 2). The prevalence of SNV was similar for the San Miguel Island helipad population in both sampling periods and for the two Santa Rosa Island populations sampled in March 1996, despite densities ranging from 13 to 104 mice per hectare (Figure 2). The lowest prevalence occurred in the densest population, and the highest prevalence, found in the same population, occurred at a density similar to that of other locations (Figure 2). These results indicate that infection rates may be independent of mouse population dynamics. Monitoring mouse population size may not be an effective predictor of infection, but it may still be the best monitoring tool because it indicates the likelihood of exposure to deer mice and their feces and urine and thus the potential risk for SNV.

Chi-square analysis of the proportions of seropositive female and male mice was done for San Miguel Island populations (each site for each sampling period, total numbers tested in 1995 and in 1996, and all mice tested from San Miguel Island during the study). All mice tested from Santa Rosa Island were also analyzed for differences in infection rates by sex; no differences were found.

In 1996, we caught only adult mice, but in September 1995, we caught juvenile, subadult, and adult mice on San Miguel Island. Significantly more adult mice (16/39) were seropositive than expected, and fewer juvenile (2/38) and subadult (0/39) were seropositive at the helipad web (chi-square = 29.6, $p < 0.0001$), and for all mice (juvenile: 2/52, subadult: 1/54, adult: 17/44) tested on San Miguel Island (chi-square = 34.6, $p < 0.0001$). Douglass et al. (8) found a similar

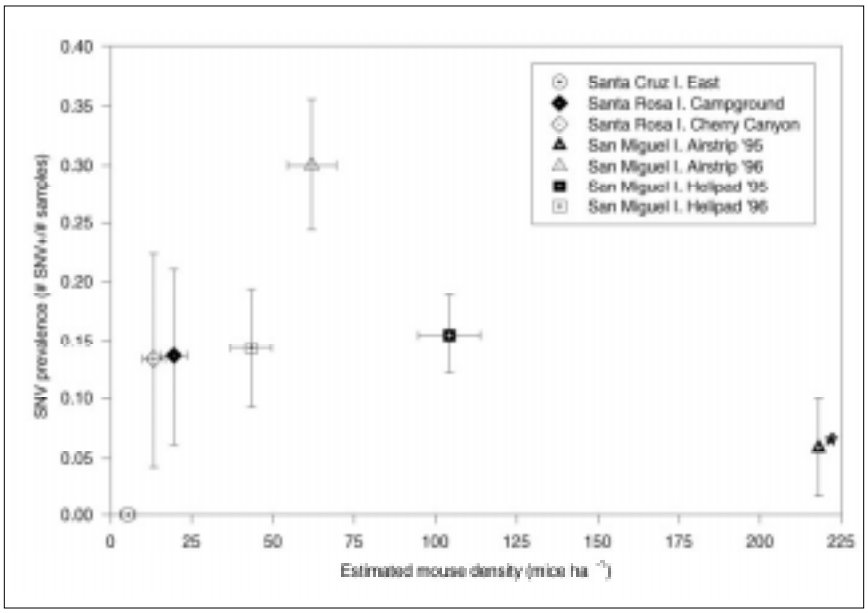


Figure 2. Deer mouse densities (±SE) vs Sin Nombre virus (SNV) prevalence (±SE) for three Channel Islands.*Density estimated directly as total number of mice caught per area of web.

pattern in Montana populations, but Jay et al. (6) did not find age differences in California mice.

We tried to trap near previous sampling locations, but only the San Miguel Island helipad site overlapped an area sampled in 1994. SNV prevalence was roughly the same at this site for our two sampling dates (16% and 14%), despite a large decline in numbers of mice. Both estimates were similar to the prevalence (18%) found in January 1994.

Our data indicate that the prevalence of SNV in mouse populations is different on different islands and that various locations on a single island may have specific dynamics. For example, density declined in both the helipad and airstrip populations from September 1995 to February 1996; SNV prevalence remained essentially the same in the helipad population but increased from 6% to 30% in the airstrip population (Figure 2). Prevalence estimates from our work were generally different from earlier testing, which implies at least spatial variation and perhaps temporal changes. Deer mice on the islands should be tested for SNV prevalence throughout the annual population cycle to determine how SNV is maintained in populations and how it is transferred from mouse to mouse.

Acknowledgments

We thank Cathy Schwemm, Tim Coonan, and Greg Austin for valuable assistance with planning and logistics throughout the project; Jim Mills, Jamie Childs, and Pierre Rollin for advice and serology on the 1995 San Miguel Island blood samples; Jerry Johnson for his assistance with the funding process; Ken Yamada and Michele Jay for advice and safety equipment; Michele Jay for fieldwork; students from UC Davis, UC Santa Barbara, and Prescott College for their assistance with fieldwork; NPS and U.S. Navy boat crews for transportation; and NPS and Santa Cruz Island reserve staff for help on the islands. Funding from CDC was provided through the National Park Service.

Tim B. Graham* and Bruno B. Chomel†
*USGS, Biological Resources Division, Moab, Utah, USA; †University of California, Davis, California, USA

References

1. Childs J, Ksiazek T, Spiropoulou C, Krebs J, Morzunov S, Maupin G, et al. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. J Infect Dis 1994;169:1271-80.
2. Nichol S, Spiropoulou C, Morzunov S, Rollin P, Ksiazek T, Feldmann H, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 1993;262:914-7.
3. Centers for Disease Control and Prevention. Hantavirus pulmonary syndrome—United States, 1995-1996. MMWR Morb Mortal Wkly Rep 1996;45:291-5.
4. Hjelle B, Chavez-Giles F, Torrez-Martinez N, Yamada T, Sarisky J, Ascher M, Jenison S. The dominant glycoprotein epitope of Four Corners hantavirus is conserved across a wide geographic area. J Gen Virol 1994;75:2881-8.
5. Drost C, Fellers G. Density cycles in an island population of deer mice, *Peromyscus maniculatus*. Oikos 1991;60:351-64.
6. Jay M, Ascher M, Chomel B, Madon M, Sesline D, Enge B, et al. Seroepidemiologic studies of hantavirus infection among wild rodents in California. Emerg Infect Dis 1997;3:183-90.
7. Parmenter R. Pecos National Historic Park mammal survey data help solve hantavirus mystery. Park Science 1995;15:12-3.
8. Douglass R, Van Horn R, Coffin K, Zanto S. Hantavirus in Montana deer mouse populations: preliminary results. J Wildl Dis 1996;32:527-30.
9. Anderson D, Burnham K, White J, Otis D. Density estimation of small-mammal populations using a trapping web and distance sampling methods. Ecology 1983;64:674-80.
10. Buckland S, Anderson D, Burnham K, Laake J. Distance sampling. New York: Chapman and Hall, 1993.

Emerging Quinolone-Resistant *Salmonella* in the United States

We conducted a national survey of antimicrobial resistance in human clinical isolates of *Salmonella* between July 1, 1994, and June 30, 1995. Every tenth nontyphoidal *Salmonella* isolate received at state public health laboratories in the United States during this period was tested for resistance to 12 antimicrobial agents, including two quinolones, nalidixic acid, and ciprofloxacin. Emerging quinolone resistance was detected; of 4,008 isolates tested, 21 (0.5%) were resistant to nalidixic acid, and one (0.02%) was resistant to ciprofloxacin. Continued surveillance for quinolone-resistant *Salmonella* is necessary, particularly after the recent approval of a fluoroquinolone for use in animals intended for food in the United States.

Each year, an estimated 2 to 4 million human *Salmonella* infections occur in the United States (1,2). Although most of these infections cause a mild, self-limiting illness, serious sequelae, including invasive infections and death can occur (1,2). Antimicrobial therapy is not recommended for routine treatment of salmonellosis; however, appropriate antimicrobial therapy can be life-saving for patients with invasive disease. Since antimicrobial agents are essential for treating some *Salmonella* infections, isolates from such infections should be monitored for antimicrobial resistance, particularly resistance to fluoroquinolones (e.g., ciprofloxacin). Fluoroquinolones have been in human clinical use in the United States since the mid-1980s and are recommended for treating invasive *Salmonella* infections in adults (3,4). Resistance to nalidixic acid—the prototypic quinolone—has been found in some instances to precede resistance to fluoroquinolones (5).

To determine the prevalence of antimicrobial resistance among *Salmonella* isolates in the United States, we conducted a study between July 1, 1994, and June 30, 1995. All state public health laboratories sent every tenth nontyphoidal *Salmonella* isolate they received to the Centers for Disease Control and Prevention for antimicrobial testing. Isolates were tested by the disk diffusion method for resistance to 12 antimicrobial agents, including two quinolones, nalidixic acid, and ciprofloxacin. For ciprofloxacin-resistant isolates, the minimum inhibitory concentration for ciprofloxacin was also determined.

Antimicrobial resistance patterns were determined for 4,008 *Salmonella* isolates received from 51 states and territories. Emerging quinolone resistance was detected; 21 isolates (0.5%) were resistant to nalidixic acid, and one (0.02%) was resistant to ciprofloxacin. The 21 nalidixic acid-resistant strains included 13 different serotypes from 15 states. The most common serotypes were Typhimurium (5 isolates), Enteriditis (3), and Virchow (2).

The ciprofloxacin-resistant strain, *Salmonella* serotype Schwarzengrund, was isolated in January 1995 from the stool of a woman referred to a hospital in the United States for treatment of complications caused by factor VIII deficiency. She had been hospitalized in the Philippines in September 1994 for "amoebic colitis," which was treated with antimicrobial agents; the patient could not recall the names of the agents. At that time, she also received a blood transfusion for severe anemia. Examination in the U.S. hospital showed a factor VIII level of 5% with a factor VIII inhibitor titer of 20 Bethesda units. The patient was afebrile and did not have diarrhea or other gastrointestinal symptoms, however, blood was observed in her stool on the second day of hospitalization. Colonoscopy showed angiodysplasia of the right colon. She was not treated with antimicrobial agents and was discharged from the hospital after 15 days.

To our knowledge, this is only the second reported isolation of fluoroquinolone-resistant *Salmonella* in the United States; the first reported isolate was from a patient who had been treated with three courses of ciprofloxacin for 8 weeks (6). The resistant pathogen found in our study was probably acquired in the Philippines, where quinolones have been available without prescription at least since 1987 (7) and where, according to a 1992 survey, 2.5% of nontyphoidal *Salmonella* isolates were resistant to

fluoroquinolones (5). Fluoroquinolone-resistant *Salmonella* have also been reported in Asia and Europe (5,8,9). Prior hospitalization, prior antimicrobial treatment (6), and travel outside the United States have been shown to increase the risk of being infected with *Salmonella* resistant to antimicrobial agents (10).

Compared with the previous national study, conducted in 1989-1990, which found one in 758 (0.1%) *Salmonella* isolates resistant to nalidixic acid, we found a fivefold increase in the prevalence of nalidixic acid resistance. Although increasing, the low prevalence of quinolone resistance in *Salmonella* in the United States and the lack of domestically acquired fluoroquinolone-resistant strains, is in sharp contrast to the situation in England and Wales, where increasing prevalence has been reported (8), particularly among isolates of *S*. Typhimurium, *S*. Virchow, and *S*. Hadar (E.J. Threlfall, L.R. Ward, J.A. Skinner, B. Rowe, unpub. obs.). Among one particular *Salmonella* strain, *Salmonella* Typhimurium Definitive Type 104 (DT 104), which was the second most frequently isolated *Salmonella* strain from humans in the United Kingdom in 1995, the incidence of fluoroquinolone resistance has increased from 0% in 1993 to 6% in 1995 (11), and has more than doubled in 1996 (Public Health Laboratory System, unpub. data). An important factor contributing to this increase may be the licensing in 1993 of the fluoroquinolone antimicrobial enrofloxacin for general veterinary use in that country (11). Although the only fluoroquinolone-resistant *Salmonella* isolate identified in our study was apparently acquired outside, DT 104 has emerged widely in the United States (12). The recent approval of a fluoroquinolone for use in animals intended for food in the United States (sarafloxacine in poultry) (13) may contribute to the emergence and circulation of fluoroquinolone-resistant strains in a way analogous to that already observed in the United Kingdom. Since fluoroquinolones are important in treating invasive *Salmonella* infections and most DT 104 isolates are already resistant to ampicillin, chlorampenicol, streptomycin, sulphonamides, and tetracyclines, continued monitoring of salmonellas for resistance to fluoroquinolone antimicrobial drugs is warranted.

Acknowledgments

We thank the numerous microbiologists at the state public health laboratories who provided the isolates included in the study.

Hallgeir Herikstad,* Peggy Hayes,* Mohamed Mokhtar,* Margaret L. Fracaro, E. John Threlfall,‡ and Frederick J. Angulo*

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; The Presbyterian Hospital in the City of New York, New York, USA; ‡Public Health Laboratory Service, Central Public Health Laboratory, London, United Kingdom

References

1. Cohen ML, Tauxe RV. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. Science 1986;234:964-9.
2. Tauxe RV. *Salmonella*: a postmodern pathogen. Journal of Food Production 1991;54:563-8.
3. Wilcox MH, Spencer RC. Quinolones and salmonella gastroenteritis. J Antimicrob Chemother 1992;30:221-8.
4. Conte JE. Manual of antibiotics and infectious diseases. Baltimore: Williams & Wilkins; 1995.
5. Turnidge J. Epidemiology of quinolone resistance. Eastern hemisphere. Drugs 1995;49:43-7.
6. Cherubin CE, Eng RHK. Quinolones for the treatment of infections due to *Salmonella*. Rev Infect Dis 1991;13:343-4.
7. Lansang MA, Lucas-Aquino R, Tupasi TE, Mina VS, Salazar LS, Juban N, et al. Purchase of antibiotics without prescription in Manila, the Philippines. Inappropiate choices and doses. J Clin Epidemiol 1990;43:61-7.
8. Frost JA, Kelleher A, Rowe B. Increasing ciprofloxacin resistance in salmonellas in England and Wales 1991-1994. J Antimicrob Chemother 1996;37:85-91.
9. Hof H, Ehrhard I, Tschape H. Presence of quinolone resistance in a strain of *Salmonella typhimurium*. Eur J Clin Microbiol Infect Dis 1991;10:747-9.
10. Lee LA, Puhr ND, Maloney EK, Bean NH, Tauxe RV. Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989-1990. J Infect Dis 1994;170:128-34.
11. Threlfall EJ, Frost JA, Ward LR, Rowe B. Increasing spectrum of resistance in multiresistant *Salmonella typhimurium*. Lancet 1996;347:1053-4.
12. Centers for Disease Control and Prevention. Multidrug-resistant *Salmonella* serotype Typhimurium—United States, 1996. MMWR Morb Mortal Wkly Rep 1997; 46:308-10.
13. Centers for Disease Control and Prevention. Establishment of a national surveillance program for antimicrobial resistance in *Salmonella*. MMWR Morb Mortal Wkly Rep 1996;45:110-1.

Multidrug-Resistant Enteroaggregative *Escherichia coli* Associated with Persistent Diarrhea in Kenyan Children

To study the association of multidrug-resistant enteroaggregative *Escherichia coli* with persistent diarrhea in Kenyan children, stool specimens were obtained from 862 outpatients under 5 years of age from July 1991 to June 1993. *E. coli* O44 was identified as the sole bacterial pathogen in four patients experiencing at least 14 days of fever, vomiting, and diarrhea. Disk diffusion testing showed *E. coli* O44 resistance to tetracycline, ampicillin, erythromycin, trimethoprim-sulphamethoxazole, and amoxicillin/clavulanate and sensitivity to chloramphenicol, nalidixic acid, azithromycin, and cefuroxime. Further studies are needed to clarify the epidemiology, clinical spectrum, and pathogenesis of enteroaggregative *E. coli* infection.

Escherichia coli infection is an important cause of illness and death in infants in developing countries (1). On the basis of patterns of adherence to tissue culture cells (HEp-2 or HeLa), *E. coli* strains can be classified into three groups: localized, diffuse, and aggregative (2). Much remains unknown about these strains. Enteroaggregative *E. coli* (EAggEC), which exhibits aggregative adherence, has been associated with diarrhea in children in Chile (3) and with persistent diarrhea in children in India (4). We report the first evidence of multidrug-resistant EAggEC associated with persistent diarrhea in Kenyan children.

From July 1991 to June 1993, stool specimens from 862 outpatients under 5 years of age at Malindi Hospital were examined for pathogenic organisms. Standard methods for isolating enteric pathogens were used. Laboratory tests to detect pathogenic factors, e.g., verotoxins (VT) in cultures of all *E. coli* isolates, were done by applying the conventional tissue culture method (which uses the Vero cell line [5] and the VT1 or VT2 genes [6]) and polymerase chain reaction (7). The genes for heat-labile enterotoxin and heat-stable enterotoxin, VT, and invasiveness were tested by DNA probes on all *E. coli* strains. The strains were further examined for adherence to HEp-2 cells (8) and tested by the disk diffusion method (9) for susceptibility to the antibiotics chloramphenicol, erythromycin, ampicillin, nalidixic acid, cefuroxime, trimethoprimsulphamethoxazole, amoxicillin/clavulanate, tetracycline, and azithromycin.

Bacterial pathogens were found in 27.7% of the samples; 119 *E. coli* isolates were obtained. The results indicated that many of the bacteria, e.g., pathogenic *E. coli*, *Salmonella* spp., and *Shigella* spp. (Table), had been transmitted by the fecal-oral route. *E. coli* O44 was isolated from four patients; the isolates occurred in an aggregative adherence pattern as chains and nearly random aggregates on HEp-2 cells.

The case descriptions of the four patients from whom the O44 strains were isolated are as

Table. Identification of enteric pathogens in children with diarrhea.

Pathogens (number tested)	Number	Percentage %
Bacteria (862)	239	28.0
Escherichia coli		
enteropathogenic *E. coli* (EPEC)	71	8.0
EAgg *E. coli (ETEC)*	4	0.5
enterotoxigenic *E. coli*	43	5.0
enterohemorrhagic *E. coli*	1	0.1
Salmonella spp.	63	7.3
Shigella spp.	56	6.5
Campylobacter spp.	42	4.9
Vibrio parahaemolyticus	4	0.5
Parasites (862)	109	12.6
Entamoeba histolytica	67	7.8
Giardia lamblia	42	4.9
Viruses (427)		
Rotavirus	69	16.2

Mixed infections: ETEC/EPEC = 12, EPEC/*Campylobacter* = 8, *Salmonella*/ETEC = *7*, *Salmonella*/EPEC = *3*, *Shigella*/*Campylobacter* = *2*, *Vibrio*/*Shigella* = 2, *Shigella*/*Salmonella* = 1, EPEC /*Shigella* = 6, ETEC/*Shigella* = 4.

follows: Patient 1, age 28 months, had fever, gross blood in stool, vomiting, and diarrhea lasting 14 days; Patient 2, age 35 months, had fever, abdominal pain, nausea, vomiting, and diarrhea lasting 15 days; Patient 3, age 24 months, had fever, gross blood in stool, vomiting, and diarrhea lasting 14 days; and Patient 4, age 26 months, had fever, abdominal pain, vomiting, and diarrhea lasting 14 days. The patients were not related and lived in different communities. These particular strains of *E. coli* O44 had similar patterns of resistance to tetracycline, ampicillin, erythromycin, trimethoprimsulphamethoxazole, and amoxicillin/clavulanate; they were all sensitive to chloramphenicol, nalidixic acid, azithromycin, and cefuroxime.

Persistent diarrhea is increasingly recognized as an important public health problem among children in developing countries (10) and is a research priority of the Diarrhoeal Diseases Control Programme of the World Health Organization (11). In the four patients from whom it was isolated, EAggEC was the sole bacterial pathogen recovered. However, tests for parasitic causes of persistent diarrhea, such as *Cyclospora* and *Cryptosporidium*, were not available at the time of our study and were not performed. The association of EAggEC with persistent diarrhea will be strengthened by extending this kind of study to other areas in Kenya and identifying all causes of persistent diarrhea.

Tetracycline**,** ampillicin, and trimethoprimsulphamethoxazole are recommended by the Kenyan Ministry of Health for the empiric treatment of diarrhea. These drugs were largely ineffective against *Shigella* spp. and EAggEC. Our results are consistent with the findings of Yamamoto et al., who found multidrug resistance in EAggEC strains from Thailand, Mexico, Chile, and Peru (12), and suggest that monitoring sensitivity to antibiotics in Kenya is necessary for optimum selection of effective antibiotics and elimination of antibiotics with little therapeutic value. Similarly, the epidemiology, clinical spectrum, and pathogenesis of EAggEC infection and the reservoir of the putative etiologic agent are still poorly understood or unknown. Further clinical, epidemiologic, and laboratory studies are needed to clarify these issues.

Acknowledgments

We are grateful to Dr. B.L. Smoak, U.S. Army Medical Research Unit/Kenya Medical Research Institute, for reviewing the paper. This work was supported by the Japanese International Cooperation Agency (JICA) and the Kenya Medical Research Institute.

W.K. Sang,* J.O. Oundo,* J.K. Mwituria,* P.G. Waiyaki,* M. Yoh,† T. Iida,† and T. Honda†

*Kenya Medical Research Institute, Nairobi, Kenya; and †Osaka University, Osaka, Japan

References

1. Levine MM. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. J Infect Dis 1987;15:377-89.
2. Scaletsky ICA, Silva MLM, Trabulsi LR. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. Infect Immun 1984;45:534-6.
3. Nataro JP, Kaper JB. Patterns of adherence of diarrhaegenic *Escherichia coli* to HEp-2 cells. Pediatr Infec Dis J 1987;6:829-31.
4. Bhan MK, Raj P, Levine MM. Enteroaggregative *Escherichia coli* association with persistent diarrhea in a cohort of rural children in India. J Infect Dis 1989;159:1061-4.
5. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun 1977;18:775-9.
6. Tomatsukuri S, Yamamoto K, Shibata S, Leaneo F, Honda T. Detection of a heat-labile enterotoxin gene in enterotoxigenic *Escherichia coli* by densitometric evaluation using highly specific enzyme-linked oligonucleotide probes. Eur J Clin Microbiol Infect Dis 1991;10:1048-55.
7. Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J Clin Microbiol 1990;28:540-5.
8. Cravioto AR, Gross J, Scotland SM, et al. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotype. Current Microbiology 1979;3:95-9.
9. Bauer AW, Kirby WMM, Sherris JC, Turk M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966;4:493-6.
10. Bhan MK, Arora NK, Ghai OP, Ramachandran K. Major factors in diarrhoea related mortality among rural children. Indian J Med Res 1986;83:9-12.
11. World Health Organization: Diarrhoeal Disease Control Programme: persistent diarrhoea in children—research priorities. Geneva: World Health Organization; 1985; CDD/DDM/85.1.
12. Yamamoto T, Echeverria P, Yokota T. Drug resistance and adherence to human intestines of enteroaggregative *Escherichia coli*. J Infect Dis 1992;165:744-9.

Molecular Epidemiologic Investigations of *Mycoplasma gallisepticum* Conjunctivitis in Songbirds by Random Amplified Polymorphic DNA Analyses

An ongoing outbreak of conjunctivitis in free-ranging house finches (*Carpodacus mexicanus*) began in 1994 in the eastern United States. Bacterial organisms identified as *Mycoplasma gallisepticum* (MG) were isolated from lesions of infected birds. MG was also isolated from a blue jay (*Cyanocitta cristata*) that contracted conjunctivitis after being housed in a cage previously occupied by house finches with conjunctivitis, and from free-ranging American goldfinches (*Carduelis tristis*) in North Carolina in 1996. To investigate the molecular epidemiology of this outbreak, we produced DNA fingerprints of MG isolates by random amplification of polymorphic DNA (RAPD). We compared MG isolates from songbirds examined from 1994 through 1996 in 11 states, representing three host species, with vaccine and reference strains and with contemporary MG isolates from commercial poultry. All MG isolates from songbirds had RAPD banding patterns identical to each other but different from other strains and isolates tested. These results indicate that the outbreak of MG in songbirds is caused by the same strain, which suggests a single source; the outbreak is not caused by the vaccine or reference strains analyzed; and MG infection has not been shared between songbirds and commercial poultry.

Mycoplasma gallisepticum (MG), a well-known cause of diseases of domesticated chickens and turkeys worldwide, most notably causes chronic respiratory disease in chickens and infectious sinusitis in turkeys (1). Although MG is a known pathogen of other gallinaceous birds and has been isolated from ducks and geese, it has not been considered a natural pathogen of wild birds, including songbirds (1). Conjunctivitis in house finches (*Carpodacus mexicanus*) was first reported in February 1994 (2,3). Since then, many ill house finches have been observed at feeders and submitted to wildlife care facilities or veterinary diagnostic laboratories in the Middle Atlantic and Southeastern regions of the United States (2-4). Slow-growing mycoplasmas (mean incubation time = 25 days) were isolated from lesions in clinically ill birds and identified as MG by direct immunofluorescence (3). These findings suggested that MG was the likely cause of this outbreak of conjunctivitis in house finches. MG was also isolated from a blue jay (*Cyanocitta cristata*) that contracted conjunctivitis after being housed in a cage previously occupied by infected house finches (3). This observation suggested that house finches infected with MG may be capable of transmitting the infection horizontally and that other avian species are susceptible to infection and disease. Recently, conjunctivitis has been observed in American goldfinches (*Carduelis tristis*), and we have made two isolates of MG from these birds. This finding suggests that the infection can be transmitted naturally to an additional host species.

To examine the epidemiologic relationships among MG isolates from this outbreak, we used two primer systems previously described for subspecies typing of avian mycoplasmas (5,6) to conduct random amplification of polymorphic DNA (RAPD). MG isolates available for this study represented several presumed epidemiologic relationships: time (1994 through 1996), geographic location (n = 11 states), and host species (n = 3). We compared these isolates with each other, with MG reference and vaccine strains, and with MG isolates from commercial poultry.

Isolation and Identification of MG Strains

Mycoplasmas were isolated from conjunctival and infraorbital sinus swabs from clinically ill birds (Figure 1) by using Frey's broth medium with 15% swine serum (7). Mycoplasma colonies

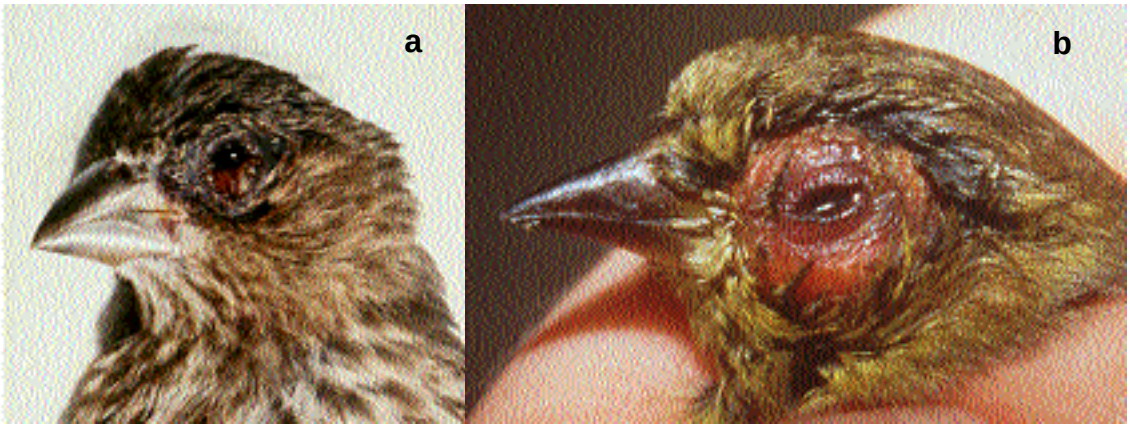


Figure 1. MG isolates have been made from songbirds with clinical signs and gross lesions characterized by mild to severe unilateral or bilateral conjunctival and periorbital swelling with serous to mucopurulent drainage and nasal exudate. Typical gross lesions in a) a female house finch (*Carpodacus mexicanus*) (photo courtesy of D. Earl Green, State of Maryland, Department of Agriculture, College Park, MD) and b) an American goldfinch (*Carduelis tristis*) (photo by K. Joyner, College of Veterinary Medicine, North Carolina State University, Raleigh, NC).

on agar plates were identified as MG by direct immunofluorescence (7), which used fluorescein-conjugated rabbit antiserum provided by S.H. Kleven (Department of Avian Medicine, University of Georgia, Athens, GA). MG strains were isolated from house finches (*C. mexicanus*), American goldfinches (*C. tristis*), and a blue jay (*C. cristata*) with conjunctivitis (Table). Other MG strains analyzed included reference strains S6, R, A5969; vaccine strains F, 6/85 (Intervet Inc., Millsboro, DE), and ts-11 (Select Laboratories, Gainesville, GA); and field isolates from commercial poultry (Table). *Mycoplasma imitans* type strain 4994 was provided by J.M. Bradbury (Department of Veterinary Pathology, University of Liverpool, South Wirral, England).

Preparation of Mycoplasma DNA

DNA for RAPD analyses was prepared from log phase MG broth cultures. Two to 3 ml of culture containing approximately 10^9 CFU were centrifuged at 16,000 x g for 6 min, washed two times with phosphate-buffered saline (PBS), and resuspended in 25 µl PBS. The cells were lysed by boiling for 10 min,

Table. *Mycoplasma gallisepticum* isolates from songbirds with conjunctivitis analyzed by random amplification of polymorphic DNA (RAPD)

Isolate no.	Host species	State of origin	Date isolated (mo/yr)	Isolated by
7994	House finch	VA	6/94	NCSU[a]
11394	Blue jay	VA	7/94	NCSU
16094-1	House finch	PA	9/94	NCSU
16094-5	House finch	DE	9/94	NCSU
16994	House finch	NC	9/94	NCSU
17494	House finch	DE	9/94	NCSU
17694	House finch	DE	9/94	NCSU
17794	House finch	VA	9/94	NCSU
K3839	House finch	MD	11/94	SCWDS/UGA[b]
95-11-14C	House finch	NY	4/95	UW[c]
13295	House finch	NC	8/95	NCSU
K4013	House finch	PA	8/95	SCWDS/UGA
K4058	House finch	GA	11/95	SCWDS/UGA
K4094	House finch	TN	1/96	SCWDS/UGA
1596-3	House finch	NC	2/96	NCSU
1596-5	Am. goldfinch	NC	2/96	NCSU
1695	Am. goldfinch	NC	2/96	NCSU
K4117	House finch	KY	2/96	SCWDS/UGA
1652442	House finch	MI	3/96	MSU[d]
K4269	House finch	OH	7/96	SCWDS/UGA

[a]D. H. Ley, North Carolina State University, College of Veterinary Medicine, Raleigh, NC.
[b]P. Luttrell and J. F. Fischer, Southeastern Cooperative Wildlife Disease Study and S. H. Kleven, Department of Avian Medicine, The University of Georgia, Athens, GA.
[c]C. B. Thomas, School of Veterinary Medicine, University of Wisconsin, Madison, WI.
[d]R. M. Fulton, Animal Health Diagnostic Laboratory, Michigan State University, East Lansing, MI.

placed on ice for 5 to 10 min, and centrifuged at 16,000 x g for 2 min. The resultant supernatant was stored at 4°C for RAPD testing.

RAPD Analyses

Two previously described RAPD methods (5,6) were used with modification. Method I (5) used a single primer in a total reaction volume of 100 µl, 2.5 units Taq polymerase (Promega, Madison, WI) in the manufacturer's recommended buffer with 2 mM $MgCl_2$, 250 µM each dNTP (Promega), 500 ng primer 1254 (5′CCGCAGCCAA 3′) (Life Technologies, Gaithersburg, MD) and 0.5 µl DNA extract containing 50 to 100 ng DNA. The amplification conditions were four cycles of 94°C for 5 min, 36°C for 5 min, and 72°C for 5 min, followed by 30 cycles of 94°C for 1 min, 36°C for 1 min, and 72°C for 1 min. These were followed by one cycle of 72°C for 10 min.

Method II (6) used three primers (M16SPCR5í = 5′AGGCAGCAGTAGGGAAT 3′, M13F = 5′GTAAAACGACGGC 3′, S1OLIGO 3′ = 5′CATAACTAACATAAGGGCAA 3′) in a total reaction volume of 100 µl, 2.5 units Taq polymerase (Promega) in the manufacturer's recommended buffer with 1.5 mM $MgCl_2$, 250 µM each dNTP (Promega), 500 ng each primer (Life Technologies), and 3.0 µl DNA extract containing 300 to 500 ng DNA. The amplification conditions were three cycles of 94°C for 15 sec, 28°C for 2 min, and 74°C for 3 min, followed by 35 cycles of 94°C for 15 sec, 45°C for 2 min, and 74°C for 3 min. These were followed by one cycle of 72°C for 10 min.

Gel Electrophoresis

Amplified DNA was separated by electrophoresis in 2% agarose (Pharmacia Biotech AB, Uppsala, Sweden) gels, poststained with ethidium bromide, illuminated with ultraviolet light, and photographed with an FCR-10 camera (Fotodyne Inc., Hartland, WI) and Polaroid 667 film (Polaroid Corp., Cambridge, MA).

Both RAPD methods I (5) and II (6) resulted in DNA banding patterns (Figures 2, 4, and data not shown) that clearly resolved differences among MG vaccine (F, 6/85, ts-11) and reference (S6, R, A5969) strains, thus demonstrating the usefulness of these assays for MG strain identification. Because RAPD analyses differentiated among known MG strains, we were confident that these DNA fingerprinting methods could identify strains. Additionally, RAPD banding patterns of known MG strains constitute the beginning of an MG strain database of DNA fingerprints with which other strains and field isolates can be compared.

RAPD analyses were performed on MG isolates obtained over 2 years from 17 house finches, two American goldfinches, and one blue jay from 11 states in the United States (Table). Upon inspection, all MG isolates from songbirds had essentially identical RAPD banding patterns by either RAPD method, and the patterns differed from those of the reference and vaccine strains tested (Figures 2-5, and data not shown). Therefore, the ongoing outbreak of MG conjunctivitis in songbirds appears to be caused by a single strain or very closely related strains of MG. This suggests the possibility of a single source for the outbreak, probably first involving house finches and more recently American goldfinches, the only two songbird species known to have acquired this disease naturally. These findings demonstrate that the MG strain involved is not host-species specific under natural conditions. Infection in the blue jay was apparently nosocomial, most likely resulting from exposure to MG-infected house finches or fomites while the birds were housed at a wildlife care facility. Isolation of what appears to be the same strain of MG from a blue jay exposed to infected house finches demonstrates the potential for this outbreak to spread to additional host species.

MG isolates from songbirds had RAPD banding patterns that differed from *M. imitans* (Figure 4, and data not shown). *M. imitans*, isolated from wild birds (duck, goose, and partridge) in Europe, cross-reacts with MG by immunofluorescence and growth inhibition tests but has only approximately 40% to 46% genetic homology with MG (type strain PG31) by DNA-DNA hybridization (8). Therefore, to rule out the possibility that isolates identified as MG by immunofluorescence tests were not *M. imitans*, we compared the RAPD banding pattern of the *M. imitans* strain: it was markedly different from that of the finch isolates and other MG strains tested (Figure 4, and data not shown). In addition, we confirmed the finch isolates as MG (2,3) by using a commercially available MG-specific polymerase chain reaction-based test (FlockChek MG DNA Probe, IDEXX Laboratories, Inc., Westbrook, ME). Differential diagnoses of conjunctivitis in songbirds should also include the

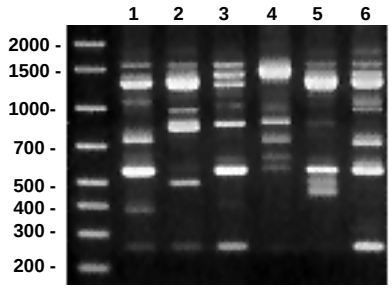


Figure 2. RAPD (method I) patterns of MG vaccine (lanes 1-3) and reference (lanes 4-6) strains. DNA base pair size standards are shown on the left. Lane 1 = ts-11; lane 2 = F; lane 3 = 6/85; lane 4 = R; lane 5 = S6; and lane 6 = A5969. Use of RAPD method I on these MG strains resulted in unique banding patterns that can be easily distinguished from one another.

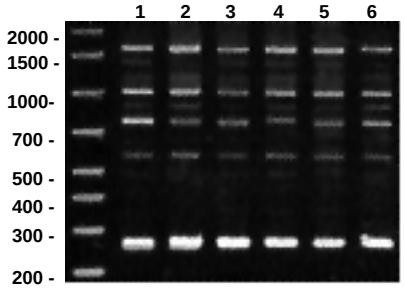


Figure 3. RAPD (method I) patterns of MG isolates from songbirds. DNA base pair size standards are shown on the left. Lane 1 = 7994 (house finch); lane 2 = 11394 (blue jay); lane 3 = 16094-1 (house finch); lane 4 = 16994 (house finch); lane 5 = 13295 (house finch); and lane 6 = 1596-6 (American goldfinch).

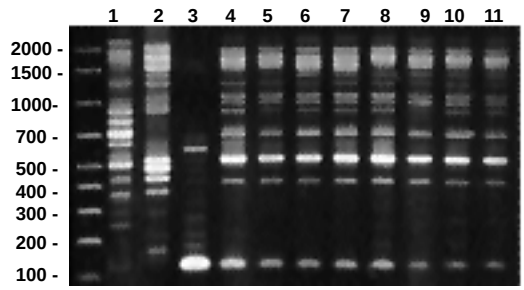


Figure 4. RAPD (method II) patterns of MG vaccine strains (lanes 1-2) and isolates from house finches (lanes 4-11), and *M. imitans* type strain (lane 3). DNA base pair size standards are shown on the left. Lane 1 = ts-11; lane 2 = 6/85; lane 3 = *M. imitans*; lane 4 = K3839; lane 5 = K4013; lane 6 = K4013; lane 7 = K4117; lane 8 = 7994; lane 9 = 1652442; lane 10 = K4058; lane 11 = K4269. An additional RAPD primer set (method II) was used to determine whether method I accurately determined MG strain identities.

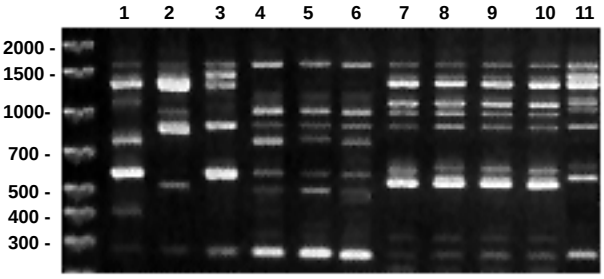


Figure 5. RAPD (method I) patterns of MG vaccine strains (lanes 1-3), isolates from songbirds (lanes 4-6), and isolates from commercial poultry (lanes 7-11). DNA base pair size standards are shown on the left. Lane 1 = ts-11; lane 2 = F; lane 3 = 6/85; lane 4 = 7994 (house finch); lane 5 = 17794 (house finch); lane 6 = 1596 (American goldfinch); lanes 7-10 = separate isolates made from commercial turkeys; lane 11 = isolate from commercial chickens.

newly characterized *Mycoplasma sturni* recovered from a European starling (*Sturnus vulgaris*) and a mockingbird (*Mimus polyglottos*) in Connecticut (9,10). We have recovered *M. sturni* from conjunctival swabs collected from one blue jay and six mockingbirds from Florida (Ley, Berkhoff, Levisohn, unpub. obs.) However, there is no evidence that either *M. imitans* or *M. sturni* is involved in the present epidemic of conjunctivitis in American goldfinches and house finches. The possible diagnostic complications that these *Mycoplasma* spp. represent should not be ignored.

Figure 5 shows RAPD banding patterns (method I) of MG vaccine strains and isolates from songbirds and commercial poultry. The poultry isolates include four made in 1996 from an outbreak in Missouri turkeys and one made in 1994 from North Carolina chickens. The MG isolates tested from commercial turkeys had RAPD banding patterns essentially identical to each other, which would be the result if a single or dominant strain were responsible for the outbreak. The isolate from commercial chickens appears similar to that from turkeys, and isolates from both are clearly different from vaccine strains. As previously observed (Figures 2-4), songbird MG RAPD banding patterns were essentially identical to each other and clearly different from the vaccine strains. Figure 5 further shows that the songbird MG RAPD banding

pattern was different from the patterns of isolates from chickens and turkeys. This initial, limited comparison of isolates from poultry and songbirds provided no evidence of shared MG strains. We are now applying RAPD analyses to additional MG isolates, both contemporary and archival, from commercial poultry to search for MG strains shared by songbirds and poultry or other birds.

RAPD method I was used to conduct routine screening and most comparative tests because of its reproducibility, discriminating capability, and ease of interpretation; however, the use of two different RAPD primer systems is valuable for confirming apparent relationships. In our study, a second RAPD primer set (method II) was used to confirm the MG strain identities determined by method I. Even though RAPD method II generally resulted in more bands, MG isolates from songbirds had essentially identical DNA fingerprints (Figure 4, and data not shown), thus supporting results by RAPD method I.

We standardized sample preparation and gel electrophoresis protocols to ensure reproducibility of RAPD results. Further standardization of technique and implementation of a computer-based gel analysis system should allow for comparison of results between laboratories, thus enhancing the usefulness and power of these procedures. We have found that RAPD analyses are useful for MG strain identification and molecular epidemiologic investigations. Applying this technology to the current epidemic of conjunctivitis in songbirds showed that all MG isolates from songbirds had essentially identical RAPD banding patterns to each other, but different patterns from all other strains and isolates tested. These results indicated that 1) the outbreak of MG in songbirds is caused by the same strain, suggesting a single source; 2) the outbreak is not caused by the vaccine or reference strains analyzed; and 3) MG infection does not appear to have been shared between songbirds and commercial poultry.

Fischer et al. (4) have stated that the epidemic of MG conjunctivitis in songbirds parallels emerging human diseases. However, even though this outbreak has no direct effect on humans (no evidence exists that MG causes zoonotic infections), the public has been aware of and involved in this epidemic, as observers (and feeders) of songbirds and as participants (citizen scientists) in such surveys as the House Finch Disease Survey (Project FeederWatch, Cornell Lab of Ornithology, PO Box 11, Ithaca, NY 14851-0011, and http://www.ornith.cornell.edu/CS/HOFI/main.html). Ultimately, the effects of this epidemic depend on its evolving course, the avian host species involved, the degrees to which the species are affected, and the species' relationships to the public's concerns (e.g., scientific, economic, recreational, professional). RAPD analyses of additional MG isolates from our archives and those of others and continued analysis of contemporary MG isolates from songbirds and other birds including commercial poultry will provide an exceptional opportunity to track this emerging disease.

Acknowledgments

We thank Chris York, Douglas Anderson, Gina Neri, Rebecca Packer, and Tyler Lund for valuable technical assistance; L. Degernes, K. Joyner, and L. Powers for their cooperation and submission of specimens; The Wildlife Center of Virginia; Tri-State Bird Rescue and Research, Inc.; New York State Department of Environmental Conservation; Southeastern Cooperative Wildlife Disease Study and Department of Avian Medicine, University of Georgia; School of Veterinary Medicine, University of Wisconsin; and Animal Health Diagnostic Laboratory, Michigan State University. Resources to support this research were provided by the State of North Carolina and a grant from the U. S. Poultry and Egg Association.

David H. Ley,* J. Edward Berkhoff,* and Sharon Levisohn†

*College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA; †Visiting Research Scientist, Kimron Veterinary Institute, Bet Dagan, Israel

References

1. Yoder HW Jr. *Mycoplasma gallisepticum* infection. In: Hofstad MS, Barnes HJ, Calnek BW, Reid WM, Yoder HW Jr, editors. Diseases of poultry. 9th ed. Ames (IA): Iowa State University Press; 1991. p. 198-212.
2. Luttrell MP, Fischer JR, Stallknecht DE, Kleven SH. Field investigation of *Mycoplasma gallisepticum* infections in house finches (*Carpodacus mexicanus*) from Maryland and Georgia. Avian Dis 1996;40:335-41.
3. Ley DH, Berkhoff JE, McLaren JM. *Mycoplasma gallisepticum* isolated from house finches (*Carpodacus mexicanus*) with conjunctivitis. Avian Dis 1996;40:480-3.
4. Fischer JR, Stallknecht M, Luttrell P, Dhondt AA, Converse KA. Mycoplasmal conjunctivitis in wild songbirds: the spread of a new contagious disease in a mobile host population. Emerg Infect Dis 1997;3:69-72.

5. Geary SJ, Forsyth MH, Aboul Saoud S, Wang G, Berg DE, Berg CM. *Mycoplasma gallisepticum* strain differentiation by arbitrary primer PCR (RAPD) fingerprinting. Mol Cell Probes 1994;8:311-6.
6. Fan HH, Kleven SH, Jackwood MW. Application of polymerase chain reaction with arbitrary primers to strain identification of *Mycoplasma gallisepticum*. Avian Dis 1995;39:729-35.
7. Kleven SH, Yoder HW Jr. Mycoplasmosis. In: Purchase HG, Arp LH, Domermuth CH, Pearson JE, editors. A laboratory manual for the isolation and identification of avian pathogens. 3rd ed. Kennett Square (PA): American Association of Avian Pathologists; 1989. p. 57-62.
8. Bradbury JM, Abdul-Wahab OM, Yavari CA, Dupiellet JP, Bove JM. *Mycoplasma imitans* sp. nov. is related to *Mycoplasma gallisepticum* and found in birds. Int J Syst Bacteriol 1993;43:721-8.
9. Forsyth MH, Tully JG, Gorton TS, Hinckley L, Frasca SJ, Van Kruiningen J, et al. *Mycoplasma sturni* sp. nov., from the conjunctiva of a European starling (*Sturnus vulgaris*). Int J Syst Bacteriol 1996;46:716-9.
10. Frasca SJ, Hinckley L, Forsyth MH, Groton TS, Geary SJ, Van Kruiningen HJ. Mycoplasmal conjunctivitis in a European Starling. J Wildl Dis 1997;33:336-9.

Reevaluating the Molecular Taxonomy: Is Human-Associated *Cyclospora* a Mammalian *Eimeria* Species?

Human-associated *Cyclospora* is a coccidian parasite that causes diarrheal disease. A reevaluation of the parasite's molecular taxonomy that takes into account newly published data for seven *Eimeria* species shows that *Cyclospora* belongs to the *Eimeria* clade (Eimeriidae family). The *Cyclospora* branch on the phylogenetic tree is between the branches of the eight avian and two mammalian *Eimeria* species that have been evaluated to date. Furthermore, preliminary results indicate that *Cyclospora* and *Isospora belli*, another coccidian parasite that causes diarrheal disease in humans, belong to different families. To improve our understanding of the taxonomy of human-associated *Cyclospora*, molecular evaluation of isolates of additional *Cyclospora* and *Eimeria* species is needed.

In 1996 and 1997, human-associated *Cyclospora*, a protozoan apicomplexan parasite, caused outbreaks of diarrheal disease in the United States and Canada that were associated with consumption of various types of fresh produce (1,2). Human-associated *Cyclospora* was previously referred to as "cyanobacteriumlike body" or "coccidialike body" and "big (or large) *Cryptosporidium*" (3). In 1993, Ortega et al. (3) proposed, on the basis of morphologic and sporulation characteristics, that this parasite be placed in the genus *Cyclospora* (Schneider, 1881) in the coccidian family Eimeriidae (Minchin, 1903). Although the species designation *Cyclospora cayetanensis* was given in 1994 to Peruvian isolates of human-associated *Cyclospora* (4), it is not yet known whether all human *Cyclospora* isolates belong to the same species.

Phylogenetic analyses by Relman et al., which included human-associated *Cyclospora* and three *Eimeria* species (two avian and one mammalian), supported the conclusion that *Cyclospora* and *Eimeria* belong to the same family of coccidian parasites (5). However, the authors noted that this apparent relatedness should be reevaluated when molecular data became available for additional *Eimeria* species and for *Isospora belli*, another coccidian parasite that causes diarrheal disease in humans.

Recently, the complete sequences of the small subunit ribosomal RNA (SSU-rRNA) gene of isolates of seven additional *Eimeria* species (six avian and one mammalian) were submitted to GenBank (6). We used these sequences, in addition to those previously available for human-associated *Cyclospora* and several *Eimeria* species (5), to reevaluate the phylogenetic relatedness of *Cyclospora* and *Eimeria*. Both of the previous phylogenetic analyses included sequences for *E. tenella* and *E. mitis* isolates; thus, we used two sequences for each of these species.

The structurally aligned sequences were retrieved from the Antwerp rRNA database (7); Mitchell L. Sogin kindly provided the alignment of the sequences used for the original molecular classification of *Cyclospora* (5). In addition to these two alignments, sequences were aligned with the ClustalW program (8). The aligned sequences were subjected to phylogenetic analysis with DNAPARS, NEIGHBOR, and DNAML programs from the PHYLIP package (9), using *Cryptosporidium parvum* (a coccidian parasite) or *Oxytricha granulifera* (a ciliate) as outgroups (all alignments are available from the authors upon request).

The topology of the phylogenetic tree obtained with all three methods was equivalent for all alignments. The maximum likelihood tree generated by the DNAML program from an alignment based on Sogin's approach is shown in the Figure. The topology of this tree, which includes human-associated *Cyclospora* and 12 isolates of 10 *Eimeria* species, is similar to that of the trees reported previously for *Cyclospora* and three species of *Eimeria* (5) and for nine species of *Eimeria* (6). The *Cyclospora* branch on the tree is between the branches of the eight avian and two mammalian *Eimeria* species that have been evaluated to date.

The results of the phylogenetic analysis strongly suggest that *Cyclospora* should be considered a member of the genus *Eimeria*, which is particularly noteworthy, since no organism

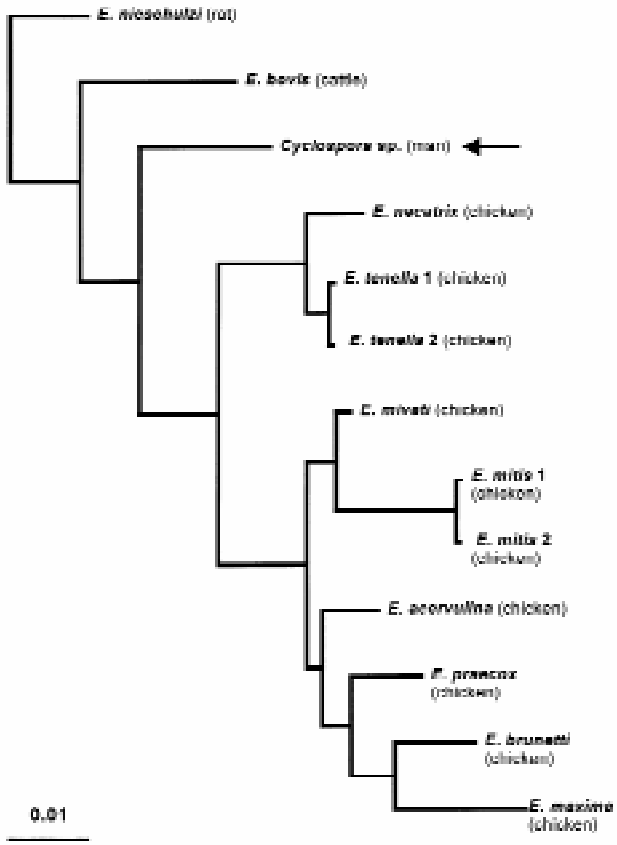


Figure. Phylogenetic tree for small subunit ribosomal RNA (SSU-rRNA) sequences of *Cyclospora* (marked by an arrow) and 12 isolates of 10 *Eimeria* species. Maximum likelihood analysis results using *Cryptosporidium parvum* as an outgroup are shown (ln likelihood = -5,421.96594). After analysis, the outgroup branch was removed to improve the readability of the tree. GenBank accession numbers for the sequences: human-associated *Cyclospora* sp. – U40261, *C. parvum* – L16996, *E. acervulina* – U67115, *E. bovis* – U77084, *E. brunetti* – U67116, *E. maxima* – U67117, *E. mitis* 1 – U67118, *E. mitis* 2 – U40262, *E. mivati* – U76748, *E. necatrix* – U67119, *E. nieschulzi* – U40263, *E. praecox* – U67120, *E. tenella* 1 – U67121, *E. tenella* 2 – U40264. Scale bar indicates an evolutionary distance of 0.01 nucleotides per position in the sequence.

currently classified as an *Eimeria* species is known to be pathogenic for humans. *Eimeria* is the largest genus of coccidian parasites and reportedly includes more than 1,500 named species (10). However, the current criteria (11) for naming "new" species of *Eimeria* (e.g., host specificity, morphologic characteristics of oocysts, duration of prepatent and patent periods, location of the infection in the host, and pathogenicity) are suboptimal, and the available data for some *Eimeria* species named in the past are incomplete. Thus, some *Eimeria* species may be synonymous, and some organisms thought to belong to the same species may not. The possibility even exists that human-associated *Cyclospora* is synonymous with a previously named *Eimeria* species. No molecular data are available for the type species of the *Cyclospora* genus or for the *Cyclospora* species that are not known to be human-associated. Reclassification, on the basis of phylogenetic analysis, of human-associated *Cyclospora* as an *Eimeria* species may stimulate productive research by suggesting possible animal reservoirs of human-associated *Cyclospora* (which may or may not infect other animals). In addition, animal models and cell culture systems that have been developed for *Eimeria* may prove useful for *Cyclospora*. However, it remains to be seen whether the biologic characteristics of *Cyclospora* are similar to those of the *Eimeria* species to which *Cyclospora* is closely related on the basis of phylogenetic criteria.

We also have preliminary data indicating that *I. belli* and human-associated *Cyclospora* do not belong to the same genus or family. *I. belli* oocysts (kindly provided by Alison Grant of Project RETRO-CI in Abidjan, Côte d'Ivoire) were gradient-purified, *I. belli*-specific DNA was extracted, and the SSU-rRNA gene was polymerase chain reaction-amplified and sequenced (Pieniazek et al., unpub. data). Sequence similarity searches of GenBank and preliminary phylogenetic analysis indicate that *I. belli* shares a more inclusive clade with members of the family Sarcocystidae than with the Eimeriidae (data not shown).

Molecular methods are arguably the best techniques available for studying the relatedness among organisms (11). To avoid confusion, reports of identification of *Cyclospora* (*Eimeria*) in animal hosts or in the environment should be supported by molecular data. Reports based on morphologic features alone (12-14) may suffer from poor resolution of features needed for classification of closely related organisms. To improve our understanding of the taxonomy of human-associated *Cyclospora*, molecular evaluation of isolates of additional *Cyclospora* and *Eimeria* species, especially other mammalian species, is needed.

Norman J. Pieniazek and Barbara L. Herwaldt
Centers for Disease Control and Prevention,
Atlanta, Georgia, USA

References

1. Herwaldt BL, Ackers M-L, and the Cyclospora Working Group. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. N Engl J Med 1997;336:1548-56.
2. Centers for Disease Control and Prevention. Update: outbreaks of cyclosporiasis—United States and Canada, 1997. MMWR Morb Mortal Wkly Rep 1997;46:521-3.
3. 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.
4. Ortega YR, Gilman RH, Sterling CR. A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. J Parasitol 1994;80:625-9.
5. Relman DA, Schmidt TM, Gajadhar A, Sogin M, Cross J, Yoder K, et al. Molecular phylogenetic analysis of *Cyclospora*, the human intestinal pathogen, suggests that it is closely related to *Eimeria* species. J Infect Dis 1996;173:440-5.
6. Barta JR, Martin DS, Liberator PA, Dashkevicz M, Anderson JW, Feighner SD, et al. Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. J Parasitol 1997;83:262-71.
7. Van der Peer Y, Jansen J, De Rijk P, De Wachter R. Database on the structure of small ribosomal subunit RNA. Nucleic Acids Res 1997;24:111-6.
8. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673-80.
9. Felsenstein J. PHYLIP—Phylogeny inference package. Cladistics 1989;5:164-6.
10. Levine ND. Phylum II. Apicomplexa Levine, 1970. In: Lee JJ, Hutner SH, Bovee EC, editors. An illustrated guide to the protozoa. Lawrence (KS): Society of Protozoologists; 1985. p. 322-74.
11. Sogin ML. Evolution of eukaryotic microorganisms and their small subunit ribosomal RNA. American Zoologist 1989;29:487-99.
12. Zerpa R, Uchima N, Huicho L. *Cyclospora cayetanensis* associated with watery diarrhoea in Peruvian patients. J Trop Med Hyg 1995;98:325-9.
13. García-López HL, Rodríguez-Tovar LE, Medina de la Garza CE. Identification of *Cyclospora* in poultry. Emerg Infect Dis 1996;2:356-7.
14. Smith HV, Paton CA, Girdwood RWA, Mtambo MMA. *Cyclospora* in non-human primates. Vet Rec 1996;138:528.

Rapid Increase in the Prevalence of Metronidazole-Resistant *Helicobacter pylori* in the Netherlands

The prevalence of primary metronidazole resistance of *Helicobacter pylori* was studied in one Dutch hospital from 1993 to 1996 and in two additional Dutch hospitals in 1993 and 1996. All cultures of antral biopsy specimens yielding *H. pylori* in the study period were evaluated, except those from patients who had received anti-*H. pylori* treatment; 1,037 *H. pylori* strains, all from different patients were included. Metronidazole resistance was determined by disk diffusion in 1993 and by Epilipsometer-test in 1994 to 1996. Metronidazole resistance increased from 7% (18/245) in 1993 to 32% (161/509) in 1996. More patients with nonulcer dyspepsia and more non-Western European patients were seen in 1996 than in 1993, but age and sex differences were not observed. A comparable increase in metronidazole resistance was observed in both nonulcer dyspepsia patients and peptic ulcer patients, and the prevalence of metronidazole resistance in Western Europeans increased from 5% in 1993 to 28% in 1996.

Since the first description (1) of *Helicobacter pylori* and the acceptance of its role in the pathogenesis of peptic ulcer disease (PUD) (2), different regimens to eradicate this microorganism have been used in clinical practice (3). Metronidazole has frequently been used as a component in these treatment regimens. *H. pylori* resistance to metronidazole has been associated with treatment failure (4-7). Recently, an increase of metronidazole resistance has been reported from different parts of the world (8-13). This retrospective study describes the prevalence of primary metronidazole resistance occurring in *H. pylori* strains in 1993 and 1996 in three regional hospitals in the northern part of the Netherlands and in 1994-95 in one of these hospitals.

Sampling

All cultures of antral biopsy specimens yielding growth of *H. pylori* in the study period were considered for evaluation. Previous anti-*H. pylori* treatment was the only reason for exclusion. All 1,037 *H. pylori* strains evaluated were isolated from different patients. Biopsy specimens for culture were taken within 3 cm of the pylorus. Endoscopes and biopsy equipment were thoroughly cleaned with a detergent and disinfected with 2% glutaraldehyde in an automatic washing machine between procedures. Culture was performed as described elsewhere (14).

Susceptibility Testing

Susceptibility to metronidazole was determined by disk diffusion in 1993 and the Epilipsometer-test (E-test) in 1994-1996. For these tests, plates were injected with a suspension adjusted to a turbidity approximating that of a McFarland No. 3 standard (15). For disk diffusion, a 5-µg disk (Mast Laboratories, Liverpool, United Kingdom) was used and read after at least 3 days of incubation. Strains with an inhibition zone of 10 mm or more were regarded as susceptible (16). The E-test (AB Biodisk, Solna, Sweden)(17) was performed according to the instructions of the manufacturer and read after at least 3 days. The strains were considered metronidazole resistant when the minimum inhibitory concentration was above 8 g/ml (18).

To test the equivalence of the two methods of susceptibility testing, a prospective study compared the E-test and disk diffusion. In 124 different *H. pylori* strains, results were concurrent in all but six.

Prevalence of Metronidazole-Resistant *H. pylori*

In one of the hospitals (Hospital C), data were available on endoscopic diagnosis, age, and ethnic background of the patients from whom the strains were isolated in 1993 and 1996. In this hospital, it was possible to compare the prevalence

of metronidazole resistance in PUD patients with that in nonulcer dyspepsia (NUD) patients and to look at resistance rates in patients of different ethnic backgrounds. Statistical analysis was performed by Fisher's exact test on binomial data and Student's t test on continuous data. Differences were considered significant when $p < 0.05$.

The number of *H. pylori* strains isolated in the three hospitals was 245 in 1993 and 509 in 1996. In Hospital C, an additional 137 strains from 1994 and 146 strains from 1995 were studied. Looking at the sex of the study population, we found a male-to-female ratio in 1993 of 1.75:1, in 1994 of 1.36:1, in 1995 of 1:1, and in 1996 of 1.18:1 (the total male-to-female ratio was 1.28:1). The proportion of women in the population examined was higher in 1996 (46%) than in 1993 (36%)($p = 0.02$). The prevalence of metronidazole resistance did not differ, however, between men and women. The prevalence of metronidazole resistance increased significantly from 1993 to 1996 in the total sample group (Figure 1) and also among men (from 7% to 30%, $p < 0.0001$) and women (from 8% to 33%, $p < 0.0001$).

In Hospital C, the number of strains isolated from PUD patients decreased from 83% to 38%. No significant difference, however, was noted in the prevalence of resistance between NUD patients and PUD patients in 1993 or in 1996 (Figure 2, $p < 0.0001$). In Hospital C more strains from non-Western European patients were included in 1996 than in 1993 ($p = 0.04$), and the prevalence of metronidazole resistance was higher in this group than in the total population. Exclusion of this patient group still resulted in an increase in the prevalence among the Western Europeans ($p < 0.0001$). The mean age of the patients from whom *H. pylori* strains were isolated in Hospital C was the same in 1993 and 1996 (55 ± 14 [mean ± standard deviation] and 54 ± 16 years, respectively.)

Our study shows a rapidly increasing prevalence of metronidazole resistance in *H. pylori* in the Netherlands. This increase was observed in all three hospitals included in the study. Our results are consistent with the findings of some investigators (8-13) but not with those of others (19,20). It confirms our own previous experience of increasing resistance in this part of the Netherlands (21,22).

We explored the possibility that the observed rise in metronidazole resistance was due to some known confounding factor such as age, sex, endoscopic diagnosis, or ethnicity. More *H. pylori* strains were isolated from NUD patients in 1996 than in 1993. In 1996, the proportions of women and foreigners in the examined populations were also higher. In contrast with the results of Ching et al. (23), however, we found that the prevalence of metronidazole resistance in NUD patients and in PUD patients was the same. Furthermore, the prevalence of metronidazole resistance was comparable among men and women in both 1993 and 1996. Exclusion of the non-Western European patients from the analysis still showed a rapid increase in metronidazole resistance. Several authors have suggested that the prevalence of metronidazole resistance is higher in the young and middle-aged (19,20,24-26). In our study population, however, the mean age was the same in 1993 and 1996.

The use of different techniques to measure metronidazole susceptibility could confound the validity

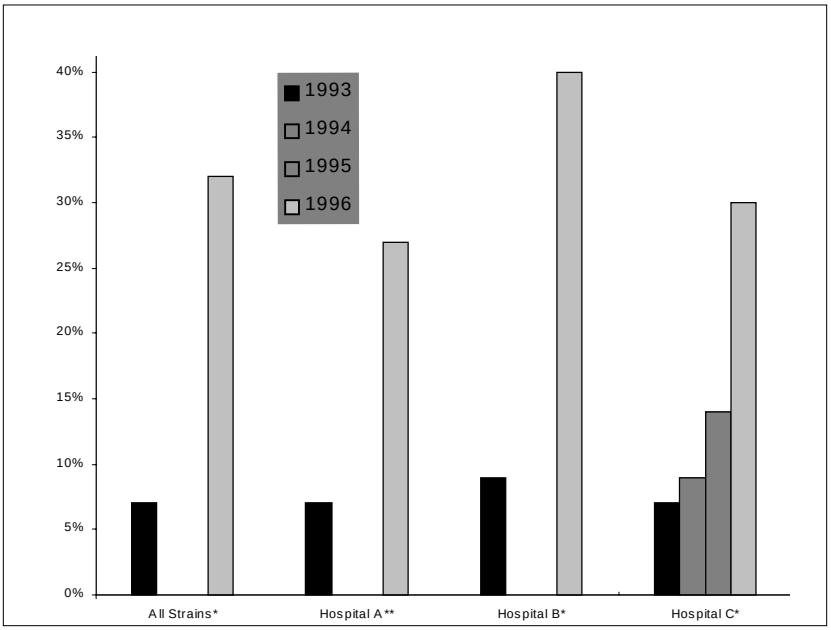


Figure 1. The increase in prevalence of metronidazole resistance of *H. pylori* from 1993 to 1996 in three different hospitals. Data presented as percent of strains that were resistant.
*p< 0.0001 1993 vs. 1996
**p<0.001 1993 vs. 1996

of our results (26). However, our prospective study comparing the E-test and disk diffusion, as well as other studies (27,28), show a very high intertest agreement when using the above-stated criteria for metronidazole resistance. We cannot exclude the possibility that other methodologic factors are involved. However, because procedures were standardized and the increase was observed in three different hospitals, each with its own laboratory, we consider this unlikely. Therefore, the observed rapid increase seems real and is relevant for clinical practice (4-7).

Possible Causes of Resistance

Several authors have suggested that the use of imidazoles for other indications, such as gynecologic infections, could account for the resistance increase (6,22-24,28,29). This would also explain the higher prevalence of metronidazole resistance in women that has been observed in several studies (6,19,21,25). Our study, however, did not show a significant difference between men and women or a more apparent increase in women. Moreover, out-of-hospital prescription of metronidazole in the Netherlands increased only slightly from 1989 until 1995 (Figure 3). Some authors have suggested that imidazole-containing regimens themselves could be the cause (25,29,30). However, we consider this unlikely. First, we excluded all strains that were isolated after known anti-*H. pylori* treatment. We cannot completely exclude the possibility that some of the patients had been treated by their general practitioner without our knowledge. We are, however, confident that this is a rare occurrence because in our region most physicians prescribe their treatment on the basis of endoscopic findings and culture of the biopsy specimens, and we purposely excluded all patients from whom *H. pylori* was previously isolated. In our region, breath testing is not available for general practitioners, and serologic tests are rarely used. Moreover, imidazole-containing anti-*H. pylori* regimens are highly effective (3,7), and metronidazole resistance could be induced only in the few *H. pylori* strains escaping eradication. Finally, as infection is rare during adulthood, it is unlikely that strains rendered resistant in that way spread in the population (31,32). Therefore, although general practitioners may have been treating *H. pylori* infections more frequently in recent years, it seems unlikely that this could have caused the observed fourfold increase in the prevalence of resistance.

The cause of the rapid increase in metronidazole resistance in *H. pylori* that we observed can only be a matter of speculation. Apparently, metronidazole-resistant *H. pylori* strains somehow have a survival advantage, and the increase in metronidazole resistance may be the result of some as yet unknown environmental pressure. Our study suggests that the prevalence of

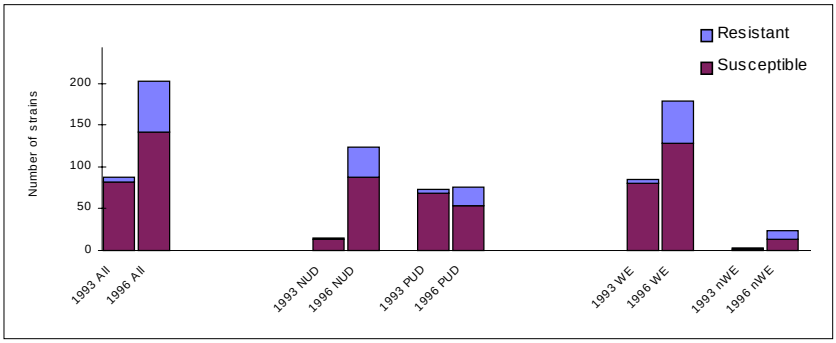


Figure 2. Distribution of metronidazole-resistant strains of *H. pylori* in 1993 and 1996 in Hospital C. All = total number, NUD = Nonulcer dyspepsia patients, PUD = peptic ulcer disease patients, nWE = non-Western Europeans.

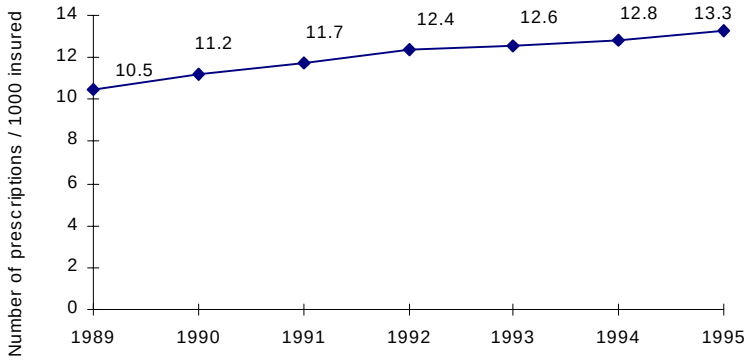


Figure 3. The out-of-hospital use of metronidazole in the Netherlands in the years 1989 to 1995. Data derived from the Health Insurance Council (Ziekenfondsraad). Drug Information Project, Amstelveen, the Netherlands.

metronidazole resistance in *H. pylori* is rapidly increasing in the Netherlands. The cause of this increase, however, is still elusive.

E.J. van der Wouden,* A.A. van Zwet,† J.C. Thijs,* G.D.C. Vosmaer,‡ J.A.J. Oom,§ A. de Jong,† and J.H. Kleibeuker¶

*Bethesda Hospital, Hoogeveen, The Netherlands;
†Regional Public Health Laboratory Groningen/ Drenthe, Hoogeveen, The Netherlands;
‡Scheper Hospital, Emmen, The Netherlands;
§Diaconessenhuis, Meppel, The Netherlands; and
¶University Hospital, Groningen, The Netherlands

References

1. Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983;i:1273.
2. National Institutes of Health. Consensus conference.*Helicobacter pylori* in peptic ulcer disease. JAMA 1994;272:65-9.
3. Van der Hulst RWM, Keller JJ, Rauws EAJ, Tytgat GNJ. Treatment of *Helicobacter pylori* infection: review of the world literature. Helicobacter 1996;1:6-19.
4. Van Zwet AA, Thijs JC, Oom JAJ, Hoogeveen J, Düringshoff BL. Failure to eradicate *Helicobacter pylori* in patients with metronidazole resistant strains. Eur J Gastroenterol Hepatol 1993;5:185-6.
5. Bell GD, Powell K, Burridge SM, Pallecaros A, Jones PH, Gant PW, et al. Experience with "triple" anti-*Helicobacter pylori* eradication therapy: side effects and the importance of testing the pretreatment bacterial isolate for metronidazole resistance. Aliment Pharmacol Ther 1992;6:427-35.
6. Rautelin H, Seppäla K, Renkonen OV, Vainio U, Kosunen TU. Role of metronidazole resistance in therapy of *Helicobacter pylori* infections. Antimicrob Agents Chemother 1992;36:163-6.
7. Thijs JC, van Zwet AA, Thijs WJ, Van der Wouden EJ, Kooy A. One week triple therapy with omeprazole, amoxicillin, and tinidazole for *Helicobacter pylori* infection: the significance of imidazole susceptibility. Aliment Pharmacol Ther 1997;11:305-9.
8. Reddy R, Osato M, Gutiérrez O, Kim JG, Graham DY. Metronidazole resistance is high in Korea and Colombia and appears to be rapidly increasing in the U.S [abstract]. Gastroenterology 1996;110:A238.
9. Ling TWK, Cheng AFB, Sung JJY, Yiu PYL, Chung SSC. An increase in *Helicobacter pylori* strains resistant to metronidazole: a five year study. Helicobacter 1996;1:57-61.
10. Xia HX, Keane CT, O'Morain CA. A 5-year survey of metronidazole and claritromycin resistance in clinical isolates of *Helicobacter pylori* [abstract]. Gut 1996;39:A6.
11. Lopez-Brea M, Martinez MJ, Domingo D, Sanchez Romero I, Sanz JC, Alarcon T. Evolution of the resistance to several antibiotics in *Helicobacter pylori* over a four year period [abstract]. Gut 1995;37:A97.
12. Teo EK, Fock KM, Ng TM, Chia SC, Khor CJL, Tan AL, et al. Primary and secondary metronidazole resistant *Helicobacter pylori* in an urban asian population [abstract]. Gut 1996;39:A23-4.
13. Weissfeld AS, Simmons DE, Vance PH, Trevino E, Kidd S, Greski-Rose P. In vitro susceptibility of pretreatment isolates of *Helicobacter pylori* from two multicenter United States clinical trials [abstract]. Gastroenterology 1996;110:A295.
14. Van Zwet AA, Thijs JC, Roosendaal R, Kuipers EJ, Pena S, de Graaff J. Practical diagnosis of *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol 1996;8:501-7.
15. Berger SA, Gorea A, Moskowitz M, Santo M, Gilat T. Effect of inoculum size on antimicrobial susceptibility of *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis 1993;12:782-3.
16. DeCross AJ, Marshall BJ, McCallum RW, Hoffman SR, Barrett LJ, Guerrant RL. Metronidazole susceptibility testing for *H. pylori*: comparison of disk, broth and agar dilution methods and their clinical relevance. J Clin Microbiol 1993;31:1971-4.
17. Graham DY, Börsch GM. The who's and when's of therapy for *Helicobacter pylori* [editorial]. Am J Gastroenterol 1990;85:1552-5.
18. National Committee for Clinical Laboratory Standards. Standard MF-A. Villanova, PA: National Committee for Clinical Laboratory Standards; 1990.
19. Karim QN, Logan RPH. *Helicobacter pylori (H. pylori)* antimicrobial resistance in the UK [abstract]. Gut 1996;39:A15.
20. De Koster E, Cozzoli A, Jonas C, Ntounda R, Butzler JP, Deltenre M. Six years resistance of *Helicobacter pylori* to macrolides and imidazoles [abstract]. Gut 1996;39:A5.
21. Thijs JC, Van Zwet AA, Oey HB. Efficacy and side effects of a triple drug regimen for eradication of *Helicobacter pylori*. Scand J Gastroenterol 1993;28:934-8.
22. Van Zwet AA, De Boer WA, Schneeberger PM, Weel J, Jansz AR, Thijs JC. Prevalence of primary *Helicobacter pylori* resistance to metronidazole and claritromycin in The Netherlands. Eur J Clin Microbiol Infect Dis 1996:15:861-4.
23. Ching CK, Leung KP, Yung RWH, Lam SK, Wong BC, Lai KC, Lai CL. Prevalence of metronidazole resistant *Helicobacter pylori* strains among Chinese peptic ulcer disease patients and normal controls in Hong Kong. Gut 1996;38:675-8.
24. Banatvala N, Davies GR, Abdi Y, Clements L, Rampton DS, Hardie JM, et al. High prevalence of *Helicobacter pylori* metronidazole resistance in migrants to east London: relation with previous nitroimidazole exposure and gastroduodenal disease. Gut 1994;35:1562-6.
25. Glupczynski Y, Burette A, De Koster E, Nyst JF, Deltenre M, Cadranel S, et al. Metronidazole resistance in *Helicobacter pylori* [letter]. Lancet 1990;335:976-7.
26. European Study Group on Antibiotic Susceptibility of *Helicobacter pylori*. Results of a multicentre European survey in 1991 of metronidazole resistance in *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis 1992;11:777-81.

27. Hirschl AM, Hirschl MM, Rotter ML. Comparison of three methods for the determination of the sensitivity of *Helicobacter pylori* to metronidazole. J Antimicrob Chemother 1993;32:45-9.
28. Midolo PD, Turnidge J, Lambert JR, Bell JM. Validation of a modified Kirby-Bauer disk diffusion method for metronidazole susceptibility testing of *Helicobacter Pylori*. Diagn Microbial Infect Dis 1995;21:135-40.
29. Becx MCJM, Janssen AJHM, Clasener HAL, de Koning RW. Metronidazole-resistant *Helicobacter pylori* [letter]. Lancet 1990;335:539-40.
30. Weil J, Bell GD, Powell K, Jobson R, Trowell JE, Gant P, Jones PH. *Helicobacter pylori* and metronidazole resistance [letter]. Lancet 1990;336:1445.
31. Walt RP. Metronidazole resistant *H. pylori*—of questionable clinical importance. Lancet 1996;348:489-90.
32. Megraud F. Epidemiology of *Helicobacter pylori* infection: where are we in 1995? Eur J Gastroenterol Hepatol 1995;7:292-5.

An Increase in Hookworm Infection Temporally Associated With Ecologic Change

This report describes a significant increase in the prevalence of hookworm infection in an area of Haiti where intestinal parasites are common, but hookworm has not been common. Changing environmental conditions, specifically deforestation and subsequent silting of a local river, have caused periodic flooding with deposition of a layer of sandy loam topsoil and increased soil moisture. We speculate that these conditions, conducive to transmission of the infection, have allowed hookworm to reemerge as an important human pathogen.

Infectious disease patterns in human populations are influenced by changes in human behavior, socioeconomic conditions, and environmental factors. Technologic advances can bring about global changes in climate and disease patterns, often with adverse effects (1,2). Shifts in land and water use as a consequence of economic development or environmental degradation may increase contact between disease agents and human populations (3). Environmental changes may directly increase exposure to infectious agents or indirectly increase disease transmission by expanding vector habitat. Altered or fluctuating ecologic conditions have resulted in a marked increase in infection rates and associated diseases for many infectious organisms, including parasites (1,2,4). As an example, the marked changes in schistosomiasis patterns in Egypt have been attributed to the construction of the Aswan High Dam (5,6). More clearly, in the Senegal River basin, construction of irrigation canals and dams and a rapid influx of workers to the area resulted in an unprecedented increase of schistosomiasis from 0% to >95% over a 3-year period (7). Changing ecologic conditions have also been responsible for marked increases in malaria transmission and drastic shifts in anopheline vector populations (2,8). In Brazil, for example, deforestation and migration of people into the interior have resulted in a fivefold increase in malaria prevalence and a shift from *Plasmodium vivax* to *P. falciparum* as the predominant species (9,10). Further evidence of the effect of climatic changes on infectious disease patterns is seen in outbreaks of malaria after floods or heavy monsoon rains associated with El Niño-Southern oscillations (11-13).

Typically, intestinal helminth infections would not be considered new or reemerging as they are highly prevalent throughout the developing world. Infection levels tend to be stable because of the lack of sanitary waste disposal and proper hygiene practices (14). However, under appropriate conditions, geohelminth infections can reemerge in areas of low prevalence. We report here a rapid increase in hookworm prevalence that coincided with ecologic change from political turmoil in the Haitian community of Leogane. The increase may be an indirect consequence of deforestation, which led to silt accumulation in the local river, subsequent flooding, altered water drainage patterns, and saturation of soil near homes.

Leogane is a small, urban community surrounded by extensive sugar cane fields (15). Access to piped water is limited, and there is no system for sewage disposal. Most people live in mud or concrete block dwellings with no electricity or indoor plumbing and few latrines. Children are exposed early in life to many parasites, including intestinal helminths. Peak prevalence of *Ascaris lumbricoides* and *Trichuris trichiura*, as well as other parasites, occurs by 24 to 30 months of age (data not shown).

Data were collected in an ongoing longitudinal study of risk factors for Bancroftian filariasis in children. Open enrollment of children under 2 years of age from several neighborhoods in Leogane took place in 1990 (approximately 60 children) and continued through 1996 (more than 250 children). Stool samples were collected at approximately 6- to 9-month intervals and were examined by the Formalin-ethyl acetate concentration technique (16). Children found infected with helminths were treated with mebendazole.

Reinfection was common, necessitating retreatment. No other systematic treatment or deworming programs were ongoing in Leogane during this period.

Throughout the 6-year follow-up, the prevalence of *Ascaris* and *Trichuris* remained relatively stable (Figure). In contrast, the prevalence of hookworm infection increased markedly from 0% to 12%-15% over the 6-year period; most of this increase took place in 3 years, beginning in 1993 through 1995.

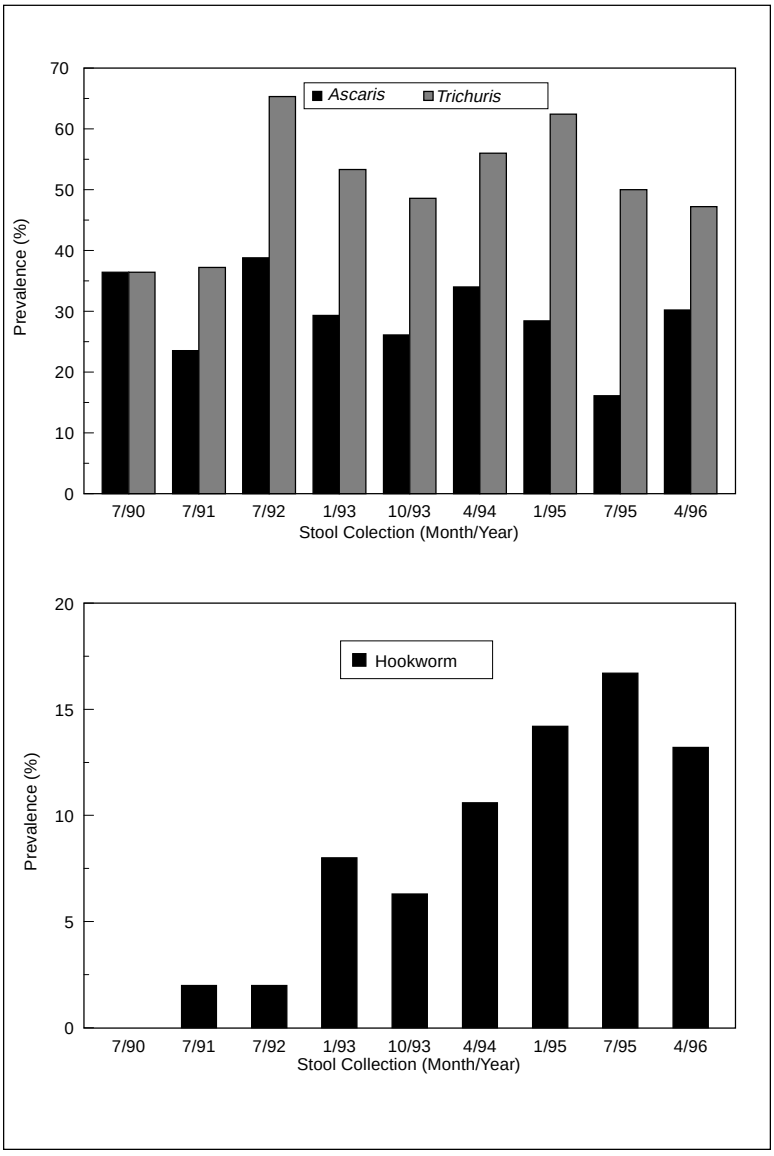


Figure. Upper panel: The prevalence of *Ascaris* (solid bars) and *Trichuris* (hatched bars) for each of the indicated stool collection periods. Lower panel: The prevalence of hookworm infection for the same collection periods. A total of 881 stools were examined after Formalin-ethyl acetate concentration (mean 98 per collection period, range 33-174).

We postulate that changes in local environmental conditions favored hookworm transmission and allowed it to increase rapidly. Hookworm transmission is favored by moist conditions that allow optimum development and survival of the larvae. Hookworms differ from other geohelminths such as *Ascaris* and *Trichuris* in which the infective eggs are more desiccation-resistant than the infective larvae. The sandy loam soil deposited by the flooding may also have been more conducive to hookworm development and survival, thus heightening the effects of the increased soil moisture.

Conditions that favored the increase in hookworm prevalence may have occurred as a result of continued flooding of the River Royone, which runs south through the town of Leogane. Because of extensive deforestation of this river's watershed, the channel accumulates silt rapidly and must be maintained by heavy equipment. After political upheaval in 1990, maintenance of the river channel and its tributaries was no longer possible and beginning in 1992, the river frequently flooded Leogane and surrounding areas. Altered river drainage patterns turned much of the community into a delta; possibly changed the soil type, especially the surface deposits; and allowed the soil to remain moist and conducive to hookworm transmission. Thus, the increase in hookworm prevalence coincides temporally with the lack of maintenance of the river channel. We noted no other dramatic changes in rainfall, temperature, or other environmental conditions during this period. Additionally, although the cohort aged over this period, the average age of the children in the survey increased only from 21 to 44 months because of continuous enrollment of younger children. Thus, increases in hookworm prevalence are not likely to be age-related.

In summary, our observations illustrate the value of longitudinal surveillance data for monitoring disease prevalence. They further highlight the effects that

environmental changes (the result of planned human activity or as in the present case, the indirect consequence of political strife) may have in shifts in prevalence of infectious diseases, including intestinal helminth infections.

Bruce Lilley,* Patrick Lammie,† Jennifer Dickerson,† and Mark Eberhard†
*University of Alabama at Birmingham, Birmingham, Alabama, USA; and
†Centers for Disease Control and Prevention, Atlanta, Georgia, USA

References

1. Wilson M. Infectious diseases: an ecological perspective. BMJ 1995;311:1681-4.
2. Patz JA, Epstein PR, Burke TA, Balbus JM. Global climate change and emerging infectious diseases. JAMA 1996;275:217-23.
3. Stanley NF, Alpers MP, editors. Man-made lakes and human health. New York: Academic Press, 1975.
4. Jackson EK. Climate change and global infectious disease threats. Med J Aust 1995;163:570-4.
5. El Alamy MA, Cline BL. Prevalence and intensity of *Schistosoma haematobium* and *S. mansoni* infection in Qalyub, Egypt. Am J Trop Med Hyg 1997;26:470-2.
6. Abdel-Wahab MF. Schistosomiasis in Egypt. Boca Raton: CRC Press, 1982.
7. Gryseels B, Stelma FF, Talla I, van Dam GJ, Polman K, Sow S, et al. Epidemiology, immunology and chemotherapy of *Schistosoma mansoni* infections in a recently exposed community in Senegal. Trop Geogr Med 1994;46:209-19.
8. Coluzzi M. Malaria and the Afrotropical ecosystems: impact of man-made environmental changes. Parassitologia 1994;36:223-7.
9. McGreevy PB, Dietze R, Prata A, Hembree SC. Effects of immigration on the prevalence of malaria in rural areas of the Amazon basin of Brazil. Mem Inst Oswaldo Cruz 1989;84:485-91.
10. Sawyer D. Economic and social consequences of malaria in new colonization projects in Brazil. Soc Sci Med 1993;37:1131-6.
11. Mathur KH, Harpalani G, Kalra NL, Murthy GGK, Narasimham MVVL. Epidemic of malaria in Barmer District (Thar Desert) of Rajasthan during 1990. Indian J Malariol 1992;29:1-10.
12. Bouma MJ, Sondrop HE, van der Kay HJ. Climate change and periodic epidemic malaria. Lancet 1994;343:1440.
13. Bouma MJ, van der Kay HJ. Epidemic malaria in India and the El Nino southern oscillation. Lancet 1994;344:1638-9.
14. World Health Organization. Prevention and control of intestinal parasitic infections. World Health Organ Tech Rep Ser 1987;749:8.
15. Raccurt C, Lowrie RC, Katz SP, Duverseau YT. Epidemiology of *Wuchereria bancrofti* in Leogane, Haiti. Trans R Soc Trop Med Hyg 1988;82:721-5.
16. Markell EK, Voge M, John DT. Medical parasitology, Philadelphia: W.B. Saunders, 1992.

Using Laboratory-Based Surveillance Data for Prevention: An Algorithm for Detecting *Salmonella* Outbreaks

By applying cumulative sums (CUSUM), a quality control method commonly used in manufacturing, we constructed a process for detecting unusual clusters among reported laboratory isolates of disease-causing organisms. We developed a computer algorithm based on minimal adjustments to the CUSUM method, which cumulates sums of the differences between frequencies of isolates and their expected means; we used the algorithm to identify outbreaks of *Salmonella* Enteritidis isolates reported in 1993. By comparing these detected outbreaks with known reported outbreaks, we estimated the sensitivity, specificity, and false-positive rate of the method. Sensitivity by state in which the outbreak was reported was 0%(0/1) to 100%. Specificity was 64% to 100%, and the false-positive rate was 0 to 1.

Effective surveillance systems provide baseline information on incidence trends and geographic distribution of known infectious agents. The ability to provide such information is a prerequisite to detecting new or reemerging threats (1). Laboratory-based surveillance can provide data on the location and frequency of isolation of specific pathogens, which can be used to rapidly detect unusual increases or clusters. These data can be transmitted electronically from multiple public health sites to a central location for analysis.

Many acute outbreaks of infectious diseases are detected by astute clinical observers, local public health authorities, or the affected persons themselves. However, outbreaks dispersed over a broad geographic area, with relatively few cases in any one jurisdiction, are much more difficult to detect locally. Rapid analysis of data to detect unusual disease clusters is the first step in recognizing outbreaks. We developed an algorithm for the Public Health Laboratory Information System (PHLIS) (2) that detects unusual clusters by using a statistical quality control method called cumulative sums (CUSUM), a method commonly used in manufacturing. CUSUM has also been applied to medical audits of influenza surveillance in England and Wales (3,4).

The Algorithm

The statistical problem of detecting unusual disease clusters in public health surveillance is similar to that of detecting clusters of defective items in manufacturing. In both cases, the aim is to detect an unusual number of occurrences. Manufacturing operations use several existing quality control methods, e.g., Shewhart Charts, moving average control, and CUSUM, to indicate abnormalities in data collected (5,6). Of these methods, CUSUM has two unique attributes that make it especially suitable for disease outbreak detection. CUSUM detects smaller shifts from the mean, and it detects similar shifts in the mean more quickly (6-8). The computational simplicity of this method also makes it especially well suited for use on personal computers. Other published methods (9-11) require more personal interactions, e.g., model building, and use more intense computations.

Applying the Algorithm to Surveillance Data

To evaluate how well the CUSUM algorithm detects unusual clusters of disease, we applied it to the Centers for Disease Control and Prevention (CDC) National *Salmonella* Surveillance System dataset. Since 1962, this surveillance system has collected reports of laboratory-confirmed *Salmonella* isolates from human sources from all U.S. state public health laboratories and the District of Columbia (12). The laboratories serotype clinical isolates of *Salmonella* by the Kauffman-White methods, which subdivide this diverse bacterial genus into more than 2,000 named serotypes (13). Each week, laboratories report to CDC each *Salmonella* strain they have serotyped, along with the age, sex, county of residence of the person from whom

it was isolated, and date of specimen collection. The algorithm uses date of specimen collection, which we consider the nearest reliable date to the date the infection began.

A one-sided CUSUM was calculated for every reported *Salmonella* serotype and week by using several values for the expected mean. Different expected means were used in the algorithm to identify which value accurately represented the historical data. First we calculated the mean of 5 weeks and the median of 5 weeks for each *Salmonella* serotype for the same week over the previous 5 years. We then calculated the mean of 15 weeks, which is the mean over a 3-week interval over the past 5 years. For example, for surveillance of the sixth week of 1993, we would use weeks 5 through 7 for each year from 1988 through 1992 to calculate the mean over a 3-week interval. The results of each calculation were compared to identify which value for the expected mean provided the best sensitivity, specificity, and false-positive rate. To minimize the time needed to process the outbreak detection algorithm for each reported serotype for each reported week, the algorithm was processed only for those *Salmonella* serotypes having a potential outbreak, an expected mean greater than zero, and counts greater than the expected mean (Figure 1). Since the entire algorithm is processed when the count for a given serotype exceeds the expected mean, the probability structure of CUSUM is not affected.

Testing the Algorithm

The outbreak detection algorithm was tested retrospectively to determine how well it discovered known outbreaks. To identify outbreaks, 52 weekly counts were calculated by serotype for each of the reporting sites over 5 years. The algorithm compared x_t, the current weekly count of each *Salmonella* serotype reported to the National *Salmonella* Surveillance System, with summary information from the same week over the previous 5 years. The summary information includes N_t, the total number of each *Salmonella* serotype reported over the past 5 years for a given week, and the expected mean over the past 5 years for a serotype for a given week. Each week, except week 52, was defined to contain 7 days. The first week of each year included January 1 through January 7; the last week contained 9 days on a leap year and 8 days otherwise.

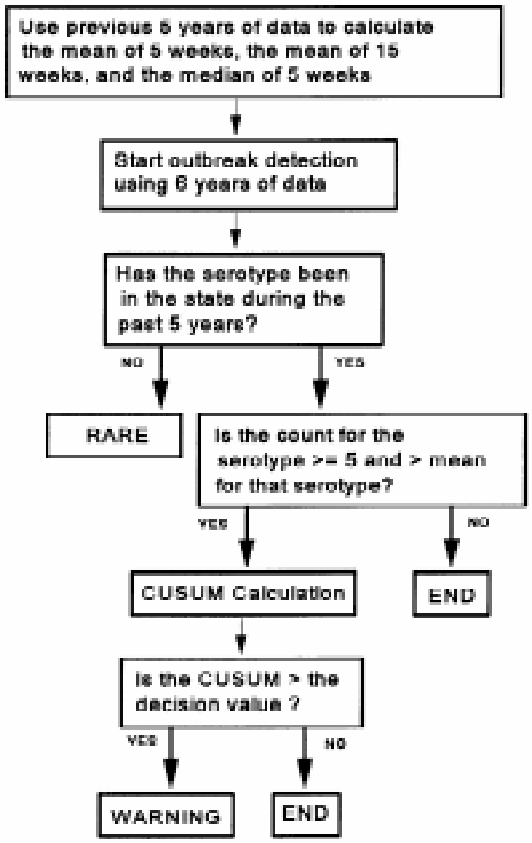


Figure 1. Algorithm for outbreak detection for one serotype for 1 week.[a]

[a]Since we are interested in detecting only increases in the number of isolates of *Salmonella* serotypes, we based our algorithm on a one-sided CUSUM. The numbers vary by serotype, and we assume the numbers of individual serotypes to be normally distributed for any given week in the past 5 years. A one-sided CUSUM determines a positive shift from the expected mean. The

CUSUM (S_t) is $S_t = \max(0, S_{t-1} + (z_t - k))$

where $z_t = (\overline{X}_t - \mu_0)/(\sigma/\sqrt{N_t})$, $S_0 = 0$, and $k > 0$.

This simplifies to $S_t = \max(0, S_{t-1} + \frac{\overline{X}_t - (\mu_0 + k\sigma_{\overline{X}_t})}{\sigma_{\overline{X}_t}})$ (8).

The standard deviation was used in our calculations instead of the standard error. S_t cumulates both the positive deviations of counts greater than k standard deviations from the mean and zero for the negative deviation of counts (8,10,14). The central reference, k, determines how many standard deviations are added to the mean. Setting k=1 helped control the variability in counts due to reporting errors, seasonality, and outbreaks.

To detect any count above delta standard deviations from the mean, a CUSUM decision value, h, was set to ensure an appropriate average run length (ARL). The values h=0.5, k=1, and delta=0.5 yielded an ARL=6 years. This ARL allowed consideration of 5 past years of counts and the count for the current year before the CUSUM signals become out of control (15,16).

A rare or uncommon serotype, i.e., a serotype that had not been reported from a state during the past 5 years, was flagged immediately as a serotype of interest. We compared flags generated by the algorithm by state and week with occurrences of reported outbreaks. We considered the sensitivity, specificity, and false-positive rate for three outbreak sizes: 1) any isolates, 2) at least three isolates, and 3) at least five isolates. Data were limited to reports during 1993 and, because we had information about previously reported outbreaks involving this serotype, CDC's *Salmonella* serotype Enteritidis (SE) Outbreak Surveillance System (17). Sensitivity was calculated as the number of outbreaks flagged by the algorithm that matched SE outbreaks reported to CDC by state and by week. Because an outbreak could have received several flags corresponding to different weeks, flags in consecutive weeks were counted as both being correct. Specificity was defined as the number of weeks without flags that corresponded to weeks without reported outbreaks. The false-positive rate was defined as the proportion of flags that did not correspond to outbreaks.

Results of the Test

The SE Outbreak Surveillance System had 63 outbreaks reported during 1993 from 20 states and one U.S. territory. Of these 63 outbreaks, 38 reports included date of collection. Two of the reported 38 SE outbreaks occurred in the same state in the same week, and multiple outbreaks occurred 1 week apart in the same state. Therefore, it is difficult to distinguish all 38 reported outbreaks as individual outbreaks.

When we used the mean of 5 weeks as the expected mean in the algorithm, 35 states had 230 flags for clusters with ≥3 isolates (Table 1). For clusters of ≥5 isolates, 25 states had 121 flags. Sensitivity calculations on these flags were 0% (0/1) to 100%, specificity was 64% to 100%, and the overall false-positive rate was 77% (Table 2).

When the median of 5 weeks was used for the expected mean in the algorithm, the algorithm flagged SE in 35 states with 380 unusual clusters with ≥3 isolates. Twenty-five states had 210 flags with ≥5 isolates (these states were the same ones that were flagged when the mean of 5 weeks and counts of ≥5 isolates were used). In each instance in which using the median of 5 weeks resulted in an unusual cluster being flagged that had not been flagged using the mean of 5 weeks, the median of 5 weeks was smaller than the mean of 5 weeks. Clusters flagged by using the median of 5 weeks but not flagged by using the mean of 5 weeks were three to 37 isolates, with a mean of seven per cluster. Three of these clusters with five or more isolates were known outbreaks. Thus, using the median of 5 weeks would have detected three more outbreaks than using the mean of 5 weeks, but at the expense of lower specificity.

Evaluating the algorithm by using the mean of 15 weeks for the expected mean, we found 125 SE flags in 25 states on clusters with ≥5 isolates. These were the same states flagged when the mean of 5 weeks was used for the expected mean. Each time a flag occurred using the mean of 15 weeks, while no corresponding flag occurred using the mean of 5 weeks, the mean of 15 weeks was smaller than the mean of 5 weeks. In this scenario, the sizes of the clusters were 3 to 8 isolates, with an average of 5 isolates per cluster. In comparison, the mean of 5 weeks was associated with a higher specificity than the mean of 15 weeks.

Without a way to calculate an overall specificity for all serotypes, the decision about which value to use as the expected mean in the algorithm was based on the data gathered about SE. Using the median of 5 weeks produced the largest number of flags and the lowest specificity; a mean of 15 weeks generated the second highest number of flags and the second lowest specificity; and using the mean of 5 weeks produced the fewest flags and the highest specificity. Even though using both the median of 5 weeks or the mean of 15 weeks produced additional early flags, this negligible increase in sensitivity was associated with a decrease in specificity. Therefore, we elected to use the mean of 5 weeks for the expected mean in the algorithm, to obtain the highest specificity.

An Assessment of the Algorithm

The CUSUM algorithm provides a simple method to evaluate surveillance data as they are being gathered and provides sensitive and rapid identification of unusual clusters of disease. In this algorithm, a mean of 5 weeks was a better value for the expected mean than a median of 5 weeks or a mean of 15 weeks. Using a mean of 5 weeks, the algorithm failed to flag reported outbreaks only three times. In addition, a median of 5 weeks and a mean of 15 weeks were associated

Table 1. Number of flags produced for *Salmonella* serotype Enteritidis, 1993

State	All Isolates			Three or more isolates			Five or more isolates		
	Mean 5 wks	Mean 15 wks	Median 5 wks	Mean 5 wks	Mean 15 wks	Median 5 wks	Mean 5 wks	Mean 15 wks	Median 5 wks
Alaska	3	3	3	0	0	0	0	0	0
Arizona	12	13	18	4	4	4	1	1	1
Arkansas	3	3	3	0	0	0	0	0	0
Colorado	21	23	35	13	13	15	1	1	1
Connecticut	12	13	31	11	12	26	9	10	21
Delaware	7	8	12	1	1	2	0	0	0
District of Columbia	4	4	8	1	1	1	0	0	0
Florida	15	15	15	1	1	1	1	1	1
Georgia	2	2	2	2	2	2	0	0	0
Hawaii	7	7	7	0	0	0	0	0	0
Idaho	12	12	14	0	0	0	0	0	0
Illinois	12	12	23	12	12	23	12	12	23
Indiana	22	22	33	14	14	17	7	7	8
Iowa	10	11	16	5	5	5	1	1	1
Kansas	12	12	16	1	1	1	1	1	1
Kentucky	10	10	13	0	0	0	0	0	0
Louisiana	16	17	18	7	7	7	0	0	0
Maryland	8	9	25	8	9	25	4	5	20
Massachusetts	5	5	9	5	5	9	5	5	9
Michigan	11	11	34	9	9	22	7	7	9
Minnesota	20	24	31	17	19	20	6	6	6
Missouri	17	19	30	5	5	6	1	1	1
Nevada	6	6	6	2	2	2	1	1	1
New Hampshire	19	22	23	3	3	3	0	0	0
New Jersey	7	7	25	7	7	25	7	7	24
New Mexico	14	14	17	7	7	7	4	4	4
New York	11	12	28	10	11	26	7	7	18
North Dakota	7	7	8	0	0	0	0	0	0
Ohio	11	13	25	10	12	19	7	8	11
Oklahoma	3	3	3	0	0	0	0	0	0
Oregon	16	17	21	4	4	4	0	0	0
Pennsylvania	1	1	1	1	1	1	1	1	1
Rhode Island	18	18	22	7	7	8	2	2	2
South Carolina	10	10	13	6	6	6	1	1	1
South Dakota	15	15	18	1	1	1	0	0	0
Tennessee	4	4	14	3	3	6	0	0	0
Texas	15	16	24	9	9	10	5	5	5
Utah	13	14	16	2	2	2	0	0	0
Vermont	28	29	30	15	15	16	9	9	10
Virginia	12	13	37	12	13	32	8	9	16
West Virginia	3	3	5	1	1	1	0	0	0
Wisconsin	14	15	31	14	14	25	13	13	15
Total	468	494	763	230	238	380	121	125	210

with lower specificity than the mean of 5 weeks. Therefore, to achieve the best specificity we used a mean of 5 weeks.

The sensitivity, specificity, and false-positive rate results indicate that the algorithm works well. However, there are several potential limitations to calculating sensitivity, specificity, and the false-positive rate as we did. Some of these include outbreak size, lack of reporting of isolates, duplicate isolate reports, and under-reporting of outbreaks. Constraints on public health resources may limit investigation of small outbreaks of SE. Therefore, we did not include these in the calculation of sensitivity. Under-reporting of isolates could cause the algorithm to miss an outbreak, regardless of its size. Under-reporting of known SE outbreaks could also inflate our estimates of specificity.

Table 2. Sensitivity and specificity for 1993 of the *Salmonella* outbreak detection algorithm and of known outbreaks of *Salmonella* serotype Enteritidis

ST	Sensitivity no. flagged wks/ no. outbreaks (%)			Specificity no. nonflagged wks/ no. possible nonflagged wks (%)			False-positive (P_{f+}) nonoutbreak flags/ no. flags (%)		
	Mean 5 wks	Mean 15 wks	Median 5 wks	Mean 5 wks	Mean 15 wks	Median 5 wks	Mean 5 wks	Mean 15 wks	Median 5 wks
AK	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
AZ	0/0	0/0	0/0	51/52 (98)	51/52 (98)	51/52 (98)	1/1 (1)	1/1 (1)	1/1 (1)
AR	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
CO	0/0	0/0	0/0	51/52 (98)	51/52 (98)	51/52 (98)	1/1 (1)	1/1 (1)	1/1 (1)
CT	5/6 (83)	5/6 (83)	5/6 (83)	39/46 (85)	39/46 (85)	36/46 (78)	4/9 (44)	5/10 (50)	16/21 (76)
DE	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
DC	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
FL	0/1	0/1	0/1	51/52 (98)	51/52 (98)	51/52 (98)	1/1 (1)	1/1 (1)	1/1 (1)
GA	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
HI	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
ID	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
IL	1/1 (100)	1/1 (100)	1/1 (100)	16/25 (64)	16/25 (64)	6/25 (24)	11/12 (92)	11/12 (92)	22/23 (96)
IN	0/0	0/0	0/0	45/52 (87)	45/52 (87)	44/52 (85)	7/7 (1)	7/7 (1)	8/8 (1)
IA	1/1 (100)	1/1 (100)	1/1 (100)	51/51 (100)	51/51 (100)	51/51 (100)	0/1 (0)	0/1 (0)	0/1 (0)
KS	1/1 (100)	1/1 (100)	1/1 (100)	51/51 (100)	51/51 (100)	51/51 (100)	0/1 (0)	0/1 (0)	0/1 (0)
KY	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
LA	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
MD	3/4 (75)	3/4 (75)	4/4 (100)	44/48 (92)	43/48 (90)	31/48 (65)	1/4 (25)	2/5 (40)	16/20 (80)
MA	0/0	0/0	0/0	47/52 (90)	47/52 (90)	43/52 (83)	5/5 (1)	5/5 (1)	9/9 (1)
MI	0/0	0/0	0/0	45/52 (87)	45/52 (87)	43/52 (83)	7/7 (1)	7/7 (1)	9/9 (1)
MN	0/0	0/0	0/0	46/52 (88)	46/52 (88)	46/52 (88)	6/6 (1)	6/6 (1)	6/6 (1)
MO	0/0	0/0	0/0	51/52 (98)	51/52 (98)	51/52 (98)	1/1 (1)	1/1 (1)	1/1 (1)
NV	0/0	0/0	0/0	51/52 (98)	51/52 (98)	51/52 (98)	1/1 (1)	1/1 (1)	1/1 (1)
NH	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
NJ	1/2(50)	1/2(50)	2/2 (100)	44/50 (88)	44/50 (88)	28/50 (56)	6/7 (86)	6/7 (86)	22/24 (92)
NM	0/0	0/0	0/0	48/52 (92)	48/52 (92)	48/52 (92)	4/4 (1)	4/4 (1)	4/4 (1)
NY	7/10 (70)	7/10 (70)	8/10 (80)	41/42 (98)	40/42 (95)	35/42 (83)	7/7 (0)	7/7 (0)	10/18 (56)
ND	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
OH	0/0	0/0	0/0	45/52 (87)	44/52 (85)	41/52 (79)	7/7 (1)	8/8 (1)	11/11 (1)
OK	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
OR	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
PA	1/1 (100)	1/1 (100)	1/1 (100)	3/3 (100)	3/3 (100)	3/3 (100)	0/1 (0)	0/1 (0)	0/1 (0)
RI	0/0	0/0	0/0	50/52 (96)	50/52 (96)	50/52 (96)	2/2 (1)	2/2 (1)	2/2 (1)
SC	1/1 (100)	1/1 (100)	1/1 (100)	51/51 (100)	51/51 (100)	51/51 (100)	1/1 (0)	1/1 (0)	1/1 (0)
SD	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
TN	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
TX	2/2 (100)	2/2 (100)	2/2 (100)	48/50 (96)	48/50 (96)	48/50 (96)	3/5 (60)	3/5 (60)	3/5 (60)
UT	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
VT	5/6 (83)	5/6 (83)	5/6 (83)	43/46 (93)	43/46 (93)	43/46 (93)	4/9 (44)	4/9 (44)	5/10 (50)
VA	0/1 (0)	0/1 (0)	0/1 (0)	43/51 (84)	43/51 (84)	35/51 (69)	8/8 (1)	9/9 (1)	16/16 (1)
WV	0/0	0/0	0/0	52/52 (100)	52/52 (100)	52/52 (100)			
WI	1/1 (100)	1/1 (100)	1/1 (100)	40/51 (78)	40/51 (78)	38/51 (75)	12/13 (92)	12/13 (92)	14/15 (93)
Total	29/38 (76)	29/38 (76)	32/38 (82)	1978/2072 (95)	1975/2072 (95)	1909/2072 (92)	92/121 (76)	96/125 (77)	178/210 (85)

An outbreak detection algorithm must have high specificity (i.e., few false flags). The algorithm can be adjusted to achieve better specificity, which would benefit state health departments that may choose to investigate small clusters.

Seasonal shifts in the incidence of *Salmonella* can interfere with the sensitivity of the outbreak detection algorithm. In our study, we examined only unusual clusters of *Salmonella* that were above the normal seasonal patterns. Thus, we may have missed smaller outbreaks that were obscured by seasonality. For example, we could have overlooked an outbreak of three cases if it occurred in a season with a high background number of reported cases.

The ability of the algorithm to detect outbreaks rapidly is also affected by the speed

with which serotyping is done and the results reported by state public health laboratories.

In early spring 1995, we implemented the algorithm on a weekly basis, looking for unusual clusters at the state, regional, and national levels among *Salmonella* isolate data reported each week from state public health laboratories to CDC. An international outbreak of *Salmonella* serotype Stanley was flagged in May 1995 (Figure 2). *S*. Stanley is an unusual serotype in the United States, with only 219 cases reported in 1994. The ensuing epidemiologic investigation

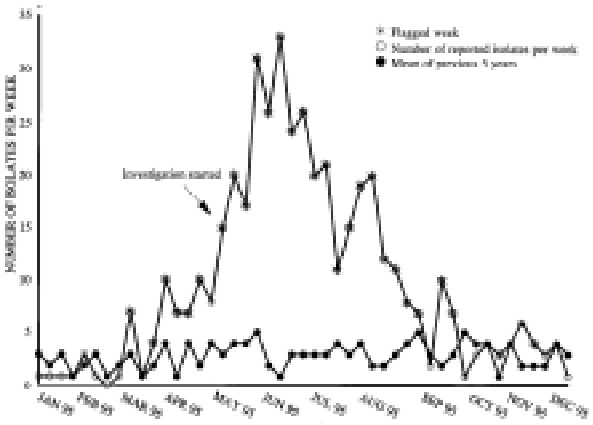


Figure 2. *Salmonella* outbreak detection algorithm *Salmonella* serotype Stanley isolates, United States, 1995.

implicated alfalfa sprouts as the vehicle of infection (18). Rapid detection of this outbreak concluded in identification of a new vehicle of salmonellosis and prompted development of prevention measures.

L. C. Hutwagner, E. K. Maloney, N. H. Bean, L. Slutsker, and S. M. Martin
Centers for Disease Control and Prevention,
Atlanta, Georgia, USA

References

1. Centers for Disease Control and Prevention. Addressing emerging infectious disease threats, a prevention strategy for the United States. Atlanta: CDC, 1994.
2. Martin SM, Bean NH. Data management issues for emerging diseases and new tools for managing surveillance and laboratory data. Emerging Infect Dis 1995;1:124-8.
3. Williams SM, Parry BR, Schlup MMT. Quality control: an application of the CUSUM. BMJ 1992;304:1359-61.
4. Tillett HE, Spencer IL. Influenza surveillance in England and Wales using routine statistics. Journal of Hygiene 1982;88:83-94.
5. Montgomery DC. Introduction to statistical quality control. New York: John Wiley and Sons; 1985.
6. Banks J. Principles of quality control. New York: John Wiley and Sons; 1989.
7. Lucas JM. The design and use of V-Mask control schemes. Journal of Quality Technology 1976;8:1-12.
8. Lucas JM. Counted data CUSUM's. Technometrics 1985;27:129-44.
9. Stroup DF, Williamson GD, Herndon JL. Detection of aberrations in the occurrence of notifiable diseases surveillance data. Stat Med 1989;8:323-9.
10. Watier L, Richardson S. A time series construction of an alert threshold with application to *S. bovismorbificans* in France. Stat Med 1991;10:1493-509.
11. Farrington CP, Andrews NJ, Beale AD, Catchpole MA. A statistical algorithm for the early detection of outbreaks of infectious disease. Journal of the Royal Statistical Society Series A 1996;159:547-63.
12. Centers for Disease Control and Prevention. Salmonella surveillance. 1993-1995 Annual Tabulation Summary. Atlanta: CDC; 1996.
13. McWhorter-Murlin AC, Hickman-Brenner FW. Identification and serotyping of *Salmonella* and an update of the Kauffmann-White Scheme. Atlanta: CDC; 1994.
14. SAS Institute Inc. SAS/QC software: reference, version 6. 1st ed. Cary (NC): SAS Institute Inc.; 1989.
15. Goel AL, Wu SM. Determination of A.R.L. and a contour nomogram for CUSUM charts to control normal mean. Technometrics 1971;13:221-30.
16. Lucas JM, Crosier RB. Fast initial response for CUSUM quality-control schemes: give your CUSUM a head start. Technometrics 1982;24:199-205.
17. Mishu B, Koeher J, Lee AL, Rodrigue D, Brenner FH, Blake P, Tauxe R. Outbreaks of *Salmonella enteritidis* infections in the United States, 1985-1991. J Infect Dis 1994;169:547-52.
18. Mahon B, Ponka A, Hall W, Komatsu K, Dietrich S, Siitonen A, et al. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seed. J Infect Dis 1997;175:876-82.

Host Genes and HIV Infection: Implications and Applications

Disease emergence often involves the introduction of a familiar microbial agent into a novel ecologic niche or the evolution of a previously unrecognized microorganism in what had ostensibly been a stable environment. So accustomed are we to emergence brought on by changes in an agent or its environment that we overlook effects of the third force of causality—the host. The easy justification for our relative indifference to the contributions of the host has been that host characteristics, especially those under genetic regulation, have less potential for rapid, epidemiologically significant evolution; moreover, the genetic mechanisms of host response have been too poorly elucidated to permit rational manipulation.

The emergence of human immunodeficiency virus (HIV), however, has been different. HIV has "emerged" so masterfully by exploiting fundamental vulnerabilities in the immune system of primates that contributions of host immunity cannot be ignored. The virus has apparently evolved from its simian cousins toward a form that is extraordinarily well adapted to humans in several ways: 1) it rapidly replicates, ensuring high mutation rates within an individual host; 2) it is readily transmissible from person to person in the absence of an animal vector; and 3) because it is not invariably lethal before the age span for most human reproduction, evolutionary pressure toward radical change, attenuation, or disappearance from the population is not strong. The enormous epidemiologic implications of these basic facts have become obvious during the decade and a half of our struggle against the virus. We cannot control it by manipulating its macroenvironment as we might a parasite carried by a vector or waterborne virus. Interrupting local transmission by setting up psychosocial or mechanical barriers has limited potential. Despite the recent highly encouraging advances in antiretroviral therapy, direct and complete pharmacologic or immunologic eradication of the virus worldwide is still an untenable prospect. So we have little choice but to search for biologic strategies that reliably interdict the host-virus relationship; to accomplish that will require insight into the fundamental mechanisms of that interaction—knowledge at the level of viral and host genetics. Indeed, modulating genetically determined features of the immune response to the virus may represent the best hope for its ultimate conquest. Recent breakthroughs have accelerated the accumulation of the knowledge necessary to accomplish that aim. In this issue of Emerging Infectious Diseases, the review of current information by McNicholl and colleagues about the genetics of virus-host interaction concentrates on the recently described variations in genes encoding the human β-chemokine receptors, appropriately providing perspectives from both laboratory and public health sciences.

The quest to identify immunogenetic determinants of the host-virus interaction in HIV infection actually began with studies of the human major histocompatibility complex (HLA) soon after the AIDS epidemic was recognized, but in the past 2 years molecular technology has been focused on promising loci in the chemokine receptor gene systems, as well as in HLA. The importance of polymorphic variants of these host genes in determining whether the infection occurs and how rapidly it proceeds has been established.

The extreme polymorphism and other related properties of HLA have made it more difficult than expected to demonstrate the full influence of products of these genes on the initiation and progression of HIV infection; however, current work on HLA is slowly confirming that expectation, which is reasonably based on 25 years of research on the role of antigen-presenting genes in a whole range of autoimmune, inflammatory, and infectious processes. In contrast, β-chemokines and the genetically mediated variation in their receptors were recognized only recently, but the initial observations and numerous confirmatory reports of their involvement in HIV infection have been compelling, and there is undoubtedly more to come.

The most important consequence of these recent discoveries has been to foster an aggressive academic and industrial enterprise aimed at developing a safe, clinically beneficial immunomodulation of β-chemokines and their receptors in both infected and uninfected persons. The relative simplicity of the gene system, the frequency of the apparently protective variant (i.e., the 32bp deletion) of CCR5, and the seemingly nonessential nature of either the wild or mutant form of the receptor for normal immune function have suggested that emulation of the unreceptive mutant state (e.g., by saturating

the normal receptor with a specific high affinity chemokinelike antibody) might interrupt viral penetration and replication. The implication here is clear. If antibodies to the normally functioning CCR5 can block viral attachment and prevent infection of the cell most critical to propagation of the agent without collateral damage to vital host immune function, a vaccine capable of inducing those antibodies without serious adverse effects could represent an adjunct to the current antiretroviral therapeutic agents and a major breakthrough toward a primary preventive strategy not dependent on changing personal behavior. The optimism and publicity that often accompany this kind of success must be tempered with caution: the strategy depends heavily on whether HIV can circumvent this hurdle by utilizing CXCR4 or other alternative pathways of entry into cells.

However, even if the promise of preventive and therapeutic intervention based on chemokine receptor manipulation is not soon fulfilled, another tangible benefit inherent in the discovery of factors like the receptor variants and HLA polymorphisms should not be overlooked. These genetic factors, however amenable or resistant to clinical manipulation they may prove to be, have true prognostic value and therefore offer a clear, immediate opportunity to refine our ongoing evaluations of other promising therapeutic or preventive measures. Consider the randomized trial of a new chemotherapeutic agent, intentionally designed to compare its average efficacy in all trial participants with the average efficacy of the conventional agent. Because HIV-1–infected persons who are heterozygous for the CCR5-deletion progress more slowly than those who carry only the wild type, stratifying the study population according to the presence or absence of the deletion, either during randomization or during analysis, should clarify whether the benefit of the experimental regimen in study participants who also carry the more favorable genetic trait is additive or even synergistic. Moreover, in clinical settings other than randomized trials, the additional information about receptor deletion status may be essential to analyzing the effects of interventions under evaluation or to customizing patient care.

The possibility that the genotype information might be used to refine the observations from current clinical research and to individualize the management of HIV-infected or even uninfected persons has also raised questions about whether typing more routinely might be appropriate. Although the concept of identifying a predisposing factor and modifying recommendations for treatment or prophylaxis accordingly is well established in the management of infectious diseases, screening for a particular genetic trait is not. So another implication of the research on host genetics in HIV infection is that it will probably draw health professionals into many of the same opportunities, obligations, and ultimately controversies that already surround the discovery of genes predisposing to cancer or chronic metabolic diseases like hemochromatosis. What may distinguish genetic screening in the context of infectious diseases from the rest, and even impose greater urgency for decisions about genetic testing, is that carriers of a genetic trait conferring relatively high risk may be readily capable of taking explicit precautions to avoid exposure to an identifiable etiologic agent. In short, in some situations the payoff may be more immediate.

The discovery of host genes that exert major influence on the acquisition and progression of HIV infection has radically altered our thinking about the pathogenesis of retroviral infection. The prognostic value of these genetic factors should be incorporated into the assessment of interventions to control the infection. The intense effort under way to translate knowledge of these human genetic traits into clinical benefit for HIV-infected and uninfected persons reflects a new rationale for research on emerging infectious diseases: consider the host, as well as the agent and the environment.

Richard A. Kaslow
University of Alabama at Birmingham
Birmingham, Alabama, USA

Controversies in the Management of Cysticercosis

Cysticercosis, an infection caused by larvae of the pork tapeworm *Taenia solium* in human tissues, is a common cause of neurologic disease in most non-Muslim developing countries, where it accounts for more than one-third of adult-onset epilepsy cases (1). Cysticercosis is increasingly diagnosed in patients in industrialized nations; persons who have never left the United States as well as visitors to disease-endemic regions are at risk. Traditionally considered an exotic disease, this infection now accounts for up to 2% of neurologic/neurosurgical admissions in southern California (2) and more than 1,000 cases per year in the United States (3). Further away from disease-endemic regions, an outbreak of cysticercosis among orthodox Jews living in New York City was reported after food was contaminated with *T. solium* eggs by immigrant cooks infected with the pork tapeworm (4); these carriers may have been completely unaware of their infections. Neurocysticercosis has been reported in AIDS patients, but immuno-suppression does not appear to increase the incidence of this infection. Once cysticercosis is diagnosed, treatment may be necessary, but optimal therapy and particularly the role of cestocidal drugs is controversial. In this commentary, we discuss current options in the treatment of established cysticercosis.

The clinical and pathologic features of neurocysticercosis vary, depending on the inflam-matory response around cysticerci, their number, size, and location. The presence of viable, living cysticerci in the central nervous system usually does not cause symptoms (5). In contrast, inflammation around degenerating cysticerci may have severe consequences, including focal encephalitis, edema, and vasculitis. The most frequent symptom is epilepsy. However, neurocysticercosis can cause a wide variety of clinical syndromes—from chronic meningitis and cranial nerve palsies to spinal infarction and symptoms due to either a mass effect or, particularly in racemose disease, raised intracranial pressure. Such variable clinical features necessitate further investigations to make a diagnosis before treatment.

The diagnosis may be made by excision biopsy of subcutaneous cysticerci, which are found in 4% to 25% of patients with neurocysticercosis (the percentages are higher in Asia than in Latin America). However, radiologic and serologic tests are usually required for diagnosis unless biopsy of a central nervous system lesion is possible. Computed tomography visualizes living cysticerci as hypodense lesions not enhanced with intravenous contrast; a small, hyperdense scolex may be observed within a living cyst (6). Degenerating cysticerci which are more often symptomatic are isodense or hyperdense, and edematous inflammation around them usually causes ring or nodular enhancement by intravenous contrast (5). Magnetic resonance imaging provides detailed images of living and degenerating cysticerci, as seen in a heavily infected patient (Figure), but may not detect calcified, destroyed cysticerci (3). An immunoblot diagnostic test on serum has been shown to have greater than 98% sensitivity and specificity (7). However, in patients with single ring-enhancing lesions, sensitivity falls to 60% to 80%. Sensitivity is also reduced if cerebrospinal fluid rather than serum immunoblot is used.

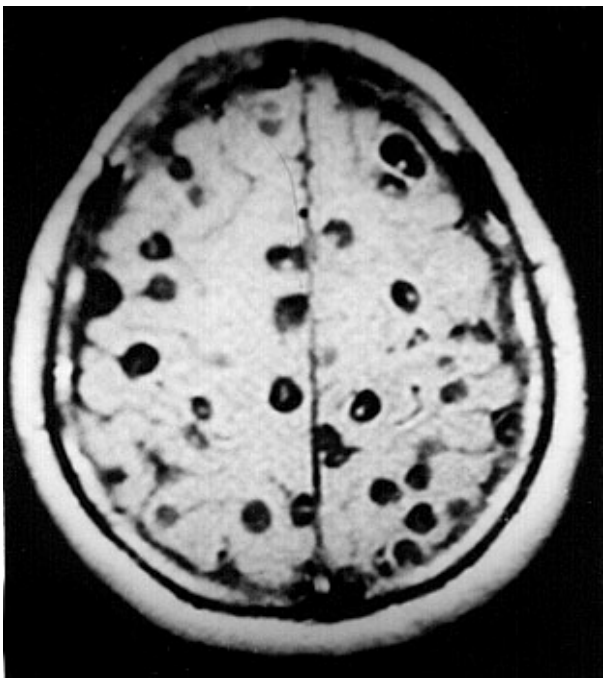


Figure. Magnetic resonance image of a patient with neurocysticercosis demonstrating multiple cysticerci within the brain.

The treatment of established neurocysticercosis is controversial and probably depends on the associated inflammatory reaction as well as clinical and pathologic features. Symptomatic

therapy with conventional anticonvulsant drugs is indicated to control epilepsy. Symptoms often result from self-limiting inflammation around a degenerating cysticercus (3,5). Raised intracranial pressure caused by this local reaction usually responds to oral corticosteroids. Steroids have been given chronically in occasional cases of persistent intracranial inflammation. Surgery also has a role: a ventriculoperitoneal shunt relieves obstructive hydrocephalus, although shunt blockage is common when the cerebrospinal fluid protein is elevated. Because inflammation associated with medical therapy may threaten vision, surgery has been used to excise intraocular cysticerci. Asymptomatic subcutaneous or intramuscular cysticerci do not require treatment.

Cestocidal therapy with praziquantel (50 mg/kg/day tid orally for 14 days) or albendazole (15 mg/kg orally tid/bid for 8 to 15 days) accelerates radiologic disappearance of viable intracranial cysticerci. Albendazole may have slightly greater efficacy and is generally less expensive than praziquantel. Cestocidal treatment combined with symptomatic care is associated with a good clinical outcome (8,9). However, these nonrandomized trials were not optimally controlled, and a similarly benign clinical course has been described after symptomatic treatment alone in both adults (5) and children (10). Furthermore, randomized placebo controlled trials with selected patients have shown no clinical (11) or radiologic (12,13) benefit from the addition of cestocidal therapy to symptomatic care. A problem with cestocidal therapy is that it causes influx of inflammatory cells around cysticerci, which is often associated with transient clinical deterioration (8). Rarely, this may be fatal in heavy infections, despite administration of corticosteroids, a common practice to minimize adverse effects (6). Although coadministering corticosteroids reduces blood levels of praziquantel and increases those of albendazole, these effects do not appear to be relevant clinically. Therefore, the immunologic basis has yet to be determined for the inflammation around cysts when they die or are killed by cestocidal treatment (14).

Although recommendations cannot yet be definitive, available evidence suggests that viable, intact cysticerci that cause epilepsy or other symptoms can be treated with cestocidal therapy, especially if they are causing mass effect. If cestocidal treatment is instituted, there is no reason to avoid the use of steroids. These should always be administered before cestocidal therapy is given to patients with multiple viable intracranial cysticerci because the sudden and simultaneous death of these parasites would otherwise cause inflammation, which can be fatal. In contrast, when untreated patients have neurologic symptoms and radiologic evidence of inflammation around a degenerating cysticercus, the parasite has probably already died, and cestocidal therapy is unlikely to be of benefit. In such cases, an expectant policy is reasonable: symptomatic therapy alone for 6 to 12 weeks, unless the patient's condition worsens. A repeat computed tomography scan then usually shows reduction in size or disappearance of a degenerating cysticercus (12,13,15). If improvement has not occurred, then empirical cestocidal chemotherapy may be considered, and possible alternative diagnoses such as tuberculosis should be entertained. Intracranial calcifications and lesions that show ring enhancement on neuroimaging are not living parasites and probably do not warrant cestocidal therapy.

This approach to cestocidal therapy is controversial, and the results of at least one ongoing, double-blind, randomized placebo-controlled trial are keenly awaited. Even when the value of cestocidal therapy is firmly established or refuted, new antiinflammatory treatments will require therapeutic approaches to be reevaluated. A greater understanding of the pathogenesis of this condition is a prerequisite to developing effective therapy to control inflammation around degenerating cysticerci.

Acknowledgments

The ongoing collaboration between the authors is funded in part by the Wellcome Trust of the United Kingdom, STD4 project PL 950004 from the European Commission, and grant number 1-U01 A135894-01 from the National Institutes of Health, USA.

Carlton Evans,* Hector H. Garcia,†‡ Robert H. Gilman,‡§ and Jon S. Friedland¶[1]

*University of Cambridge Clinical School, Cambridge, United Kingdom; †Instituto de Ciencias Neurologicas, Lima, Peru; ‡Universidad Peruana Cayetano Heredia,Lima, Peru; §Johns Hopkins University School of Hygiene and Public Health, Baltimore, USA; and ¶Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom

[1]Corresponding author

[1]Corresponding author

References

1. Garcia HH, Gilman R, Martinez M, Tsang VCW, Pilcher JB, Herrera G et al. Cysticercosis as a major cause of epilepsy in Peru. Lancet 1993;341:197-200.
2. McCormick GF. Cysticercosis: review of 230 patients. Bulletin of Clinical Neurosciences 1985; 50:76-101.
3. Shandera WX, White AC, Chen JC, Diaz P, Armstrong R. Neurocysticercosis in Houston, Texas. Medicine 1994; 73:37-52.
4. Schantz PM, Moore AC, Munoz JL, Hartman BJ, Schaefer JA, Aron AM, et al. Neurocysticercosis in an orthodox Jewish community in New York City. N Engl J Med 1992; 327:692-5.
5. Kramer LD, Locke GE, Byrd SE, Daryabagi J. Cerebral cysticercosis: documentation of natural history with CT. Radiology 1989; 171:459-62.
6. Wadia N, Desai S, Bhatt M. Disseminated cysticercosis: new observations, including CT scan findings and experience with treatment by praziquantel. Brain 1988; 111:597-614.
7. Tsang VCW, Garcia HH. Immunoblot diagnostic test (EITB) for *Taenia solium* cysticercosis and its contribution to the definition of this under-recognized but serious public health problem. In: Garcia HH, Martinez SM (editors). Teniasis/ Cisticercosis por *T. solium*. Lima: Ed. Universo; 1996. p. 259-69.
8. Sotelo J, Escobedo F, Rodriguez J, Torres B, Rubio F. Therapy of parenchymal brain cysticercosis with praziquantel. N Eng J Med 1984; 310:1001-7.
9. Vasquez V, Sotelo J. The course of seizures after treatment for cerebral cysticercosis. N Engl J Med 1992; 327:696-702.
10. Mitchell WG, Crawford TO. Intraparenchymal cerebral cysticercosis in children: diagnosis and treatment. Pediatrics 1988; 82:76-82.
11. Carpio A, Santillan F, Leon P, Flores C, Hauser A. Is the course of neurocysticercosis modified by treatment with antihelminthic agents? Arch Intern Med 1995; 155:1982-8.
12. Padma MV, Behari M, Misra NK, Ahuja GK. Albendazole in neurocysticercosis. Neurology 1994; 44:1344-6.
13. Padma MV, Behari M, Misra NK, Ahuja GK. Albendazole in single CT ring lesions in epilepsy. Natl Med J India 1995; 8:255-8.
14. Evans C, Gonzalez AE, Gilman RH, et al. Cysticercosis: immunology and immunotherapy. In: Rose FC (editor). Recent advances in tropical neurology. Amsterdam: Elsevier; 1996. p. 155-64.
15. Singhal BS, Ladiwala U. Neurocysticercosis in India. In: Rose FC (editor). Recent advances in tropical neurology. Amsterdam: Elsevier; 1996. p. 99-109.

Paratyphoid Fever Due to *Salmonella enterica* Serotype Paratyphi A

To the Editor: An outbreak of paratyphoid fever caused by *S.* Paratyphi A occurred during September and October 1996 in a residential area of New Delhi, India.

S. Paratyphi A has been responsible for 3% to 17% of cases of enteric fever in India (1). We suspected an outbreak because the *S.* Paratyphi A isolation rates exceeded the expected frequency based on the blood culture-positive rates from the cases of enteric fever reported by the Department of Microbiology at the All India Institute of Medical Sciences, New Delhi, the previous September and October (nine cases in 1995, 36 cases in 1996).

Thirty-six cases of culture-positive enteric fever due to *S.* Paratyphi A were reported on the basis of blood cultures received by the Department of Microbiology at the All India Institute of Medical Sciences Hospital, New Delhi, during September and October 1996. All the patients lived in the same residential area of 428 homes. The male to female ratio was 2:1, and most cases were in young adults (mean age = 20.1 yrs). All patients had a history of fever of 3 to 5 days' duration. The first culture-confirmed case was reported on September 12, 1996. After the initial case, 14 cases were reported in week 1, 10 cases in week 2, five cases in week 3, three cases in week 4, two cases in week 5, and two in week 6. Four households reported two cases each; the rest reported only one case per household. All the patients responded to ciprofloxacin treatment. All the isolates were sensitive to chloramphenicol, amoxycillin, cotrimoxazole, ciprofloxacin, gentamicin, and ceftriaxone. All the strains belonged to phage type 1.

The first suspected source of infection was contaminated food because two important Hindu festivals were celebrated on August 28 and September 5, 1996, respectively, just before the first culture-positive report on September 12. Investigators visited the affected households and distributed a questionnaire regarding demographic information, history of fever, food consumption from a common source, festival attendance, and type of water supply used. All the household contacts were also questioned. The information gathered did not indicate a foodborne outbreak. The second suspected source of infection was the water supply. The residential area receives water intermittently from a central reservoir. The water and sewage pipelines lie close to each other; the sewer line has many joints close to the water pipes, so the water may become contaminated with human excreta from the sewer line. New Delhi had a heavy rainfall toward the end of August and the beginning of September 1996, which led to waterlogging in the residential area. The contaminated soil might have entered the water pipes (because of negative pressure inside the pipes created by intermittent water supply) and contaminated the water supplied to these households. Water samples from these households during the last week of September did not contain fecal coliform. Soil samples from different sites did not contain salmonellae. Since *S. typhi* does not survive long in the environment, isolating the organism from the source is difficult by the time the outbreak is suspected (2). This may also be true for *S.* Paratyphi A.

An outbreak of enteric fever due to *S.* Paratyphi A has never been reported. Although we could not isolate the organism from the water or the soil by the time the outbreak was suspected, epidemiologic evidence suggests a waterborne outbreak.

Arti Kapil, Seema Sood, V.P. Reddaiah, Bimal Das, and Pradeep Seth
All India Institute of Medical Sciences,
New Delhi, India

References

1. Saxena SN, Sen R. *Salmonella* Paratyphi A infection in India-Incidence and phage types. Trans Roy Soc Trop Med Hyg 1966;603:409-11.
2. Smith GR. Enteric infections: typhoid and paratyphoid fever. In: Wilson G, Miles A, Parker MT, editors. Principles of bacteriology, virology and immunity. 7th ed., vol. 3. London: Edward Arnold Ltd.; 1982. p. 407-33.

MHC and Infectious Diseases

To the Editor: The review on the importance of the major histocompatibility complex (MHC) in infectious diseases by Singh et al. (Emerg Infect Dis 1997;3:41-9) failed to mention the potential role of human leukocyte antigen (HLA)-DM in conferring susceptibility to infectious diseases. HLA-DM is an MHC class II-like molecule essential for normal antigen processing and presentation (1). HLA-DM has been shown to function as a peptide editor, in that it influences the repertoire of peptides bound to HLA-DR.

Furthermore, this influence occurs in an allele-specific fashion (2). In addition, HLA-DM polymorphisms have been reported to confer an increased relative risk for such varied entities as rheumatoid arthritis (3), kidney transplant rejection (4), and membranous nephropathy (5). Since HLA-DM is important in determining which peptides are immunogenic, it may be as important as MHC class II molecules in regulating the immune response and therefore in conferring susceptibility to infectious diseases.

Victor S. Sloan
University of Medicine and Dentistry of New Jersey
New Brunswick, New Jersey, USA

References

1. Busch R, Mellins ED. Developing and shedding inhibitions: how MHC class II molecules reach maturity. Curr Opinion Immunol 1996;8:51-8.
2. Sloan VS, Zaller DM. Allelic specificity of the influence of HLA-DM on peptide repertoire. Arthritis Rheum 1996;39:S310.
3. Pinet V, Combe B, Avinens O, Caillat-Zucman S, Sany J, Clot J, Eliaou JF. Polymorphism of the HLA-DMA and HLA-DMB genes in rheumatoid arthritis. Arthritis Rheum 1997;40:854-8.
4. Chevrier D, Giral M, Bignon JD, Muller JY, Soulillou JP. Impact of the "new" MHC-encoded genes (HLA-DMA, -DMB and LMP2) on kidney graft outcome. Hum Immunol 1996;47:O717.
5. Giral M, Chevrier D, Muller JY, Bignon JD, Soulillou JP. TAP1*0201 and HLA-DMA*0103 markers and severe forms of membranous nephropathy. Hum Immunol 1996;47:0140.

Reply to V.S. Sloan: Dr. Sloan has rightly pointed out the importance of HLA-DM in regulating the immune response in rheumatoid arthritis, kidney transplant rejection, and membranous nephropathy. We did not mention the role of HLA-DM because our review dealt solely with infectious diseases that have well-established HLA associations.

Neeloo Singh
Central Drug Research Institute
Lucknow, India

Acute Epiglottitis due to *Pasteurella multocida* in an Adult without Animal Exposure

To the Editor: *Pasteurella multocida* infection in humans usually involves animal contact, most commonly with a domestic dog or cat (1). Epiglottitis due to human *P. multocida* infection associated with animal contact is very rare (2-4). We report a case of epiglottitis due to *P. multocida* not associated with animal contact.

A 44-year-old patient was admitted to the hospital with fever, throat fullness, and drooling. He had been healthy until 12 hours before admission when he noticed difficulty in swallowing liquids; anterior neck discomfort and fever followed, and soon he could not swallow his saliva.

When he arrived at the Emergency Department of Montefiore Medical Center on September 23, 1996, the patient was mildly toxic and had an oral temperature of 103.2°F. Pulse was 110 and blood pressure 110/70. He was drooling. He had mild anterior neck tenderness, no cervical adenopathy, no pharyngitis on inspection of the oropharynx, and no palate deviation. The heart, lungs, abdomen, and skin showed no abnormalities. A lateral neck radiograph showed an enlarged epiglottis ("thumb sign"). Indirect laryngoscopy confirmed inflamed and edematous epiglottis and supraglottic structures. A culture of the epiglottis was not performed.

On admission, the patient had a hemoglobin of 1.9 g/dL; hematocrit was 48%; white blood cell count was 14,100/mm^3; and platelet count was 170,000/mm^3. A machine differential count showed 86% granulocytes, 9% lymphocytes, and 5% monocytes.

The patient was treated with dexamethasone and ceftriaxone. The fever abated rapidly, and all symptoms resolved. Repeat laryngoscopy on day 3 confirmed resolving epiglottitis. Blood cultures taken on admission grew gram-negative, oxidase-positive bacilli that did not grow on MacConkey agar (BBL, Cockeysville, MD) in two sets, both aerobically and anaerobically. The isolate was identified as *P. multocida* by the Vitek GNI card (BioMérieux-Vitek, Inc., Hazelwood, MO). Kirby-Bauer susceptibility testing demonstrated susceptibility to penicillin. Because of the patient's marked improvement after treatment with

ceftriaxone and convenience of outpatient parenteral therapy, this antibiotic was continued to complete a 10-day course. On extensive questioning, the patient denied contact with any cat, dog, or other animal. He had recently traveled to Nigeria but denied even transient animal contact.

Since 1966, three cases of *P. multocida* epiglottitis have been reported (2-4). Although no direct culture of the epiglottis was performed in the present case, the clinical syndrome and the absence of any other focus accounting for *P. multocida* bacteremia strongly suggest that this organism caused the epiglottitis. Including the present case, three of the four reported cases have occurred since 1993, which suggests that either earlier cases were not recognized or the incidence of this condition may be increasing. In all three previous cases of *P. multocida* epiglottitis, the patients had cats as pets. As in the current case, the clinical features of *P. multocida* epiglottitis were indistinguishable from epiglottitis secondary to more common bacterial pathogens. However, the cases were all associated with positive blood cultures. In contrast, a 23% rate of bacteremia was reported in a series of epiglottitis cases in adults (including patients with blood cultures positive for *Haemophilus influenzae* type b or Group A streptococci)(5).

The vehicle of infection for this patient remains unknown, as human-to-human transmission has not been documented. This case demonstrates that epiglottitis due to *P. multocida*, a rare condition that may be increasing in frequency, need not be accompanied by recognized exposure to animals.

Michael Glickman and Robert S. Klein
Montefiore Medical Center and the Albert Einstein College of Medicine, New York, New York, USA

References

1. Weber DJ, Wolfson JS, Swartz MN, Hooper DC. *Pasteurella multocida* infections: report of 34 cases and review of the literature. Medicine 1984;63:133-54.
2. Johnson RH, Rumans LW. Unusual infections caused by *Pasteurella multocida*. JAMA 1977;237:146-7.
3. Leung R, Jassal J. Pasteurella epiglottitis. Aust N Z J Med 1994;24:218.
4. Rydberg J, White P. *Pasteurella multocida* as a cause of acute epiglottitis. Lancet 1993;341:381.
5. MayoSmith MF, Hirsch PJ, Wodzinski SF, Schiffman FJ. Acute epiglottitis in adults. An eight-year experience in the state of Rhode Island. N Engl J Med 1986;314:1133-9.

Hemolytic Uremic Syndrome Surveillance to Monitor Trends in Infection with *Escherichia coli* O157:H7 and Other Shiga Toxin-Producing *E. coli*

To the Editor: In the past 15 years, knowledge about the role of Shiga toxin-producing *Escherichia coli* (STEC) in human disease has expanded rapidly. The most distinctive complication of STEC infection is diarrhea-associated hemolytic uremic syndrome (HUS), a major cause of acute renal failure in U.S. children. Other manifestations of STEC infection can range from mild diarrhea to severe hemorrhagic colitis, thrombotic thrombocytopenic purpura, and death (1). In the United States, O157 is the most common STEC and causes an estimated 20,000 infections and 250 deaths annually. *E. Coli* O157 outbreaks associated with beef have caused concern among public health workers, clinicians, and the public, prompting major changes in clinical and laboratory practice, meat production, and food preparation. However, critical questions remain unanswered. Have prevention measures decreased risk? Are new sources of STEC infections emerging? Is the incidence of O157 infection changing? How much illness is due to STEC of serotypes other than O157?

Diarrhea-associated HUS is associated with Shiga toxin, which is produced in quantity only by STEC and by *Shigella dysenteriae* type 1; approximately 90% of HUS cases are diarrhea-associated (2,3). In the United States, where *S. dysenteriae* type 1 infections are very rare, STEC infections are the cause of virtually all diarrhea-associated HUS. The incidence of HUS in North America is about three cases per 100,000 children under 5 years of age per year; the rate among older children is somewhat lower, and the rate among adults is not known (2-6). HUS complicates approximately 5% to 10% of O157 infections and an unknown percentage of non-O157 STEC infections (1). Except for supportive care and hemodialysis, no treatment has been shown to decrease the severity of illness or to prevent complications. The sequelae of HUS—death in 3% to 5% of cases (2,3,5) and long-term renal dysfunction in 10% to 30% of survivors (6)—and the lack of specific therapy make prevention critical.

Important changes that may decrease the incidence of STEC infection and HUS in the United States are occurring now. For example, because most outbreaks of STEC infections and HUS have been linked to the consumption of undercooked beef, raw milk, or other products contaminated by the intestinal contents of cattle (1), some U.S. meat producers have changed processing practices to decrease bacterial contamination of meat. As a result of an O157 outbreak caused by consumption of frozen precooked meat patties, federal regulations requiring that this product be cooked adequately to kill O157 were implemented. New requirements that raw meat be labeled with instructions for safe handling and that carcasses be tested for O157 have also recently been implemented nationwide. New vehicles of O157 transmission, for example, dry-cured salami, fermented sausage, and unpasteurized apple juice, have been discovered, prompting the reconsideration of manufacturing processes for these products. Because O157 can be transmitted from person to person (7,8), public health recommendations for control measures to prevent transmission from infected persons have been developed and disseminated (1,8). All these prevention measures show promise; however, their effectiveness has not been documented.

Other changes may not be so salutary. For instance, an increase in international trade in beef and other foods may increase exposure to non-O157 STEC in the United States. Argentina, which has a particularly high incidence of HUS (9), has recently gained approval to start exporting beef to the United States. In Australia, which also exports beef to the United States, a large outbreak of infections with Shiga toxin-producing *E. coli* O111:NM in 1995 was linked to a sausage product; in this outbreak, 23 children became ill with HUS, and one died (10).

Current surveillance methods are unlikely to detect the impact of any of these changes because of two fundamental problems. The first is that changing rates of reported O157 infections and outbreaks do not necessarily reflect actual changes in O157 incidence; it is impossible to tell how much of the marked increase in these reports may be due to greater awareness of rather than actual increase in the incidence of infection. As the public health importance of O157 has become clear, many states have attempted to improve surveillance by mandating reporting of O157 infections. Between 1987 and February 1997, the number of states requiring such reporting increased from 3 to 42 (CDC, unpub. data). In both 1994 and 1995, 32 outbreaks were reported to CDC, the largest numbers ever, bringing the total number of reported U.S. outbreaks to 102; these comprised 2,806 illnesses and 23 deaths (CDC, unpub. data). Both heightened clinician awareness and changes in laboratory stool screening practices (11) have dramatically improved recognition of O157 infections, which has clear public health benefits. For example, if clinical laboratories in Nevada had been screening stool specimens routinely for O157 in 1993, one of the largest clusters of O157 infections ever investigated in the United States might have been recognized and controlled more quickly (12). On the other hand, changing rates of ascertainment of these infections means that O157-based surveillance systems have not been able to show trends in incidence and may not be able to do so reliably in the future.

The second fundamental problem is that surveillance for O157 infections cannot detect trends in non-O157 STEC infections. Non-O157 STEC are not likely to be detected by plating stool specimens on sorbitol-MacConkey agar (13), the method most commonly used to screen for O157. This screening test is based on the fact that, unlike most *E. coli*, very few O157 strains ferment sorbitol rapidly; since most other STEC do ferment sorbitol, this test does not detect them. Yet the non-O157 STEC pose a threat to public health in the United States. Non-O157 STEC, including *E. coli* O111:NM and *E. coli* O104:H21, have caused recent outbreaks detected only because of unusual circumstances. Similarly, sorbitol-positive O157, which recently caused a large outbreak in Germany (pers. comm. Dr. Andrea Ammon, Robert Koch Institute, Germany), would not be detected by current screening practices. In other countries, such as Australia (10) and Argentina (9), non-O157 STEC infections appear to be more common than O157 infections, and in Germany, non-O157 STEC have replaced O157 as the STEC most commonly isolated in HUS cases since 1989 (14). As travel and international trade in food increase, Americans' risk of exposure to these other STEC also increases. However, even major non-O157 STEC outbreaks might not be detected by current U.S. surveillance.

To address these two fundamental problems with current surveillance, the Centers for Disease Control and Prevention has recently initiated

active HUS surveillance in sentinel sites, which include Connecticut, Georgia, Minnesota, Oregon, and Alameda County, California and which may be expanded in the future. HUS cases, identified by a definition designed for surveillance (15), in persons under 18 years of age will be prospectively identified through referrals to pediatric nephrologists; these sites have fairly well-defined population bases, so estimating the incidence rate of pediatric HUS will be possible. Including adult HUS may be possible in the future. Because HUS is a distinctive and serious illness, its diagnosis is not likely to be affected by the first surveillance problem, the vagaries of clinical and laboratory practices that can make interpreting O157 isolation data difficult; ascertainment will likely be fairly complete. Therefore, unlike O157-based surveillance, HUS-based surveillance will allow monitoring of trends in the incidence of STEC infection and the examination of the impact of prevention efforts and changing or emerging routes of exposure. HUS cases identified through surveillance are being linked to microbial diagnosis, by culturing patients' stool for O157 and, after screening stool for Shiga toxin-producing colonies, for non-O157 STEC (in the future sera may be tested for O157 or other STEC infections). This linkage will address the second surveillance problem—by differentiating illness caused by the various STEC, linked microbial diagnosis will allow detection of trends in the incidence of non-O157 STEC as well as O157. Because passive surveillance for HUS, which has been conducted by many states and continues on a national level, lacks the microbial diagnostic component of the active surveillance system, it cannot show these trends. Active surveillance for HUS will be an efficient approach to STEC surveillance because essentially all patients with diarrhea-associated HUS have STEC infections—HUS is a more potent indicator than any other clinical syndrome.

This surveillance effort can provide the framework for future investigations in several areas. Clinical and immunologic risk factors for HUS following O157 infection could be defined through case-control studies using as controls patients with O157 infection but without HUS. Risk factors for infection with non-O157 STEC could be characterized. Serum collected from patients with non-O157 STEC infections could be used to develop serologic tests for infection with these organisms. Methods for Shiga toxin identification in stool could be evaluated. Finally, HUS treatments could be evaluated in a well-defined patient population and study network.

Reliable surveillance data are critical to targeting prevention efforts and defining their success. A national HUS surveillance system will provide the information needed to measure the impact of new and changing vehicles of STEC transmission, evaluate the effectiveness of prevention measures, and detect illness caused by non-O157 STEC.

Barbara E. Mahon,[*] Patricia M. Griffin,[†] Paul S. Mead,[†] Robert V. Tauxe[†]

[*]UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey, USA;
[†]Centers for Disease Control and Prevention, Atlanta, Georgia, USA

References

1. Griffin PM. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL, editors. Infections of the gastrointestinal tract. New York: Raven Press, Ltd., 1995. p. 739-61.
2. Martin D, MacDonald K, White K, Soler J, Osterholm M. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. N Engl J Med 1990;323:1161-7.
3. Rowe PC, Orrbine E, Wells GA, McLaine PN, Members of the Canadian Pediatric Kidney Disease Reference Center. Epidemiology of hemolytic-uremic syndrome in Canadian children from 1986 to 1988. J Pediatr 1991;119:218-24.
4. Kinney J, Gross T, Porter C, Rogers M, Schonberger L, Hurwitz E. Hemolytic-uremic syndrome: a population-based study in Washington, DC and Baltimore, Maryland. Am J Public Health 1988;78:64-5.
5. Tarr PI, Hickman RO. Hemolytic uremic syndrome epidemiology: a population-based study in King County, Washington, 1971 to 1980. Pediatrics 1987;80:41-5.
6. Siegler R, Pavia A, Christofferson R, Milligan M. A 20-year population-based study of postdiarrheal hemolytic uremic syndrome in Utah. Pediatrics 1994;96:35-40.
7. Rowe PC, Orrbine E, Ogborn M, Wells GA, Winther W, Lior H, McLaine PN. Epidemic *Escherichia coli* O157:H7 gastroenteritis and hemolytic-uremic syndrome in a Canadian Inuit community: intestinal illness in family members as a risk factor. J Pediatrics 1994;124:21-6.
8. Belongia E, Osterholm M, Soler J, Ammend D, Braun J, MacDonald K. Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. JAMA 1993;269:883-8.
9. Lopez EL, Diaz M, Grinstein S, Devoto S, Mendilaharzu F, Murray BE, et al. Hemolytic uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. J Infect Dis 1989;160:469-75.
10. Goldwater PN, Bettelheim KA. An outbreak of hemolytic uremic syndrome due to *Escherichia coli* O157:H7: or was it? Emerg Infect Dis 1996;2:153-4.

11. Boyce TG, Pemberton AG, Wells JG, Griffin PM. Screening for *Escherichia coli* O157:H7—a nationwide survey of clinical laboratories. J Clin Microbiol 1995;33:3275-7.
12. Cieslak PR, Noble SJ, Maxson DJ, Empey LC, Ravenholt O, Legarza G, et al. Hamburger-associated *Escherichia coli* O157:H7 infection in Las Vegas: a hidden epidemic. Am J Public Health 1997;87:176-80.
13. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157 associated with hemorrhagic colitis. J Clin Microbiol 1986;23:869-72.
14. Huppertz H, Busch D, Schmidt H, Aleksic S, Karch H. Diarrhea in young children associated with *Escherichia coli* non-O157 organisms that produce Shiga-like toxin. J Pediatr 1996;128:341-6.
15. Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance. MMWR Morb Mortal Wkly Rep 1997;46:17.

Virus Hunter, by C.J. Peters and Mark Olshaker, Anchor Books, New York, 1997, $23.95.

This splendid book is part memoir, part autobiography, and part scientific history. It represents the partnership of the protagonist, C.J. Peters, with Mark Olshaker, a professional author and journalist. Dr. Peters is chief, Special Pathogens Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, the federal agency with responsibility for the public health aspects of infectious diseases in the United States.

Peters, a Hopkins-trained physician, has dedicated his professional career over the last 30 years to field and laboratory studies of hemorrhagic fever viruses. These exotic agents share several characteristics, including their extraordinary lethality in primates, their maintenance in zoonotic reservoirs that are often obscure, and their occurrence in remote regions of the world. Interest in the hemorrhagic fevers has expanded over the last quarter century because they exemplify the problem of emerging infectious diseases in its most dramatic and terrifying form. Twenty-five years ago, Michael Crichton coined the term "Andromeda strain" in a famous fictional treatment in which a rogue microbe threatens to produce a devastating global pandemic. The hemorrhagic fevers represent a potential real world example of such an Andromeda scenario.

The emergence of new infectious diseases has captured the attention of epidemiologists and public health workers in the last decade. Many episodes are associated with increasing human invasion of wild ecosystems, human-made alterations of existing ecosystems through the construction of dams, massive deforestation, agricultural revolutions, the search for fossil fuels, or the importation of wild animals into new and artificial habitats. These events may expose people to zoonotic viruses of animals that can cross the species barrier resulting in human infections and disease. The bad news is that viruses that cause relatively benign infections in their natural hosts often are much more virulent in a new species. The good news is that only rarely can zoonotic viruses be transmitted from person to person; therefore, the extent of such outbreaks is limited. A remote but alarming contingency is a novel pathogenic virus that could spread by direct human-to-human contact. In a few instances, this has happened with the hemorrhagic fever viruses, but all such outbreaks have been limited to a few generations of person-to-person spread, partly because of the use of barrier nursing and partly because of biologic forces that are poorly understood. If such a devastating infection were to transmit readily and escape efforts at containment, we could face unthinkable consequences, AIDS on a fast track.

Peters, in this chronicle of his personal encounters with hemorrhagic fever viruses, develops this thesis, and effectively explains the problems of emerging viral diseases. Furthermore, his often understated accounts convey an immediate sense of the dangers of emerging infections more dramatically than would any recitation of numbers and facts.

In my view, this book is a landmark memoir in the annals of infectious diseases. It has the immediacy of a personal account, stitching together a series of vignettes that build an unforgettable picture of the heroic aspect of microbiology at the end of the 20th century. Mark Olshaker, a professional writer, permits the author's personality to shine so that the book captures the essence of all great portraits: it conveys a true picture of an individual, "warts and all."

Finally, although the text may seem a bit rough in places, it develops a single consistent theme, which will be a major one in the next millennium, namely the potential dangers of unconsidered human manipulation of the fragile ecosystems on which depend our very survival as a species.

Neal Nathanson
University of Pennsylvania
Philadelphia, Pennsylvania, USA

Meeting Summary

Multidrug-Resistant *Salmonella* Typhimurium Definitive Type 104

Approximately 50 representatives from the Centers for Disease Control and Prevention, U.S. Department of Agriculture, and U.S. Food and Drug Administration attended an interagency workshop to develop a collaborative agenda for the control and prevention of human illness caused by multidrug-resistant *Salmonella* serotype Typhimurium Definitive Type 104 (DT104). Invited speakers from the Minnesota Department of Health, the Schools of Veterinary Medicine at Washington State University and Cornell University, and the ministries of health of the United Kingdom, Canada, and the Netherlands attended the workshop, held in Atlanta, Georgia, in May 1997. Workshop goals were to review the available data on multidrug-resistant *S.* Typhimurium DT104, identify research needs and available resources to address this emerging public health problem, and outline a strategic plan for the control and prevention of human illness caused by this organism.

Multidrug-resistant *S.* Typhimurium DT104 resistant to ampicillin, chloramphenicol, sulfonamides, streptomycin, and tetracycline (R-type ACSSuT) were among the most common *Salmonella* isolates identified in the United States, Canada, the United Kingdom, and several other European countries in 1996. Data presented at the workshop indicate that multidrug-resistant *S.* Typhimurium DT104 recently emerged almost simultaneously in North America and Europe; mechanisms for this widespread distribution are not known. In several European countries, the organism is also frequently becoming resistant to trimethoprim and fluoroquinolones. *S.* Typhimurium DT104 R-type ACSSuT is also causing marked illness in animals, particularly cattle.

Further studies are necessary to elucidate the distribution of *S.* Typhimurium DT104 in the environment and in the human and animal food chains and to examine additional subtyping techniques, including pulsed-field gel electrophoresis, plasmid profiles, and polymerase chain reaction. In addition, laboratory procedures for *S.* Typhimurium DT104 (including phage typing techniques and interpretation) need to be standardized. Participants agreed to form an interagency working group to exchange information and assist in allocating resources to address this emerging public health problem.

Frederick J. Angulo
Centers for Disease Control and Prevention
Atlanta, Georgia, USA

Guidelines for the Prevention of Opportunistic Infections in HIV-Infected Persons

The 1997 U.S. Public Health Service (USPHS)/Infectious Diseases Society of America (IDSA) guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus (HIV) were published in the Morbidity and Mortality Weekly Report June 27, 1997, (Vol. 46, No. RR-12) and will appear in other publications within the next few months. An editorial will be published in the Journal of the American Medical Association. An update of the 1995 version, the guidelines are intended for health-care providers of HIV-infected patients. They cover preventing exposure to opportunistic pathogens, using chemoprophylaxis or vaccination to prevent the first episode of disease, and preventing recurrence for 17 opportunistic infections or groups of opportunistic infections.

The guidelines, formulated by representatives of federal agencies, universities, professional societies, community health-care providers, and patient advocates, have been endorsed by USPHS, IDSA, The American College of Physicians, American Academy of Pediatrics, Infectious Diseases Society of Obstetrics and Gynecology, Society of Healthcare Epidemiologists of America, and National Foundation for Infectious Diseases.

Single copies of the guidelines are available from the Centers for Disease Control and Prevention, National AIDS Clearinghouse, P.O. Box 6003, Rockville, MD 20849-6003. Telephone: (800) 458-5231. The document (June 27, 1997, Vol. 46/ No. RR-12) is also available on the Internet at http://www.cdc.gov/epo/mmwr/mmwr_rr.html or http://www.cdc.gov/nchstp/hiv_aids/pubs/mmwr.htm.

Erratum Vol. 3, No. 2: In the article "*Rhodococcus equi* and *Arcanobacterium haemolyticum*: Two "coryneform" Bacteria Increasingly Recognized as Agents of Human Infection," by Regina Linder on page 147, the heading should read *Arcanobacterium haemolyticum*; the first line should begin with *Corynebacterium haemolyticum* instead of *A. haemolyticum*. We apologize to our readers for this error.

Editorial Policy and Call for Articles

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

The journal has an international scope and is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, demography, sociology, and other disciplines. Inquiries about the suitability of proposed articles may be directed to the editor at 404-639-3967 (tel), 404-639-3039 (fax), or eideditor@cdc.gov (e-mail).

Emerging Infectious Diseases is published in English and features three types of articles: Perspectives, Synopses, and Dispatches. The purpose and requirements of each type of article are described in detail below. Spanish translations of some articles can be accessed at (ftp://fcv.medvet.unlp.edu.ar/pub/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). Articles published in this way are translated from English into the author's native language and appear in the same issue of the journal.

Instructions to Authors

Manuscripts should be prepared according to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" [Ann Intern Med 1997:126[1]:36-47].

Begin each of the following sections on a new page and in this order: title page, abstract, text, acknowledgments, references, each table, figure legends, and figures. On the title page, give complete information about each author (full names and highest degree). Give current mailing address for correspondence (include fax number and e-mail address). Follow Uniform Requirements style for references. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations. Tables and figures should be numbered separately (each beginning with 1) in the order of mention in the text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Italicize scientific names of organisms from species names all the way up, except for vernacular names (viruses that have not really been speciated, such as coxsackievirus and hepatitis B; bacterial organisms, such as pseudomonads, salmonellae, and brucellae).

All articles are reviewed by independent reviewers. The Editor reserves the right to edit articles for clarity and to modify the format to fit the publication style of Emerging Infectious Diseases.

Documents sent in hardcopy should also be sent on diskette, or by e-mail. Acceptable electronic formats for text are ASCII, WordPerfect, AmiPro, DisplayWrite, MSWord, MultiMate, Office Writer, WordStar, or Xywrite. Send graphics documents in Corel Draw, Harvard Graphics, Freelance, or save as .TIF (TIFF), .GIF (CompuServe), .WMF (Windows Metafile), .EPS (Encapsulated Postscript), or .CGM (Computer Graphics Metafile). The preferred font for graphics files is Helvetica. If possible, convert Macintosh files into one of the suggested formats. Submit photographs as glossy, camera-ready photographic prints.

Send all manuscripts and correspondence to the Editor, Emerging Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop C-12, Atlanta, GA 30333, USA, or by e-mail to eideditor@cdc.gov.

Perspectives: Contributions to the Perspectives 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 infectious 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. Articles should be approximately 3,500 words and should include references, not to exceed 40. Use of additional 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. Provide a short abstract (150 words) and a brief biographical sketch.

Synopses: Submit concise reviews of infectious diseases or closely related topics. Preference will be given to reviews of emerging and reemerging infectious diseases; however, timely updates of other diseases or topics are also welcome. Synopses 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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch.

Dispatches: Provide brief updates on trends in infectious diseases or infectious disease research. Include descriptions of new methods for detecting, characterizing, or subtyping emerging 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 of text) should not be divided into sections. Provide a short abstract (50 words); references, not to exceed 10; and figures or illustrations, not to exceed two. To expedite publication of information of a more urgent nature, we post the journal's dispatches on the Internet as soon as they are cleared and edited. As soon as the full issue is completed, these dispatches become part of the issue.