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New Tick-borne Virus

Hantavirus: Person-to-Person Transmission?

Pertussis

Invasive Hib

Gestational Psittacosis

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Cover photo: Adult deer tick

*This article is also available in Japanese at http://www.cdc.gov/ncidod/EID/eid.htm

The Economic Impact of a Bioterrorist Attack: Are Prevention and Postattack Intervention Programs Justifiable?

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Understanding and quantifying the impact of a bioterrorist attack are essential in developing public health preparedness for such an attack. We constructed a model that compares the impact of three classic agents of biologic warfare (*Bacillus anthracis*, *Brucella melitensis*, and *Francisella tularensis*) when released as aerosols in the suburb of a major city. The model shows that the economic impact of a bioterrorist attack can range from an estimated $477.7 million per 100,000 persons exposed (brucellosis scenario) to $26.2 billion per 100,000 persons exposed (anthrax scenario). Rapid implementation of a postattack prophylaxis program is the single most important means of reducing these losses. By using an insurance analogy, our model provides economic justification for preparedness measures.

Bioterrorism and its potential for mass destruction have been subjects of increasing international concern. Approximately 17 countries (including five implicated as sponsors of international terrorism) may have active research and development programs for biologic weapons (1). Moreover, groups and individuals with grievances against the government or society have been known to use or plan to use biologic weapons to further personal causes.

Only modest microbiologic skills are needed to produce and effectively use biologic weapons. The greatest, but not insurmountable, hurdle in such an endeavor may be gaining access to a virulent strain of the desired agent. Production costs are low, and aerosol dispersal equipment from commercial sources can be adapted for biologic weapon dissemination. Bioterrorists operating in a civilian environment have relative freedom of movement, which could allow them to use freshly grown microbial suspensions (storage reduces viability and virulence). Moreover, bioterrorists may not be constrained by the need for precise targeting or predictable results.

The impact of a bioterrorist attack depends on the specific agent or toxin used, the method and efficiency of dispersal, the population exposed, the level of immunity in the population, the availability of effective postexposure and/or therapeutic regimens, and the potential for secondary transmission. Understanding and quantifying the impact of a bioterrorist attack are essential to developing an effective response. Therefore, we have analyzed the comparative impact of three classic biologic warfare agents (*Bacillus anthracis*, *Brucella melitensis*, and *Francisella tularensis*) when released as aerosols in the suburbs of a major city and compared the benefits of systematic intervention with the costs of increased disease incidence (from the economic point of view used in society).

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Analytic Approach

Scenario Assumptions

We compared the impact of a theoretical bioterrorist attack on a suburb of a major city, with 100,000 population exposed in the target area. The attack was made by generating an aerosol of an agent (*B. anthracis* spores, *B. melitensis*, or *F. tularensis*) along a line across the direction of the prevailing wind. The meteorologic conditions (thermal stability, relative humidity, wind direction and speed) were assumed to be optimal (2), and the aerosol cloud passed over the

target area within 2 hours. We projected impact on the basis of 10% and 100% of the target population being exposed to the aerosol cloud.

We assumed that, when inhaled, the infectious dose$_{50}$ (ID_{50}) was 20,000 spores for *B. anthracis* and 1,000 vegetative cells for *B. melitensis* and *F. tularensis*. The rate of physical decay for airborne particles 5 μm or less in diameter was estimated to be negligible during the 2-hour transit time. The rate of biologic decay of the particulate agents was estimated to be negligible for the *B. anthracis* spores and 2% per minute for the *B. melitensis* and *F. tularensis* vegetative cells. Viability and virulence did not dissociate. Persons who were exposed to the *B. anthracis* cloud at any point during the 2-hour transit time inhaled one ID_{50} dose, and persons who were exposed to either the *B. melitensis* or *F. tularensis* cloud inhaled one to 10 ID_{50} doses, depending on their proximity to the origination point of the aerosol cloud.

The epidemic curve for anthrax by days after exposure was assumed to be <1 day, 0% of cases; 1 day, 5%; 2 days, 20%; 3 days, 35%; 4 days, 20%; 5 days, 10%; 6 days, 5%; and 7 or more days, 5% (3-5). Case-fatality rates were also assumed to vary by the day symptoms were first noted. The case-fatality rate was estimated as 85% for patients with symptoms on day 1; 80% for patients with symptoms on day 2; 70% for those with symptoms on day 3; 50% for those with symptoms on days 4, 5, and 6; and 70% for those with symptoms on and after day 7. The increased death rate in persons with an incubation period of 7 or more days is calculated on an assumption of delayed diagnosis, with resultant delayed therapy.

When estimating days in hospital and outpatient visits due to infection, we assumed that 95% of anthrax patients were hospitalized, with a mean stay of 7 days. Patients not admitted to a hospital had an average of seven outpatient visits, and surviving hospitalized patients had two outpatient visits after discharge from the hospital. Persons who received only outpatient care were treated for 28 days with either oral ciprofloxacin or doxycycline. No significant long-term sequelae resulted from the primary infection, and no relapses occurred.

The epidemic curve for brucellosis by days after exposure was assumed to be 0 to 7 days, 4% of cases; 8 to 14 days, 6%; 15 to 28 days, 14%; 29 to 56 days, 40%; 57 to 112 days, 26%, and 113 or more days, 10% (4, 6-9). The case-fatality rate was estimated to be 0.5%. Fifty percent of patients were hospitalized, with an average stay of 7 days. Nonhospitalized patients had an average of 14 outpatient visits, and hospitalized patients had seven outpatient visits after discharge from the hospital. Outpatients received a combination of oral doxycycline for 42 days and parenteral gentamicin for the first 7 days of therapy. Five percent of patients had a relapse or long-term sequelae, and required 14 outpatient visits within 1 year.

The epidemic curve for tularemia by days after exposure was assumed to be: <1 day, 0% of cases; 1 day, 1%; 2 days, 15%; 3 days, 45%; 4 days, 25%; 5 days, 10%; 6 days, 3%; and 7 or more days, 1% (4,10-11). The estimated case-fatality rate was 7.5%; and 95% of patients were hospitalized, with an average stay of 10 days. Nonhospitalized patients had an average of 12 outpatient visits, and hospitalized patients who survived the acute illness had two outpatient visits after discharge from the hospital. Outpatients received oral doxycycline for 14 days and parenteral gentamicin for 7 days. Five percent of patients had a relapse or long-term sequelae and required an average of 12 outpatient visits.

The efficacy of intervention strategies is unknown; our projections are our best estimates based on published clinical and experimental data (4,12-14). For anthrax, the projected intervention program was either a 28-day course of oral ciprofloxacin or doxycycline (assumed to be 90% effective), or a 28-day course of oral ciprofloxacin or doxycycline plus three doses of the human anthrax vaccine (assumed to be 95% effective); for brucellosis, a 42-day course of oral doxycycline and rifampin (assumed to be 80% effective), or a 42-day course of oral doxycycline, plus 7 days of parenteral gentamicin (assumed to be 95% effective); for tularemia, the intervention program was a 14-day course of oral doxycycline (assumed to be 80% effective), or a 14-day course of oral doxycycline plus 7 days of parenteral gentamicin (assumed to be 95% effective). Only 90% of persons exposed in the target area were assumed to effectively participate in any intervention program. Because the target area cannot be precisely defined, we estimated that for every exposed person participating in the intervention program, an additional 5, 10, or 15 nonexposed persons would also participate.

Economic Analyses of Postattack Intervention

To analyze the economic factors involved in establishing an intervention program, we compared the costs to the potential savings from such an intervention. Following the recommendation of the Panel of Cost-Effectiveness in Health and Medicine (PCEHM), we used estimates of actual costs rather than financial charges or market prices, which usually incorporate profit (15). We calculated the net savings (cost reductions) by using the following formula: Net savings = (number of deaths averted x present value of expected future earnings) + (number of days of hospitalization averted x cost of hospitalization) + (number of outpatient visits averted x cost of outpatient visits) - cost of intervention.

When we calculated the costs of hospitalization and outpatient visits, we assumed that only persons with symptoms (i.e., case-patients) would use medical facilities. The remainder of the exposed and potentially exposed populace would receive postexposure prophylaxis.

Present Value of Expected Future Earnings

The cost of a premature human death was nominally valued at the present value of expected future earnings and housekeeping services, weighted by the age and sex composition of the work force in the United States (16). The undiscounted average of future earnings is $1,688,595. As recommended by PCEHM (17), the stream of future earnings was discounted at 3% and 5%, to give values of $790,440 and $544,160, respectively. The present value of expected future earnings was estimated with 1990 dollars, adjusted for a 1% annual growth in productivity (16). However, in constant terms (1982 dollars), the average hourly earnings in private industry fell from $7.52 in 1990 to $7.40 in 1994 (18); therefore, the estimate of future earnings was not adjusted upwards.

Cost of Hospitalization

In 1993, the average charge for a single day of hospitalization was $875 (19). To derive true cost, we multiplied the average charge by the cost-to-charge ratio of 0.635, (the April 1994 statewide average cost-to-charge ratio for urban hospitals in New York state) (16). On this basis, we estimated true hospitalization costs at $556/day (Table 1). Hospital costs included all professional services, drugs, x-rays, and laboratory tests. Lost productivity during hospital stay was valued at $65/day (the value of an "unspecified" day's earnings, weighted for age and sex composition of the U.S. work force) (16).

Cost of Posthospitalization Outpatient Visits

After discharge from the hospital, a patient was assumed to have follow-up outpatient visits, the number of which varied by disease (Table 1). Outpatient visit costs were valued by using the Medicare National Average Allowance (20), which was chosen to represent the equivalent of bulk purchase discounted costs (i.e., actual costs) (Table 1). The first visit has a Current Procedural Terminology (CPT) code of 99201, which is classified as a "level 1" visit, requiring a physician to spend an average of 10 minutes with a patient (20). Subsequent level 1 visits, with the physician spending an average of 5 minutes with each patient, have a CPT code of 99211 (20). During outpatient visits, a general health panel test incorporating clinical chemistry tests and complete blood counts (CPT code 80050) and a single antigen or antibody detection test (e.g., CPT code 86558) were assumed to be ordered (20). Although data on Medicare allowances for office visits and many other procedures were available, data on Medicare allowances for laboratory tests were not. Thus, to establish the costs of the tests, we arbitrarily divided the lowest allowable charge for each test in half. X-rays (CPT code 71021) were valued according to the Medicare National Average Allowance (Table 1). In terms of lost productivity, we assumed that each outpatient visit cost the equivalent of 2 hours, or one-quarter, of the value of an unspecified day (16).

Cost of Outpatient Visits of Nonhospitalized Patients

For nonhospitalized outpatients, the cost of each visit, laboratory test, x-ray, and lost productivity was the same as an outpatient visit for discharged hospital patients and varied by disease (Table 1). We assumed that one set of laboratory tests would be ordered every other visit and that two sets of x-rays (CPT code 71021) would be ordered during the therapeutic course. Drug costs are discussed below.

Cost of an Intervention

The costs of an intervention can be expressed as follows: Cost of intervention = (cost of drugs used) x ([number of people exposed x multiplication

Table 1. Costs of hospitalization and outpatient visits (OPVs) following a bioterrorist attack

	Anthrax		Tularemia		Brucellosis	
	Base	Upper	Base	Upper	Base	Upper
Hospitalized patient						
Days in hospital	7	7	10	10	7	7
Cost per day ($)[a]	556	669	556	669	556	669
Lost productivity ($/day)	65	65	65	65	65	65
Follow-up OPVs (no.)	2	2	2	2	7	7
Cost 1st OPV ($)	28	44	28	44	28	44
Cost other OPVs, ea. ($)	13	24	13	24	13	24
OPV laboratory ($)[b,c]	87	174	87	174	131	261
OPV x-rays costs ($)[d]	66	66	0	0	0	0
Lost productivity ($/OPV)[e]	16	16	16	16	16	16
Total costs ($)	4,541	5,380	6,338	7,582	4,584	5,587
Avg. costs/day ($/day)	649	769	634	758	655	798
% increase: Base to upper estimate		*18*		*20*		*22*
Nonhospitalized patient						
Number of OPVs	7	7	12	12	14	14
Cost 1st OPV ($)	28	44	28	44	28	44
Cost other OPVs, ea. ($)	13	24	13	24	13	24
Lost productivity ($/OPV)[e]	16	16	16	16	16	16
Laboratory costs ($)[b,f]	131	174	261	522	261	522
X-ray costs ($)[d]	66	66	66	66	66	66
Drugs used[g]	D	C	D+G	D+G	D+R	D+R+G
Cost of drugs ($)	6	181	29	29	220	246
Total costs ($)	422	810	722	1,120	972	1,418
Avg. costs/day ($/day)	60	116	60	93	69	101
% increase: Base to upper estimate		*93*		*55*		*46*

Notes: All costs rounded to the nearest whole dollar.
[a]Hospital costs assumed to include all costs such as drugs, laboratory tests, and x-rays.
[b]Laboratory tests consists of general health panel (CPT code 80050) and an antigen or antibody test (modeled on the cost of a *Streptococcus* screen, CPT code 86588).
[c]Follow-up OPVs for hospitalized patients included two laboratory test sets for anthrax and tularemia patients and three laboratory test sets for brucellosis patients.
[d]X-ray costs (CPT code 71021), included two sets taken at different OPVs.
[e]Productivity lost due to an OPV was assumed to be one-quarter of an unspecified day's value.
[f]For OPVs of nonhospitalized patients, one set of laboratory tests is assumed for every two visits.
[g]Drugs used: D = doxycycline; C = ciprofloxacin; R = rifampin.
Sources: See text for explanation of sources of cost estimates.

factor] - number killed - number hospitalized - number of persons who require outpatient visits).

The intervention costs per person depend directly on the costs of the antimicrobial agents and vaccines used in a prophylaxis program (Table 2). We obtained drug prices from the 1996 Drug Topics Red Book and used the lowest cost available for each drug (21). The cost of doxycycline ($0.22 per 200 mg total daily dose) was the Health Care Financing Administration cost, whereas the cost of gentamicin ($3.76 per 160 mg total daily dose), ciprofloxacin ($3.70 per 1,000 mg total daily dose), and rifampin ($5.01 per 900 mg total daily dose) were wholesale costs from pharmaceutical companies. The cost of anthrax vaccine was $3.70 per dose (Helen Miller-Scott, pers. comm., 1996). The cost of administering one vaccine dose or gentamicin injection was estimated at $10.00, on the basis of the 1992 cost of administering a vaccine in a clinical setting (Valerie Kokor, pers. comm., 1996). In estimating the cost of administering oral antimicrobial agents, we assumed weekly visits, during which the drug would be distributed and counseling would be given ($15.00 for the first visit and $10.00 for each subsequent visit).

We assumed that more people would receive prophylaxis than were actually exposed because of general anxiety and uncertainty about the boundaries of the attack, the timing of the attack, and the time it would take nonresidents to travel through the attack area. Three different multiplication factors (5, 10, and 15) were used to construct

Table 2. Costs of prophylaxis following a bioterrorist attack

Level of effectiveness	Anthrax	Tularemia	Brucellosis
Lower			
Effectiveness (%)	90	80	80
Drugs used[a]	D or C	D	D+R
Cost of drugs ($)[b]	6 or 181	3	220
No. of visits[c]	4	2	6
Total cost/ person ($)	51 or 226	28	285
Upper			
Effectiveness (%)	95	95	95
Drugs used[a]	D+V or C+V	D+G	D+G
Cost of drugs ($)[b]	17 or 193	29	36
No. of visits[c]	4	7	12
Total cost/ person ($)	62 or 238	104	161
Minimum No. participants[d]	451,912	418,094	423,440
Maximum No. participants[e]	1,492,750	1,488,037	1,488,037

Notes: All costs are rounded to the nearest whole dollar.
[a]Drugs used: D = doxycycline; C = ciprofloxacin; V = anthrax vaccine; G = gentamicin; R = rifampin.
[b]See text for explanation of drug costs.
[c]Cost of visit to drug-dispensing site: 1st visit = $15/person; follow-up visits = $10/person/visit.
[d]Estimate assumed that the prophylaxis program was initiated on postattack day 6 for anthrax and tularemia and postattack day 113 for brucellosis, that the prophylaxis program had the lower effectiveness level, and that the multiplication factor for unnecessary prophylaxis givn to unexposed persons was 5.
[e]Estimate assumed that prophylaxis was initiated on postattack day 0 (day of release), that prophylaxis had the upper effectiveness level, and that the multiplication factor for unnecessary prophylaxis given to unexposed persons was 15.

alternative cost-of-intervention scenarios that take into account persons who were not at risk but participated in the prophylaxis program. Thus, if 100,000 people were exposed, we assumed that the maximum number seeking prophylaxis was 500,000, 1,000,000, or 1,500,000.

Economic Analysis of Preparedness: Insurance

The analyses outlined above consider only the economics of an intervention after an attack and include several assumptions: First, stockpiles of drugs, vaccines, and other medical supplies would be available and could be rapidly moved to points of need. Second, civil, military, and other organizations would be in place and have the capability to rapidly identify the agent, dispense drugs, treat patients, and keep order within the population. Finally, ongoing intelligence gathering would detect possible bioterrorist threats. The cost of these prerequisite activities can be calculated if they are seen as a form of insurance, the goal of which is to "purchase" the maximum net savings through preparedness to manage the consequences of an attack and reduce the probability of an attack. The "actuarially fair premium" for the "insurance" can be defined as follows (22): Actuarially fair premium = reduction of loss probability x value of avoidable loss.

The term "reduction of loss probability" indicates that, although increased surveillance and related activities can reduce the odds of an attack, they cannot guarantee absolute protection. The term "avoidable loss" refers to the fact that, even if a postexposure prophylaxis program were implemented on the day of release (day zero), some deaths, hospitalizations, and outpatient visits would be unavoidable.

Various reductions of attack probability illustrated the impact of these estimates on the calculation of actuarially fair premiums. Such reductions included reducing the probability from 1 in 100 years (0.01) to 1 in 1,000 years (0.001), a reduction of 0.009, and reducing a probability from 1 in a 100 years (0.01) to 1 in 10,000 years (0.0001), and from 1 in 100 years (0.01) to 1 in 100,000 years (0.00001). The attack probability of 0.01 in the absence of enhanced preventive actions was selected for illustrative purposes and does not represent an official estimate.

A range of minimum and maximum values of avoidable loss was derived from the net savings calculations. The values reflect differences in effectiveness of the various prophylaxis regimens, the reduced impact of delayed prophylaxis on illness and death, and the two discount rates used to calculate the present value of earnings lost because of death.

Sensitivity Analyses

In addition to the scenarios discussed above, three sensitivity analyses were conducted. First, the impact of increasing the cost of hospitalization and outpatient visits was assessed by using a set of upper estimates (Table 1). The cost of a hospital day was increased to $669 by increasing the cost-to-charge ratio from 0.634 to 0.764 (the ratio for Maryland) (16). The costs of outpatient visits (first and follow-up) were increased by assuming each visit was a "level 2" visit, doubling the average time a physician spends with each patient. The

costs of laboratory tests were increased to the full amount of the allowable charge (20).

The second sensitivity analysis considered a reduced impact, in which only 10% of the original 100,000 target population were considered exposed. All other estimates were held constant. The third sensitivity analysis considered the threshold cost of an intervention, given differences due to the effectiveness of various drug regimens, and discount rates used to calculate the present value of expected lifetime earnings lost to a death. The threshold cost occurs when net savings equal $0. Thus, the threshold value represents the maximum that could be spent per person on an intervention without having the intervention cost more than the loss from no intervention.

Findings

Postattack Illness and Death

In our model, all three biologic agents would cause high rates of illness and death. In the absence of an intervention program for the 100,000 persons exposed, the *B. anthracis* cloud would result in 50,000 cases of inhalation anthrax, with 32,875 deaths; the *F. tularensis* cloud in 82,500 cases of pneumonic or typhoidal tularemia, with 6,188 deaths; and the *B. melitensis* cloud in 82,500 cases of brucellosis requiring extended therapy, with 413 deaths.

The speed with which a postattack intervention program can be effectively implemented is critical to its success (Figure 1). For diseases with short incubation periods such as anthrax and tularemia, a prophylaxis program must be instituted within 72 hours of exposure to prevent the maximum number of deaths, hospital days, and outpatient visits (Figure 1). Some benefit, however, can be obtained even if prophylaxis is begun as late as day 6 after exposure. The relative clinical efficacy of the intervention regimen has a lesser but definite impact on observed illness and death rates (Figure 1).

A disease with a long incubation period such as brucellosis has a similar pattern (Figure 1); an important difference is the time available to implement an intervention program. Having more time available to implement an intervention program can make a marked difference in its effectiveness. However, the prolonged incubation period creates a greater potential for panic in potentially exposed persons because of the uncertainty about their health status.

Economic Analyses of Postattack Intervention: No Program

Without a postexposure prophylaxis program, an attack with *B. anthracis* is far costlier than attacks with *F. tularensis* or *B. melitensis* (Table 3). The differences between agents in medical costs as a percentage of total estimated costs are due to the large differences in death rates attributed to each agent (Figure 1).

Net Savings Due to a Postexposure Prophylaxis Program

If the postexposure prophylaxis program is initiated early, it reduces the economic impact of all three diseases, especially anthrax (Figure 2). Regardless of drug costs, the largest cost reductions

Table 3. Costs[a] ($ millions) of a bioterrorist attack with no postexposure prophylaxis program

	Anthrax	Tularemia	Brucellosis
Direct costs			
Medical: Base estimates[b]			
Hospital	194.1	445.8	170.3
OPV[c]	2.0	10.5	48.9
Medical: Upper estimates[d]			
Hospital	237.1	543.3	211.7
OPV[c]	4.4	18.5	78.3
Lost productivity			
Illness[e]			
Hospital	21.6	50.9	18.8
OPV[c]	0.7	3.9	15.0
Death			
3% discount[f]	25,985.7	4,891.2	326.5
5% discount[f]	17,889.3	3,367.3	224.7
Total costs			
Base estimates			
3% discount[f]	26,204.1	5,402.4	579.4
5% discount[f]	18,107.7	3,878.4	477.7
Upper estimates			
3% discount[f]	26,249.7	5,507.9	650.1
5% discount[f]	18,153.1	3,983.9	548.4

[a]Assuming 100,000 exposed.
[b]Medical costs are the costs of hospitalization (which include follow-up outpatient visits) and outpatient visits (Table 1).
[c]OPV = outpatient visits.
[d]Upper estimates calculated with data in Table 1.
[e]Lost productivity due to illness is the value of time spent in hospital and during OPVs (Table 1).
[f]Discount rate applied to calculate the present value of expected future earnings and housekeeping services, weighted by age and sex composition of the United States workforce (16), lost due to premature death.

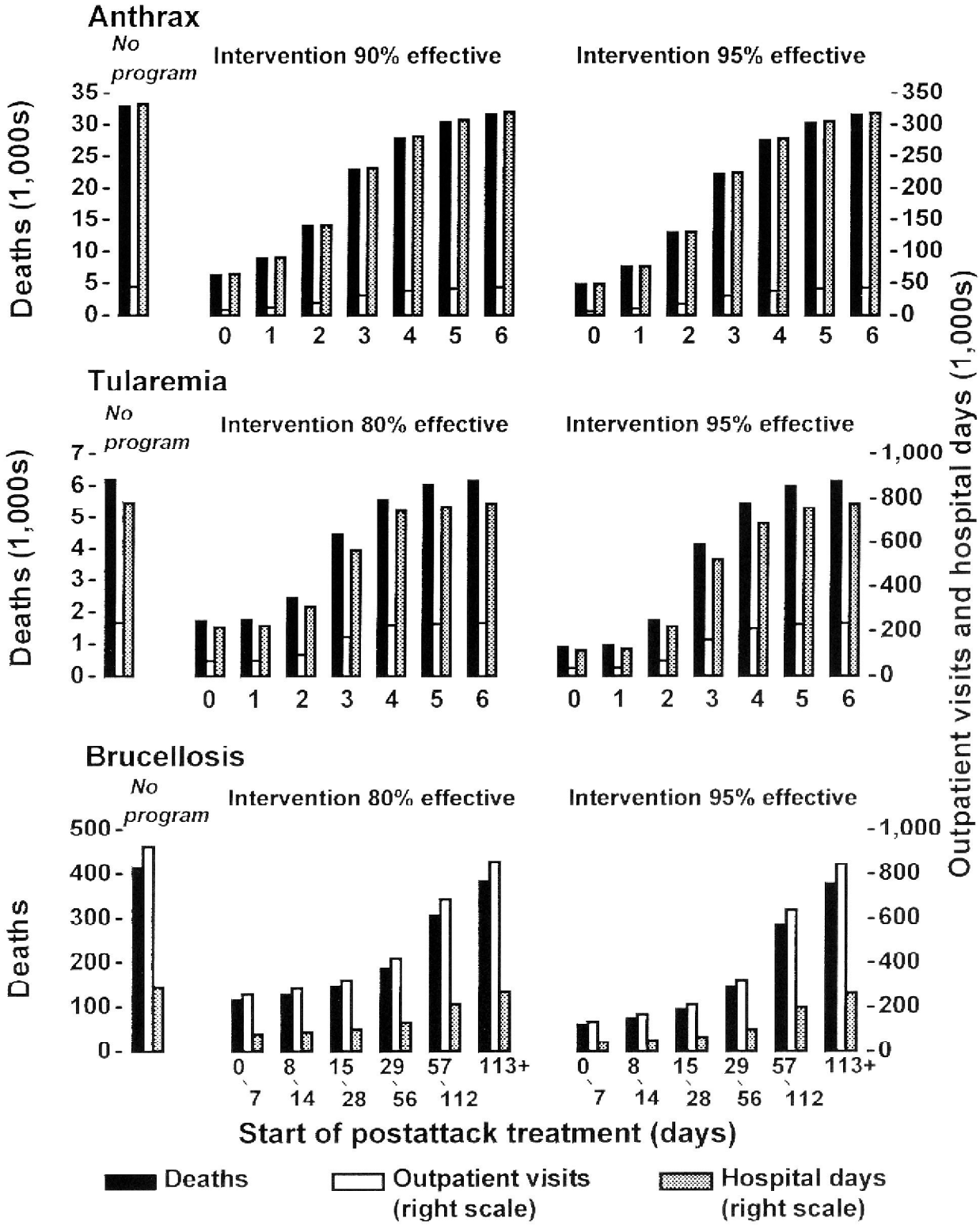

Figure 1. Total deaths, hospital days, and outpatient visits associated with aerosol releases of *B. anthracis*, *B. melitensis*, and *F. tularensis* by the postattack day of prophylaxis initiation and level of prophylaxis effectiveness.

are obtained through a combination of the most effective prophylaxis regimen (i.e., 95% effective, Table 2), the smallest multiplication factor to adjust for persons who unnecessarily receive prophylaxis, and a 3% discount rate to calculate the present value of the expected value of lifetime earnings.

In the case of anthrax, either doxycycline or ciprofloxacin could be used in the intervention program (Table 2), but the use of doxycycline generated the largest savings. The largest difference in net savings between the two drugs was approximately $261.6 million. This difference occurred when it was assumed that the program began on day zero (day of release), each drug was used in combination with the anthrax vaccine, a 3% discount rate was used, and a multiplication factor of 15 for unnecessary prophylaxis was used. This amount is equal to approximately 1.2% of the maximum total net savings generated by using a regimen of doxycycline plus the anthrax vaccine.

Some scenarios, particularly those in which prophylaxis programs were started late, generated negative net savings (i.e., net losses). In the case of tularemia, at a 5% discount rate, net losses of $10.7 to $115.1 million occurred when a postexposure program was delayed until day 6 after exposure, and a prophylaxis regimen of doxycycline and gentamicin (estimated 95% efficacy) was used. For the same scenario, but with a 3% discount, a net savings of $1,513.3 million was observed when a multiplication factor of five for unnecessary prophylaxis was used. However, multiplication factors of 10 and 15 generated net losses of $49.8 and $102.0 million, respectively. With the same drug combination, beginning the program 1 day earlier (day 5 after exposure) resulted in net savings in all scenarios except when a multiplication factor of 15 and a discount rate of 5% were used. Under the latter two assumptions, net savings result only for prophylaxis initiated by day 4 after exposure.

In the case of brucellosis, the use of a doxycycline-rifampin regimen (estimated 80% efficacy), a multiplication factor of 15 for unnecessary prophylaxis, and a discount rate of either 3% or 5% generated net losses regardless of when intervention began (Figure 2). The doxycycline-gentamicin regimen (estimated 95% efficacy) generated net losses only when it was assumed that the start of a program was delayed until 113 or more days after exposure.

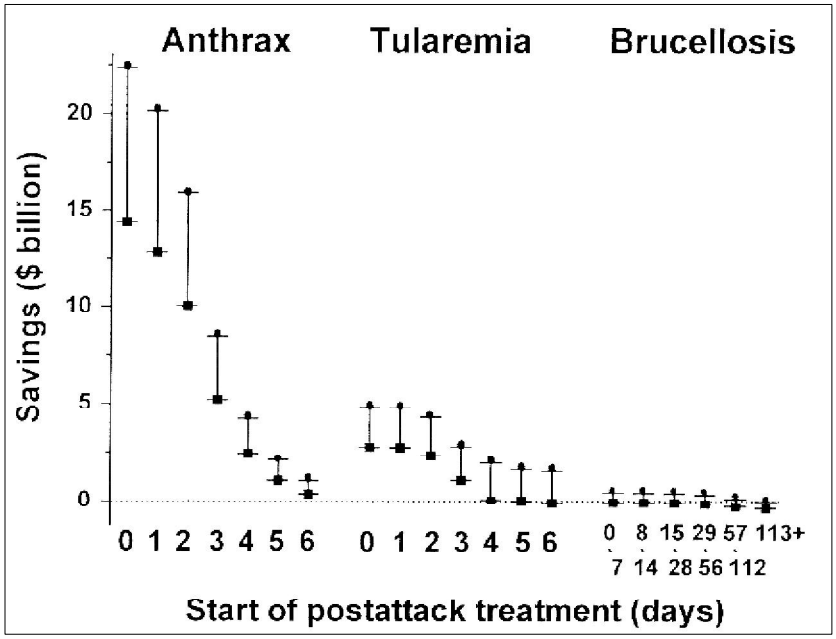

Figure 2. Ranges[a] of net savings due to postattack prophylaxis by disease and day of prophylaxis program initiation.

[a]Maximum savings (●) were calculated by assuming a 95% effectiveness prophylaxis regimen and a 3% discount rate in determining the present value of expected lifetime earnings lost due to premature death (16) and a multiplication factor of 5 to adjust for unnecessary prophylaxis. Minimum savings (■) were calculated by assuming an 80% to 90% effectiveness regimen and a 5% discount rate and a multiplication factor of 15. In tularemia prophylaxis programs initiated on days 4-7 postattack, the minimum savings were calculated by assuming a 95% prophylaxis regimen effectiveness rather than an effectiveness of 80% to 90%.

Preparedness: Insurance

The annual actuarially fair premium that can be justifiably spent on intelligence gathering and other attack prevention measures increases with the probability that a bioterrorist attack can be decreased by such measures (Table 4). However, the potential net savings attributed to reduced probability are minor compared with the potential net savings from implementing a prophylaxis program. Depending on the level of protection that can be achieved, the annual actuarially fair pre-

mium in an anthrax scenario would be $3.2 million to $223.5 million (Table 4). The lower premium would be justifiable for measures that could reduce the risk for an attack from 0.01 to 0.001 and provide the ability to mount an intervention program within 6 days of the attack. The higher premium would be justifiable for measures that could reduce the risk from 0.01 to 0.00001 and allow immediate intervention if an attack occurred.

Sensitivity Analyses

The upper estimates of the cost of hospitalization increased average costs per day by 18% to 22%, and upper estimates of the cost of outpatient visits increased average costs per day by 46% to 93% (Table 1). However, the upper estimates only increased medical costs by 1% to 6% of the total medical costs associated with a bioterrorist attack (Table 3). The largest increase was for brucellosis, for which upper estimates increased medical costs from 38% to 44% of total costs (Table 3).

Table 4. The maximum annual actuarially fair premium[a] by reduction in probability of event and size of avoided loss: Anthrax

		Actuarially fair annual premium ($ millions)		
Days post-attack[b]	Preventable loss ($millions)	0.01 to 0.001	0.01 to 0.0001	0.01 to 0.00001
Maximum loss estimate[c]				
0	22,370.5	201.3	221.5	223.5
1	20,129.4	181.2	199.3	201.1
2	15,881.5	142.9	157.2	158.7
3	8,448.0	76.0	83.6	84.4
4	4,200.1	37.8	41.6	42.0
5	2,076.1	18.7	20.6	20.7
6	1,013.8	9.1	10.0	10.1
Minimum loss estimate[d]				
0	14,372.4	128.9	141.8	143.1
1	12,820.1	115.4	126.9	128.1
2	10,049.1	90.4	99.5	100.4
3	5,200.1	46.8	51.5	51.9
4	2,429.7	21.9	24.1	24.3
5	1,004.2	9.4	10.3	10.4
6	351.2	3.2	3.5	3.5

[a]See text for definition.
[b]No. of days from attack to effective initiation of prophylaxis.
[c]Maximum loss preventable (potential net savings) occurs with the doxycycline-anthrax vaccine prophylaxis regimen, a multiplication factor of 5 for unnecessary prophylaxis, and a discount rate of 3% (Table 2).
[d]Minimum loss preventable (potential net savings) occurs with the ciprofloxacin prophylaxis regimen, a multiplication factor of 15 for unnecessary prophylaxis, and a discount rate of 5% (Table 2).

When the number of persons infected during an attack was reduced tenfold, the patient-related costs were reduced proportionately (Table 3). In most cases, however, the net savings in total costs are less than 10% of the net savings when 100% of the target population was presumed infected. The shortfall in savings is caused by an increase in the number of unexposed persons receiving prophylaxis. In the case of anthrax, when intervention programs are initiated within 3 days of exposure, savings are 4.1% to 10% of those in the original scenario (Figure 2). Delaying initiation of prophylaxis until days 4, 5, or 6 after exposure, however, results in net losses of $13.4 to $283.1 million. Losses occur regardless of prophylaxis regimen, discount rate, or multiplication factor used to adjust for unnecessary prophylaxis by unexposed persons.

In scenarios in which a multiplication factor of 15 was used to adjust for unnecessary prophylaxis, the threshold value of intervention was always above the prophylaxis cost for anthrax but not above the prophylaxis costs for tularemia and brucellosis (Table 5). For tularemia, the threshold intervention costs exceeded disease costs up to day 5 in the scenario with 95% effectiveness and a 5% discount, and for brucellosis, at all levels in the scenarios with 80% effectiveness and up to day 56 in the scenarios with 95% effectiveness. This is consistent with the lower range of estimated net savings (net losses) given in Figure 2. Reducing the number of unexposed persons receiving prophylaxis increases the cost thresholds, making the program cost beneficial. For example, changing the multiplication factors for unnecessary prophylaxis to 5 and 10 increases the cost thresholds to $659 and $319, respectively, for a brucellosis prophylaxis program initiated 15 to 28 days after exposure, with a 5% discount rate. If a discount rate of 3% is used instead of 5%, the cost thresholds increase to $799 and $387. All these cost thresholds are above the estimated prophylaxis cost of $285 per person for the doxycycline-rifampin regimen and $161 per person for the doxycycline-gentamicin regimen (Table 2).

Conclusions

The economic impact of a bioterrorist attack can range from $477.7 million per 100,000 persons exposed in the brucellosis scenario to $26.2 billion per 100,000 persons exposed in the anthrax scenario (Table 3). These are minimum

Table 5. Cost thresholds[a] of interventions ($/person) by day of intervention initiation, prophylaxis effectiveness, and discount rates.

Threshold costs for intervention ($/person, multiplication factor of 15[b])								
Anthrax			Tularemia			Brucellosis		
Post-attack day[d]	Disc. rate[c] 5%	3%	Post-attack day	Disc. rate 5%	3%	Post-attack day	Disc. rate 5%	3%
90% effectiveness[e]			*80% effectiveness[e]*			*80% effectiveness[e]*		
0	9,838	14,238	0	1,891	2,633	0-7	233*	282*
1	8,851	12,809	1	1,873	2,609	8-14	224*	272*
2	7,022	10,162	2	1,599	2,227	15-28	211*	255*
3	3,775	5,463	3	756	1,053	29-56	179*	217*
4	1,893	2,739	4	258	366	57-112	86*	104*
5	944	1,366	5	79	110	113+	24*	30*
6	468	677	6	20*	28			
Prophylaxis cost[c]	*$226*			*$28*			*$285*	
95% effectiveness[e]			*95% effectiveness[e]*			*95% effectiveness[e]*		
0	10,370	15,007	0	2,229	3,104	0-7	274	333
1	9,359	13,544	1	2,207	3,074	8-14	264	320
2	7,427	10,948	2	1,898	2,644	15-28	248	301
3	3,995	5,782	3	898	1,251	29-56	211	256
4	2,004	2,900	4	328	457	57-112	102*	124*
5	1,000	1,447	5	93*	131	113+	29*	35*
6	496	718	6	23*	32*			
Prophylaxis cost[e]	*$238*			*$104*			*$161*	

*Threshold value is below estimated cost of prophylaxis.
[a]Cost threshold is the point where cost of intervention and net savings due to the intervention are equal.
[b]Multiplication factor to adjust for persons who participated in the prophylaxis program but were unexposed.
[c]Applied to present value of expected future earnings and housekeeping services (weighted average for age and sex).
[d]Postattack day on which prophylaxis was effecively implemented.
[e]See Table 2 for prophylaxis regimens assumed to give the stated levels of effectiveness and cost/person of prophylaxis.

estimates. In our analyses, we consistently used low estimates for all factors directly affecting costs. The ID_{50} estimates for the three agents are twofold to 50-fold higher than previously published estimates (5,6,10,11), resulting in a possible understatement of attack rates. Also, in our analyses we did not include a number of other factors (e.g., long-term human illness or animal illnesses) (Table 6) whose cumulative effect would likely increase the economic impact of an attack.

Our model shows that early implementation of a prophylaxis program after an attack is essential. Although the savings achieved by initiating a prophylaxis program on any given day after exposure has a wide range, a clear trend of markedly reduced savings is associated with delay in starting prophylaxis (Figure 2). This trend was found in the analysis of all three agents studied.

Delay in starting a prophylaxis program is the single most important factor for increased losses (reduced net savings). This observation was supported by the actuarially fair premium for preparedness analysis (Table 4). Reductions in preventable loss due to early intervention had significantly greater impact on the amount of an actuarially fair premium than reductions in probability of an attack through intelligence gathering and related activities.

Although implemented at different times in a threat-attack continuum, both attack prevention measures and prophylaxis programs are forms of preventive medicine. Attack prevention measures seek to prevent infection, while prophylaxis programs prevent disease after infection has occurred.

Using an actuarially fair premium analogy in which cost and benefit are required to be equal, we find that the incremental rate of increasing prevention effectiveness (the marginal increase) declines rapidly as probability reduction targets go from 0.001 to 0.0001 to 0.00001. Because the loss probability is decreasing on a logarithmic scale, the potential increment in marginal benefit drops comparably, resulting in ever smaller increments in the protection above the preceding base level.

Conversely, delaying a prophylaxis program for anthrax, a disease with a short incubation

Table 6. Potential factors affecting the economic impact of a bioterrorist attack

Factor	Potential impact on net savings	Relative magnitude of impact
Higher than projected case-fatality rate	Increase	++++
Long term illness (physical and psychological)	Increase	++
Decontamination and disposal of biohazardous waste	Increase	++
Disruptions in commerce (local, national, and international)	Increase	++
Animal illness and death	Increase	+
Lower than projected effectiveness of prophylaxis	Decrease	- - -
Adverse drug reactions due to prophylaxis	Decrease	-
Postattack prophylaxis distribution costs, including crowd control and security	Decrease	-
Training and other skill maintenance costs	Decrease	-
Procurement and storage of antimicrobial drugs and vaccines before attack	Decrease	-
Criminal investigations and court costs	Variable	+/-

period and a high death rate, increases the risk for loss in a manner akin to a semilogarithmic scale. Arithmetic increases in response time buy disproportionate increases in benefit (prevented losses.) The potential for reducing loss is great because an attack is assumed, thus increasing the actuarially fair premium available to prepare for and implement a rapid response.

Large differences between prophylaxis costs and the threshold costs for most scenarios, particularly if prophylaxis is early (Table 5), suggest that the estimates of savings from prophylaxis programs are robust. Even with large increases in prophylaxis cost, net savings would still be achieved.

The ability to rapidly identify persons at risk would also have significant impact on costs. For example, the threshold costs for brucellosis prophylaxis are often lower than intervention costs when the ratio of unexposed to exposed persons in the prophylaxis program is 15:1 (Table 5). This finding provides an economic rationale for preparedness to rapidly and accurately identify the population at risk and reduce unnecessary prophylaxis costs.

The maximum amount of the annual actuarially fair premium varies directly with the level of risk reduction and the rapidity of postattack response (Table 4). The calculated amount of actuarially fair premiums, however, should be considered a lower bound estimate. A higher estimate (called the certainty equivalent) can also be calculated; however, this requires the determination of a social welfare function (22), and such complexity is beyond the scope of this study.

Our model provides an economic rationale for preparedness measures to both reduce the probability of an attack and increase the capability to rapidly respond in the event of an attack. The larger portion of this preparedness budget (insurance premium) should be allocated to measures that enhance rapid response to an attack. These measures would include developing and maintaining laboratory capabilities for both clinical diagnostic testing and environmental sampling, developing and maintaining drug stockpiles, and developing and practicing response plans at the local level. These measures should be developed with a value-added approach. For example, the laboratory capability could be used for other public health activities in addition to preparedness, and drugs nearing their potency expiration date could be used in government-funded health care programs. However, these secondary uses should not undermine the preparedness program's effectiveness.

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Hantaviruses: A Global Disease Problem

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Hantaviruses are carried by numerous rodent species throughout the world. In 1993, a previously unknown group of hantaviruses emerged in the United States as the cause of an acute respiratory disease now termed hantavirus pulmonary syndrome (HPS). Before then, hantaviruses were known as the etiologic agents of hemorrhagic fever with renal syndrome, a disease that occurs almost entirely in the Eastern Hemisphere. Since the discovery of the HPS-causing hantaviruses, intense investigation of the ecology and epidemiology of hantaviruses has led to the discovery of many other novel hantaviruses. Their ubiquity and potential for causing severe human illness make these viruses an important public health concern; we reviewed the distribution, ecology, disease potential, and genetic spectrum.

The genus *Hantavirus*, family Bunyaviridae, comprises at least 14 viruses, including those that cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) (Table 1). Several tentative members of the genus are known, and others will surely emerge as their natural ecology is further explored. Hantaviruses are primarily rodent-borne, although other animal species har-boring hantaviruses have been reported. Unlike all other viruses in the family, hantaviruses are not transmitted by arthropod vectors but (most frequently) from inhalation of virus-contaminated aerosols of rodent excreta (1). Human-to-human transmission of hantaviruses has not been documented, except as noted below.

The recognition of a previously unknown group of hantaviruses as the cause of HPS in 1993 is an example of virus emergence due to environmental factors favoring of the natural reservoir; a larger reservoir increases opportunities for human infection. We reviewed the global distribution of hantaviruses, their potential to cause disease, and their relationships to each other and to their rodent hosts.

History of HFRS and HPS

"Hemorrhagic fever with renal syndrome" denotes a group of clinically similar illnesses that occur throughout the Eurasian landmass and adjoining areas (2,3). HFRS includes diseases previously known as Korean hemorrhagic fever, epidemic hemorrhagic fever, and nephropathia epidemica (4). Although these diseases were recognized in Asia perhaps for centuries, HFRS first came to the attention of western physicians when approximately 3,200 cases occurred from 1951 to 1954 among United Nations forces in Korea (2,5). Other outbreaks of what is believed to have been HFRS were reported in Russia in 1913 and 1932, among Japanese troops in Manchuria in 1932 (2,6), and in Sweden in 1934 (7,8). In the early 1940s, a viral etiology for HFRS was suggested by Russian and Japanese investigators who injected persons with filtered urine or serum from patients with naturally acquired disease (2). These studies also provided the first clues to the natural reservoir of hantaviruses: the Japanese investigators claimed to produce disease in humans by injecting bacteria-free filtrates of tissues from *Apodemus agrarius* or mites that fed on the *Apodemus* mice. Mite transmission was never conclusively demonstrated by other investigators, and it was not until 1978 that a rodent reservoir for HFRS-causing viruses was confirmed by investigators who demonstrated that patient sera reacted with antigen in lung sections of wild-caught *Apodemus agrarius* and that the virus could be passed from rodent to rodent (9). The successful propagation of Hantaan (HTN) virus in cell culture in 1981 provided the first opportunity to study this pathogen systematically (10). The history of HFRS has been explored (2,11,12).

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Table 1. Members of the genus *Hantavirus*, family Bunyaviridae

Species	Disease	Principal Reservoir	Distribution of Virus	Distribution of Reservoir
Hantaan (HTN)	HFRS[a]	*Apodemus agrarius* (striped field mouse)	China, Russia, Korea	C Europe south to Thrace, Caucasus, & Tien Shan Mtns; Amur River through Korea to E Xizang & E Yunnan, W Sichuan, Fujiau, & Taiwan (China)
Dobrava-Belgrade (DOB)	HFRS	*Apodemus flavicollis* (yellow-neck mouse)	Balkans	England & Wales, from NW Spain, France, S Scandinavia through European Russia to Urals, S Italy, the Balkans, Syria, Lebanon, & Israel
Seoul (SEO)	HFRS	*Rattus norvegicus* (Norway rat)	Worldwide	Worldwide
Puumala (PUU)	HFRS	*Clethrionomys glareolus* (bank vole)	Europe, Russia, Scandinavia	W Palearctic from France and Scandinavia to Lake Baikai, south to N Spain, N Italy, Balkans,W Turkey, N Kazakhstan, Altai & Sayan Mtns; Britain & SW Ireland
Thailand (THAI)	nd[b]	*Bandicota indica* (bandicoot rat)	Thailand	Sri Lanka, peninsular India to Nepal, Burma, NE India, S China, Laos, Taiwan, Thailand, Vietnam
Prospect Hill (PH)	nd	*Microtus pennsylvanicus* (meadow vole)	U.S., Canada	C Alaska to Labrador, including Newfoundland & Prince Edward Island, Canada; Rocky Mountains to N New Mexico, in Great Plains to N Kansas, & in Appalachians to N Georgia, U.S.
Khabarovsk (KHB)	nd	*Microtus fortis* (reed vole)	Russia	Transbaikalia Amur region; E China
Thottapalayam (TPM)	nd	*Suncus murinus* (musk shrew)	India	Afghanistan, Pakistan, India, Sri Lanka, Nepal, Bhutan, Burma, China, Taiwan, Japan, Indomalayan Region
Tula (TUL)	nd	*Microtus arvalis* (European common vole)	Europe	Throughout Europe to Black Sea & NE to Kirov region, Russia
Sin Nombre (SN)	HPS[c]	*Peromyscus maniculatus* (deer mouse)	U.S., Canada, Mexico	Alaska Panhandle across N Canada, south through most of continental U.S., excluding SE & E seaboard, to southernmost Baja California Sur and to NC Oaxaca, Mexico
New York (NY)	HPS	*Peromyscus leucopus* (white-footed mouse)	U.S.	C and E U.S. to S Alberta & S Ontario, Quebec & Nova Scotia, Canada; to N Durango & along Caribbean coast to Isthmus of Tehuantepec & Yucatan Peninsula, Mexico

[a]HFRS, hemorrhagic fever with renal syndrome
[b]nd, none documented
[c]HPS, hantavirus pulmonary syndrome

Table 1. Members of the genus *Hantavirus*, family Bunyaviridae (continued)

Species	Disease	Principal Reservoir	Distribution of Virus	Distribution of Reservoir
Black Creek Canal (BCC)	HPS	*Sigmodon hispidus* (cotton rat)	U.S.	SE U.S., from S Nebraska to C Virginia south to SE Arizona & peninsular Florida; interior & E Mexico through Middle America to C Panama; in South America to N Colombia & N Venezuela
El Moro Canyon (ELMC)[d]	nd	*Reithrodontomys megalotis* (Western harvest mouse)	U.S., Mexico	British Columbia & SE Alberta, Canada; W and NC U.S., S to N Baja California & interior Mexico to central Oaxaca
Bayou (BAY)[d]	HPS	*Oryzomys palustris* (rice rat)	U.S.	SE Kansas to E Texas, eastward to S New Jersey & peninsular Florida
Probable species:[e]				
Topografov (TOP)	nd	*Lemmus sibiricus* (Siberian lemming)	Siberia	Palearctic, from White Sea, W Russia, to Chukotski Peninsula, NE Siberia, & Kamchatka; Nearctic, from W Alaska E to Baffin Island & Hudson Bay, S Rocky Mtns to C B.C., Canada
Andes (AND)[d]	HPS	*Oligoryzomys longicaudatus*[f] (long-tailed pygmy rice rat)	Argentina	NC to S Andes, approximately to 50 deg S latitude, in Chile & Argentina
To be named[d]	HPS	*Calomys laucha* vesper mouse	Paraguay	N Argentina & Uruguay, SE Bolivia, W Paraguay, and WC Brazil
Isla Vista (ISLA)[d]	nd	*Microtus californicus* (California vole)	U.S.	Pacific coast, from SW Oregon through California, U.S., to N Baja California, Mexico
Bloodland Lake (BLL)[d]	nd	*Microtus ochrogaster* (prairie vole)	U.S.	N & C Great Plains, EC Alberta to S Manitoba, Canada, S to N Oklahoma & Arkansas, E to C Tennessee & W West Virginia, U.S.; relic populations elsewhere in U.S. & Mexico
Muleshoe (MUL)[d]	nd	*Sigmodon hipidus* (cotton rat)	U.S.	See Black Creek Canal
Rio Segundo (RIOS)[d]	nd	*Reithrodontomys mexicanus* (Mexican harvest mouse)	Costa Rica	S Tamaulipas & WC Michoacan, Mexico, S through Middle American highlands to W Panama; Andes of W Colombia & N Ecuador
Rio Mamore (RIOM)[d]	nd	*Oligoryzomys microtis* (small-eared pygmy rice rat)	Bolivia	C Brazil south of Rios Solimoes-Amazon & contiguous low lands of Peru, Bolivia, Paraguay, & Argentina.

[d] not yet isolated in cell culture
[e] viruses for which incomplete characterization is available, but for which there is clear evidence indicating that they are unique
[f] suspected host, but not confirmed
Adapted from (57,72) and from (9,13,23,38,42,43,50-71)

HPS was first described in 1993 when a cluster of cases of adult fatal respiratory distress of unknown origin occurred in the Four Corners region of the United States (New Mexico, Arizona, Colorado, and Utah). The unexpected finding that sera from patients reacted with hantaviral antigens was quickly followed by the genetic identification of a novel hantavirus in patients' tissues and in rodents trapped near patients' homes (13-15).

Prevalence and Clinical Course

Approximately 150,000 to 200,000 cases of HFRS involving hospitalization are reported each year throughout the world, with more than half in China (16). Russia and Korea also report hundreds to thousands of HFRS cases each year. Most remaining cases (hundreds per year) are found in Japan, Finland, Sweden, Bulgaria, Greece, Hungary, France, and the Balkan countries formerly constituting Yugoslavia (16). Depending in part on which hantavirus is responsible for the illness, HFRS can appear as a mild, moderate, or severe disease (Table 2). Death rates range from less than 0.1% for HFRS caused by Puumala (PUU) virus to approximately 5% to 10% for HFRS caused by HTN virus (16). The clinical course of severe HFRS involves five overlapping stages: febrile, hypotensive, oliguric, diuretic, and convalescent; it is not uncommon, however, for one or more of these stages to be inapparent or absent. The onset of the disease is usually sudden with intense headache, backache, fever, and chills. Hemorrhage, if it occurs, is manifested during the febrile phase as a flushing of the face or injection of the conjunctiva and mucous membranes. A petechial rash may also appear, commonly on the palate and axillary skin folds. Sudden and extreme albuminuria, around day 4, is characteristic of severe HFRS. As the febrile stage ends, hypotension can abruptly develop and last for hours or days, during which nausea and vomiting are common. One-third of deaths occur during this phase because of vascular leakage and acute shock. Almost half of all deaths occur during the subsequent (oliguric) phase because of hypervolemia. Patients who survive and progress to the diuretic phase show improved renal function but may still die of shock or pulmonary complications. The final (convalescent) phase can last weeks to months before recovery is complete (3,5,12).

More than 250 cases of HPS have been reported throughout North and South America. Although the disease has many features (e.g., a febrile prodrome, thrombocytopenia, and leukocytosis) in common with HFRS (Table 2), in HPS capillary leakage is localized exclusively in the lungs, rather than in the retroperitoneal space, and the kidneys are largely unaffected. Most of the 174 cases of HPS in the United States and Canada have been caused by Sin Nombre (SN) virus. In HPS, death occurs from shock and cardiac complications, even with adequate tissue

Table 2. Distinguishing clinical characteristics for HFRS and HPS

Disease	Pathogens	Distinguishing Characteristics*
HFRS (moderate-severe) Death rate 1%-15%	HTN, SEO, DOB	hemorrhage +++ azotemia/ proteinuria +++/++++ pulmonary capillary leak +/++ myositis +/+++ conjunctival injection ++/++++ eye pain/myopia ++/++++
HFRS (mild) Death rate <1%	PUU	hemorrhage + azotemia/ proteinuria +/++++ pulmonary capillary leak -/+ myositis + conjunctival injection + eye pain/myopia ++/++++
HPS (prototype) Death rate >40%	SN, NY	hemorrhage + azotemia/ proteinuria + pulmonary capillary leak ++++ myositis - conjunctival injection -/+ eye pain/myopia -
HPS (renal variant) Death rate>40%	BAY, BCC, Andes	hemorrhage + azotemia/ proteinuria ++/+++ pulmonary capillary leak +++/++++ myositis ++/++++ conjunctival injection -/++ eye pain/myopia -

*Minimum/maximum occurrence of the characteristic: - rarely reported; + infrequent or mild manifestation; ++, +++, ++++ more frequent and severe manifestation.

oxygenation. Cases of HPS in the southeastern United States, as well as many in South America, are caused by a newly recognized clade (a group that shares a common ancestor) of viruses that includes Bayou (BAY), Black Creek Canal (BCC), and Andes viruses. As with HFRS, clinical differences can be observed among patients with HPS caused by different hantaviruses. For example, although HPS due to SN virus infection can sometimes be associated with renal insufficiency after prolonged hypoperfusion, renal impairment is only rarely observed early in disease, and chemical evidence of skeletal muscle inflammation (increased serum levels of the muscle enzyme creatine kinase) is rare (17). In contrast, both renal insufficiency and elevated creatine kinase levels are observed at much higher frequency, although not universally, with Andes, BAY, and BCC virus infections (18-20; J. Davis, J. Cortes, and C. Barclay, pers. comm.). In an outbreak of HPS recently described in Paraguay, a novel hantavirus, carried by *Calomys laucha*, was identified as the etiologic agent (21). The relationship of this virus to other HPS-causing hantaviruses remains to be established.

Ecology and Epidemiology

Hantavirus infection is apparently not deleterious to its rodent reservoir host and is associated with a brisk antibody response against the virion envelope and core proteins and chronic, probably lifelong infection. In natural populations, most infections occur through age-dependent horizontal route(s). The highest antibody prevalence is observed in large (mature) animals. A striking male predilection for hantavirus infection is observed in some rodent species such as harvest mice and deer mice, but not in urban rats (*Rattus norvegicus*) (22-24). Horizontal transmission among cage-mates was experimentally demonstrated (25), but vertical transmission from dam to pup is negligible or absent both in wild and experimental settings (22,24,25).

Outbreaks of hantaviral disease have been associated with changes in rodent population densities, which may vary greatly across time, both seasonally and from year to year. Cycles respond to such extrinsic factors as interspecific competition, climatic changes, and predation. Spring and summer outbreaks of HFRS in agricultural settings in Asia and Europe are linked to human contact with field rodents through the planting and harvesting of crops (16,26). PUU outbreaks in Scandinavia and the HPS outbreak in the Four Corners region of the United States were associated with natural rodent population increases, followed by invasion of buildings by rodents (27,28). The ecologic events that led to 1994 and 1996 outbreaks of Andes virus-HPS in Patagonia, a region in southern South America, are being investigated. Human interventions, such as the introduction of Old World plant species (e.g., rosas mosquetas and Scottish brougham) to Patagonia, with associated alteration in rodent population dynamics, have been suggested as possible factors. Recent fires and a mild winter in Argentina's Rio Negro and Chubut Provinces may also have had a positive effect on the carrier rodent, the colilargo, *Oligoryzomys longicaudatus* (M. Christie and O. Pearson, pers. comm.).

Although the aerosol route of infection is undoubtedly the most common means of transmission among rodents and to humans, virus transmission by bite may occur among certain rodents (29) and may also occasionally result in human infection (30) (often inside a closed space, such as a rodent-infested grain silo, garage, or outbuilding used for food storage). Epidemiologic investigations have linked virus exposure to such activities as heavy farm work, threshing, sleeping on the ground, and military exercises. Indoor exposure was linked to invasion of homes by field rodents during cold weather or to nesting of rodents in or near dwellings (16,31). Genetic sequencing of rodent- and patient-associated viruses has been used to pinpoint the precise locations of human infections, which has supported the role of indoor exposure in hantavirus transmission (32,33). Many hantavirus infections have occurred in persons of lower socioeconomic status because poorer housing conditions and agricultural activities favor closer contact between humans and rodents. However, suburbanization, wilderness camping, and other outdoor recreational activities have spread infection to persons of middle and upper incomes.

Nosocomial transmission of hantaviruses has not been documented until very recently (34) and must be regarded as rare. However, viruses have been isolated from blood and urine of HFRS patients, so exposure to bodily fluids of infected persons could result in secondary transmission. Only rarely have multiple North American HPS cases been associated with particular households or buildings. During recent outbreaks of HPS in South America, however, clustering of cases in

households and among personal contacts appeared to be more common (M. Christie, pers. comm.). During a recent outbreak of Andes-virus–associated HPS in Patagonia, a Buenos Aires physician apparently contracted the infection after minimal exposure to infected patient blood (34; D.A. Pirola, pers. comm.). An adolescent patient in Buenos Aires apparently contracted hantavirus infection from her parents, who were infected in Patagonia. This unprecedented observation of apparent person-to-person spread of a hantavirus clearly requires laboratory confirmation, especially by careful comparative analysis of the viral sequences (32,33).

Hantaviruses have also caused several laboratory-associated outbreaks of HFRS. Laboratory-acquired infections were traced to persistently infected rats obtained from breeders (35-37), to wild-caught, naturally infected rodents (38-40), or to experimentally infected rodents (39). No illnesses due to laboratory infections have been reported among workers using cell-culture adapted viruses, although asymptomatic seroconversions have been documented (40).

Hantavirus Distribution and Disease-causing Potential

The worldwide distribution of rodents known to harbor hantaviruses (Table 1) suggests great disease-causing potential. Each hantavirus appears to have a single predominant natural reservoir. With rare exception, the phylogenetic interrelationships among the viruses and those of their predominant host show remarkable concordance (Figure; 41). These observations suggest that hantaviruses do not adapt readily to new hosts and that they are closely adapted for success in their host, possibly because of thousands of years of coexistence. As many as three hantaviruses can be found in a particular geographic site, each circulating in its own rodent reservoir, with no apparent evolutionary influence on one another (42).

All known hantaviruses, except Thottapalayam (TPM) virus, have been isolated or detected in murid rodents. Because only one isolate of TPM virus was made from a shrew (Order Insectivora), it is not clear if *Suncus* is the true primary reservoir or an example of a "spillover" host, i.e., a secondary host infected through contact with the primary host. Spillover is common in sympatric murid rodents, including those identified as the predominant carrier of another hantavirus; thus, the opportunity for genetic exchange among hantaviruses is present in nature. Spillover hosts are believed to have little or no impact on hantaviral distribution or associated disease. However, rodents other than the primary reservoirs can play an important carrier role. For example, *Microtus rossiaemeridionalis* may play a role in maintenance of Tula virus in some settings (43), and *Peromyscus leucopus* and *Peromyscus boylii* can be important reservoirs for SN virus in the western United States (T. Yates and B. Hjelle, unpub. data). Apparent spillover may also be the result of laboratory errors such as polymerase chain reaction (PCR) contamination or misidentification of rodent species. However, spillover is probably underappreciated in many studies that rely on reverse transcriptasePCR for identifying specific viruses because many primer pairs may not detect an unexpected spillover virus. In either case, because mistaken identities and cell culture contaminations with other hantaviruses have occasionally been reported, investigators should verify unusual findings to prevent further confusion.

Antigenic and Genetic Diversity among Hantaviruses

Hantaviruses have been characterized by a combination of antigenic and genetic methods. For viruses propagated in cell culture, the plaque-reduction neutralization test is the most sensitive serologic assay for differentiation (44,45); nine hantaviruses have been defined by this test: HTN, Seoul (SEO), PUU, Prospect Hill, Dobrava-Belgrade (DOB), Thailand, TPM, SN, and BCC viruses (44-48). Genetic relationships among hantaviruses are mirrored in their antigenic properties. A direct correlation between genetic and antigenic relationships is difficult; however, it can be assumed that the plaque-reduction neutralization test measures differences in the M segment gene products, i.e., the G1 and G2 envelope glycoproteins. Comparing the deduced G1 and G2 amino acid sequences, therefore, may provide clues to the antigenic as well as genetic diversity among hantaviruses.

Of characterized hantavirus isolates, SEO virus is the most genetically homogeneous. Isolates of SEO virus, regardless of their geographic origin, display M segment nucleotide and deduced amino acid sequence homologies of approximately 95%, and 99%, respectively (41,47). A reported exception, the R22 isolate from China, had a slightly lower homology;

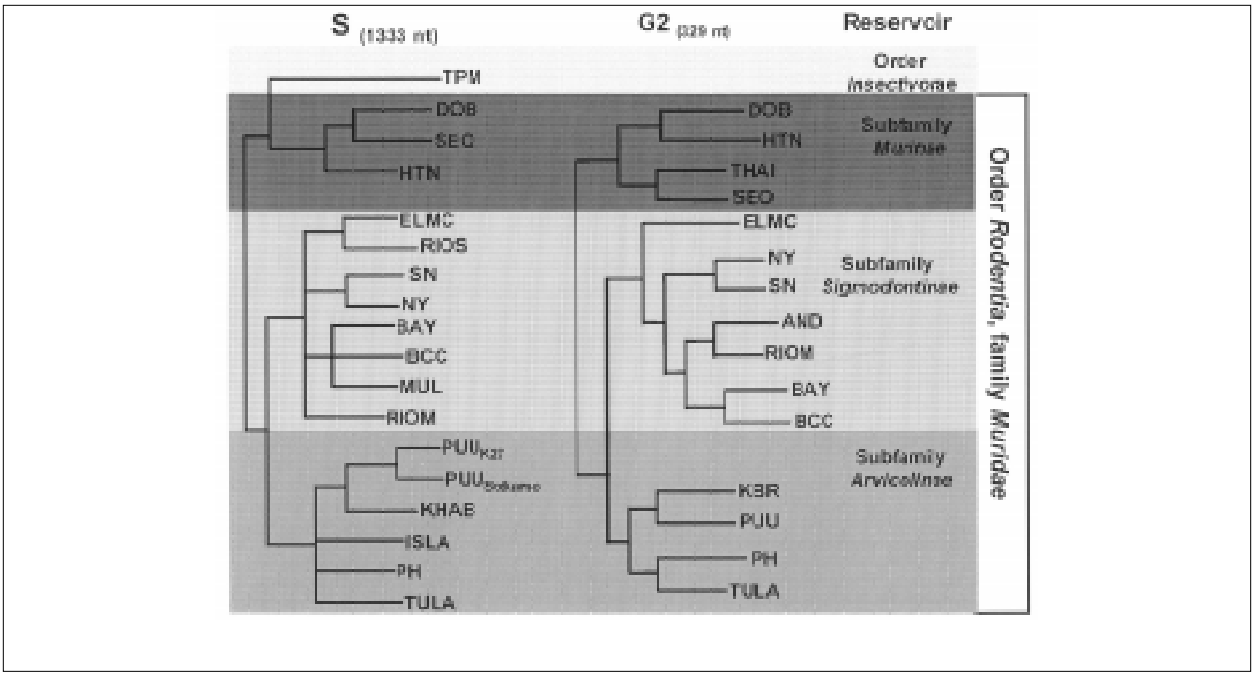

Figure. Phylogeny of hantaviruses and their relationships to natural reservoirs. The trees were constructed by comparing the complete coding regions of the S segments of hantaviruses or of 330 nucleotides corresponding to those of the M segment of Hantaan virus (strain 76118) from nucleotides 1987 to 2315. Abrreviations for viruses are as in Table 1. For each analysis, a single most parsimonious tree was derived by using PAUP 3.1.1 software. For the S segment tree, boostrap values resulting from 100 replications were all greater than 87% except for the branch leading to BCC (78%) and the branch leading to DOB (52%). The next most common placing of DOB was on a branch with HTN.

however, the original data suggest that an error in the nucleotide sequence, resulting in a frame shift reading error, may account for almost all of the additional changes. PUU virus isolates vary the most, with M segment nucleotide and amino acid sequence homologies of 83% and 94% observed between a Finnish and Russian isolate. HTN virus also appears to be quite stable in nature. Comparing the M segment sequences of prototype HTN virus (from *Apodemus*) and those of two human isolates obtained at a 6-year interval from HFRS patients in Korea produced nucleotide and deduced amino acid sequence homologies of 94% and 97%, respectively (48). For SN virus, comparing the complete M or S segment sequences of three strains from California or New Mexico produced homologies of 87% to 99%. Partial nucleotide sequence comparisons of the M or S segments of SN viruses from adjacent counties in California, detected in deer mice captured 19 years apart, were 97.5% homologous (49). Similarly, a retrospective analysis of archived tissue samples collected in Mono County, California, in 1983 showed viruses with partial M and S segment nucleotide sequence homologies of about 87% with SN from an 1993 HPS patient from New Mexico (50). In all cases, the amino acid sequences encoded by these genes differed between cognate proteins by much less than 5%. These values are similar to those observed among strains of HTN virus. Studies have just begun to appear in which the nature of quasispecies in natural rodent hosts is defined (43,51). Such investigations should provide more definitive data concerning the genetic diversity among hantaviruses in nature.

Evolution of Hantaviruses

Phylogenetic trees derived by comparing complete or partial S (Figure), M, or L segment nucleotide sequences (41,52,53) show two major lineages of hantaviruses, one leading to HTN, SEO, Thailand, and DOB viruses, and one leading to PUU, Prospect Hill, SN, and other New World hantaviruses. TPM virus, the first hantavirus isolated in cell culture (54), may be the most antigenically and genetically disparate member of the genus; however, comparison of the complete nucleotide sequence of the TPM S segment (A. Toney, B. Meyer, C. Schmaljohn, unpub. data) suggests that TPM virus is more closely related to HTN, SEO, and DOB viruses

than to any of the other viruses in the genus (Figure). Nucleotide sequence homologies of the M and S segments of any two hantaviruses have approximately the same degree of divergence between each of the three segments, which suggests similar evolutionary rates for these two gene segments. A slightly higher homology among L segments sequenced to date perhaps indicates a greater need for conservation of either RNA or protein functions (47). Point mutations appear to account for most of the genetic drift among hantaviruses. Recombination has not been reported for hantaviruses, although segment reassortment within a particular species appears common (52,55). The exchange of gene segments is suggested to be nonrandom, with a higher propensity for M segment swapping, than for S or L (55). Whether it contributes to the pathogenesis of hantaviruses is not known, but reassortment certainly provides an avenue for more rapid accumulation of changes than could occur by point mutation. There is no evidence that reassortment can occur between different species of hantaviruses; however, this has not been studied systematically.

Murid rodents have probably harbored inapparent hantavirus infections for thousands, perhaps millions of years. It is likely that the genus *Hantavirus* evolved in the Old World and that viruses were carried by rodents across the Bering land bridge when they migrated during the Oligocene, and into South America in the Pliocene (71). Humans are incidental hosts, the victims of spillover infections from the natural host rodents. One of the two major forms of hanta-viral diseases is endemic in each hemisphere. Both HFRS and HPS can be divided into distinct clinical subtypes, and the viral strain is a key determinant of the severity and nature of the clinical abnormalities. Not covered in this review are clinical studies of HFRS and HPS patients, which suggest that pathogenesis may be immunologic and may be mediated by cytokine responses (72). New outbreaks with novel hantavirus strains are still being uncovered, especially in South America. However, the largest clinical caseload and largest number of deaths occur in Asia and Europe.

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Japanese Spotted Fever: Report of 31 Cases and Review of the Literature

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This article is also available in Japanese. The Japanese version can be accessed at http://www.cdc.gov/ncidod/EID/eid.htm

Spotted fever group (SFG) rickettsioses, which are transmitted by ticks, were long thought not to exist in Japan. Three clinical cases of Japanese spotted fever (JSF) were first reported in 1984. The causative agent was isolated and named *Rickettsia japonica*. Through October 1996, 31 cases were diagnosed as JSF in Tokushima Prefecture. Infected patients typically had acute high fever, headache, and characteristic exanthema; eschar was observed in 90%. After the discovery of JSF, more than a hundred cases were reported in southwestern and central Japan. Recent surveys show ticks to be the most probable vectors. As an emerging infectious disease, JSF is not commonly recognized by clinicians; therefore, even though it has not caused fatal cases, it merits careful monitoring.

The spotted fever group (SFG) rickettsioses, which are transmitted by ticks, have a worldwide distribution. Japanese spotted fever (JSF) is one of the newcomers of this group (1); the first clinical cases were reported in 1984 (2). The causative agent was isolated and named *Rickettsia japonica* (3). Because outbreaks were sporadic and limited, clinical reports concerning JSF, especially from specialists in dermatology and physiology and from general practitioners, were scarce. JSF was first found in Tokushima Prefecture, on the island of Shikoku in southwestern Japan; Tsutsugamushi disease, an important rickettsiosis in Japan, was found there soon afterwards (4). Through October 1996, 31 clinical cases of JSF and 11 cases of Tsutsugamushi disease were diagnosed at Mahara Hospital, Tokushima Prefecture. During the same period, 45 cases of human tick bites were recorded in this JSF-endemic area in the same hospital. This article describes JSF's history, clinical characteristics, and differences from Tsutsugamushi disease and summarizes current information about the epidemiology, vectors, and causative agent of JSF.

History

In the 1980s, clinicians believed that Tsutsugamushi disease (scrub typhus) was the only rickettsial disease in Japan except for sporadic outbreaks of epidemic typhus in the 1950s. In Tokushima Prefecture, neither disease has been reported in the last two decades. In May 1984, a 63-year-old woman (the wife of a farmer) was hospitalized at Mahara Hospital with high fever and erythematous nonpruritic skin eruptions over the entire body. Antibiotics (ß-lactam and aminoglycoside) used for common febrile infections were not effective, but the patient gradually became afebrile in 2 weeks without effective treatment. In May and July 1984, two additional patients with similar symptoms were treated at the same hospital. Doxycycline was markedly effective in these cases. Before the onset of illness, the patients had collected shoots from bamboo plantations on the same mountain. In two of the patients, an eschar was observed. Tsutsugamushi disease was suspected. However, results of Weil-Felix tests showed positive OX2 serum agglutinins, and OXK were negative in all three cases. These results did not indicate Tsutsugamushi disease, but rather OX2-positive infections, i.e., SFG rickettsioses (1).

The cases were subsequently confirmed by complement fixation test with antigens of SFG rickettsiae (5,6). The name Japanese spotted fever was proposed for these infections (7) and has been commonly used since then (8-10). Oriental spotted fever (11) is a synonym for JSF. The causative agent was isolated in 1986 (12) and named *R. japonica* (3).

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Clinical Features

The clinical features of the 31 patients whose illness was diagnosed as JSF at Mahara Hospital from 1984 to October 1996 were analyzed. The disease developed abruptly, with the common symptoms of headache (25 [80%] of 31 patients), fever (31 [100%] of patients), and shaking chills (27 [87%]). Other major objective symptoms of JSF included skin eruptions (31 [100%]) and tick bite eschars (28 [90%]). Most patients (28 [90%]) complained of malaise; joint and muscle pain or numbness of the extremities was rarely mentioned. In the acute stage, remittent fever accompanied by shaking chills was frequently observed. In severe cases, high fever (40°C or more) continued for several days (Figure 1). The maximum body temperature was 38.5°C to 40.8°C (mean 39.5°C), which was higher than that seen in patients with Tsutsugamushi disease (38.5°C ~ 39.1°C). With abrupt high fever or, a few days after onset, fever of unknown origin, the characteristic erythemas developed on the extremities and spread rapidly (in a few hours) to all parts of the body including palms and soles, without accompanying pain or itching. These eruptions were the size of a grain of rice or soybean, and the margin of each of the spots was unclear (Figure 2). The erythemas became remarkable during the febrile period and tended to spread more over the extremities than the trunk. Palmar erythema, a characteristic finding not seen in Tsutsugamushi disease, disappeared in the early stage of the disease. The erythemas became petechial after 3 to 4 days, peaked in a week or 10 days, and disappeared in 2 weeks. However, in severe petechial cases, the brown pigmentation remained for 2 months or more. Eschar was observed on the hands, feet, neck, trunk, and shoulders of patients (Figure 3). This eschar generally remained for 1 to 2 weeks, but in some cases it disappeared in a few days. Eschars in JSF patients are smaller than those seen in patients with Tsutsugamushi disease and may be missed without careful observation. Regional or generalized lymphadenopathy, which is observed in almost all cases of Tsutsugamushi disease, was not remarkable in JSF patients. Swelling of the liver and spleen was observed in a few patients. One patient had cardiomegaly (5), and in another area, a patient had central nervous system involvement (13).

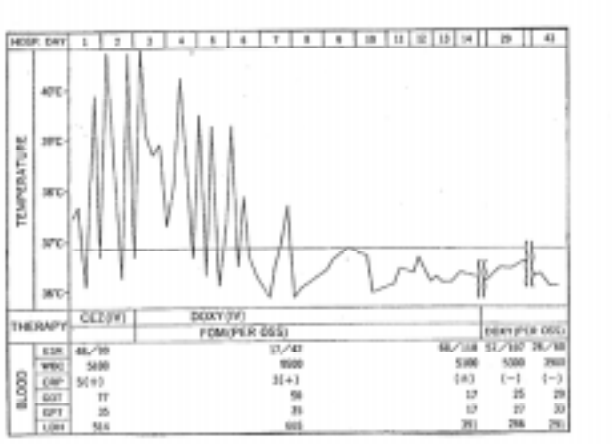

Figure 1. Fever and clinical course, 62-year-old woman.

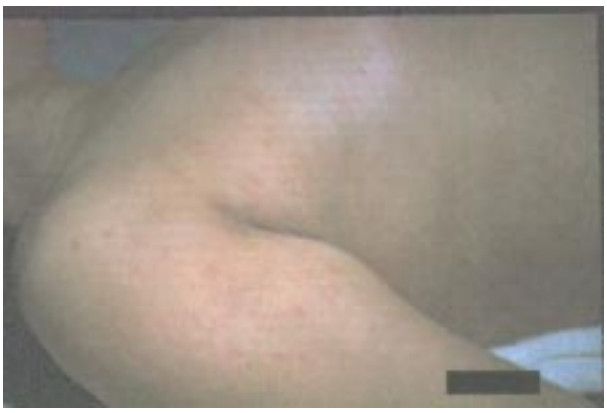

Figure 2. Skin eruptions, hospital day 3.

Laboratory Examinations

The results of laboratory examinations of JSF patients are almost the same as those of patients with common SFG rickettsioses. During clinical examinations at the initial stage of the disease, urinalyses registered a slight positive reading for protein and occult blood, which may lead to a misdiagnosis of urinary infection. In the acute stage, leukocytosis may also be found with leukopenia (3,600~12,800), and a left shift in leukocyte count was observed. Thrombocytopenia (6.8~35.3) may also be found. In week 1 to 2, leukocyte counts increased slightly, and lymphocyte counts tended to increase. Among biochemical examinations, C-reactive proteins were strongly positive, and liver functions were slightly impaired but returned to normal in 2 to 3 weeks.

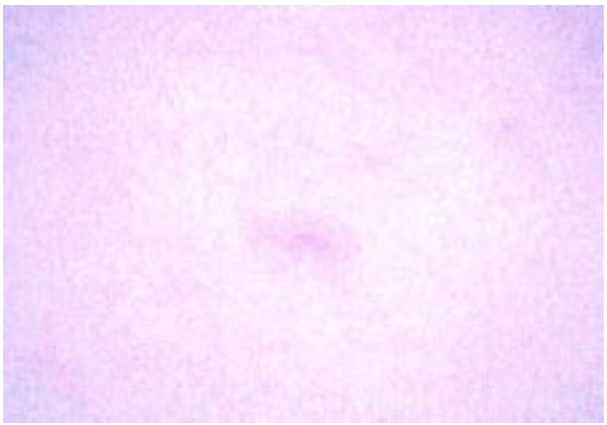

Figure 3. Small and shallow eschar on admission, which disappeared in a few days.

Serologic Results

Serodiagnosis for JSF is usually performed by the indirect immunoperoxidase (IP) or immunofluorescence (IF) techniques, with antigens prepared from *R. japonica* or other SFG rickettsiae. With the IP test, IgG and IgM antibodies were detected in the sera beginning on day 9 after the onset of fever; titers of IgG antibodies were higher than those of IgM antibodies (14). The IF test had similar results (15). In the 31 clinically diagnosed cases of JSF in Tokushima Prefecture at Mahara Hospital, all patients had significant changes in serum IP antibody titers to *R. japonica*, and 27 (87%) had significant changes in OX2 agglutinin titers by the Weil-Felix technique (10,14; F. Mahara, unpub. data).

Treatment

Antibiotics such as penicillins, ß-lactams, or aminoglycosides, commonly used in the empiric treatment of febrile disease, were completely ineffective, but doxycycline and minocycline were markedly effective in treating the JSF patients (16).

On the first day of hospitalization in one patient with severe disease (Figure 1), fever of more than 40°C with shaking chills persisted after the antibiotics cefazolin and fosfomycin were administered. The general condition of the patient worsened and on the morning of the third day, generalized edema and confusion developed. On day 3, the patient was given drip infusion of doxycycline 300 mg per day, which was dramatically effective; the fever decreased to 38°C during the first drip infusion.

In an in vitro study, minocycline was the most effective antibiotic against *R. japonica*, followed by other tetracycline antibiotics (17,18). In contrast, the sensitivity to ß-lactam and penicillin was lower or negligible, but quinolones were effective against the JSF agent (18). Three patients were treated with a new quinolone (tosufloxacin 300 mg per day in three divided doses per os), which proved effective in two cases (9). Patients with dehydration received drip infusion of doxycycline or minocycline (200 mg to 300 mg per day for 3 to 7 days), and after becoming afebrile, received 200 mg per day 2 divided doses to prevent relapse. Patients suspected of having JSF should receive empiric therapy with minocycline or doxycycline without waiting for serologic confirmation of illness.

Epidemiology

From 1984 to 1995, 144 cases of JSF were reported by the National Institutes of Health in Japan (19; Figure 4). Case reports included only the number of cases and prefectures where cases were reported, including Tokushima Prefecture. According to this information, JSF-endemic prefectures are located along the coast of southwestern and central Japan in a warm climate (Figure 5). The landscape is diverse, including bamboo plantations, crop fields, coastal hills, and forests (20). Of the 31 JSF patients in Tokushima Prefecture, nine were male, and 22 were female. Ages were 4 to 78 years, but most patients (68%) were 60 to 70 years old. The onset of the disease was 2 to 8 days after work in the fields. In this prefecture, farmers work in the forest to gather bamboo shoots in the spring and chestnuts in the autumn; cases occurred from April to October

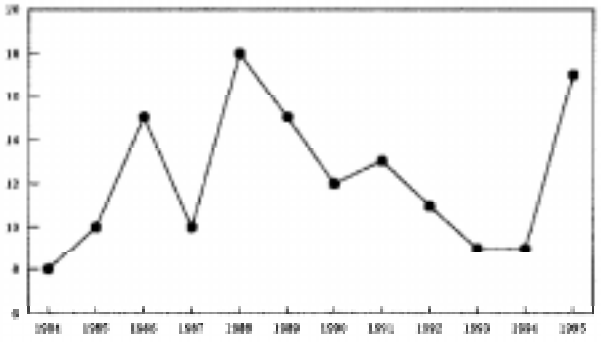

Figure 4. The number of Japanese spotted fever patients in Japan (1984–1995).

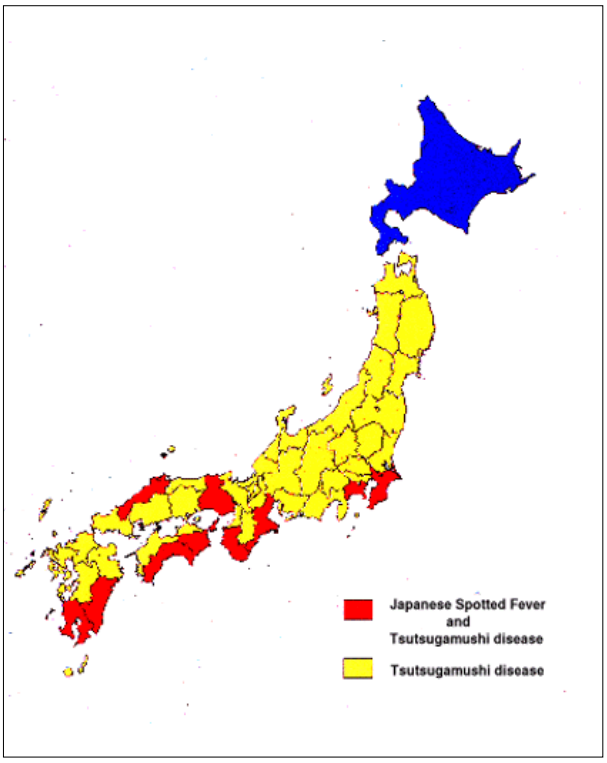

Figure 5. Geographic distributions of JSF and Tsutsugamushi disease in Japan.

(6,18; Figure 6). In this area, we can distinguish seasonal differences in the occurrence of JSF and Tsutsugamushi disease; whereas JSF occurs from spring to autumn, Tsutsugamushi disease occurs in winter (November–February). Although seasonal differences occur in the western parts of Japan, the prevalent seasons for Tsutsugamushi disease vary in other parts of Japan (21).

Like other SFG rickettsioses, JSF is presumed to be transmitted by a tick bite. The high proportion of patients with tick bite eschars supports this hypothesis. Thirteen JSF patients (28.9%) recalled tick bites before the onset of illness; however, the ticks had been lost, and no specimens from the patients were available for further study. From 1984 to October 1996, 45 persons with tick bites were recorded at Mahara Hospital (Table 1).Identified ticks included three genera and eight species.

Three genera and six species of ticks have been reported as positive for *R. japonica* in JSF-endemic areas (Table 2). Hemolymph samples from *Dermacentor taiwanensis, Haemaphysalis flava, Haemaphysalis formosensis, Haemaphysalis hystricis, Haemaphysalis longicornis,* and *Ixodes ovatus* were positive when tested by the IP technique using a species-specific monoclonal antibody against *R. japonica* (22). *R. japonica* was also detected by IF in the hemolymph of *H. longicornis* (23). A polymerase chain reaction (PCR) technique using species-specific primers detected *R. japonica* in *H. hystricis* (24), *H. flava,* and *I. ovatus* (25). The agent has also been detected in *H. longicornis* by restriction fragment length polymorphism of PCR product (23). Of these, *H. flava, H. longicornis,* and *I. ovatus* commonly feed on humans in Japan (26). Recent tick surveys in Japan have identified two serotypes or species of SFG rickettsial isolates other than *R. japonica,* which are of uncertain clinical significance (27,28).

Pathogen

The etiologic agent was first isolated from a patient (in Kochi Prefecture) in 1986 (12). In 1987, the causative rickettsia was also isolated from a JSF patient in Tokushima Prefecture (29-31). The former isolate is the type strain YH

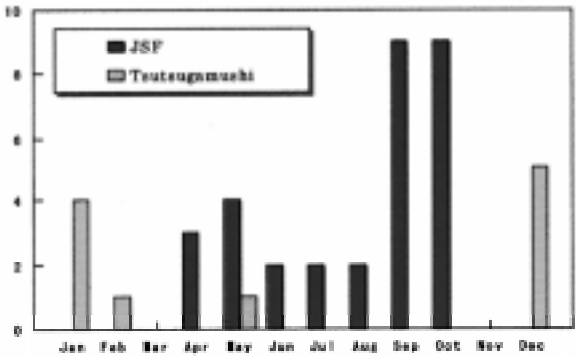

Figure 6. Seasonal prevalence of Japanese spotted fever and Tsutsugamushi disease.

Table 1. Cases of human tick bites, Mahara Hospital, 1984–1996

	Female	Male	Nymph	Larva	Total
Haemaphysalis flava	1		4		5
Haemaphysalis longicornis	8	1	3	2	14
Amblyomma testdunarium	4	1	12		17
Ixodes ovatus	1				1
Ixodes nipponensis	4				4
Ixodes persulcatus			2		2
Ixodes tanuki	1				1
Haemaphysalis kitaokai	1				1
Totals	20	2	21	2	45

Table 2. Reported tick species positive for *Rickettsia japonica* and their prevalence in endemic disease areas

	No. positive/no. examined		
Tick species	Tokushima	Kochi	Kanagawa
Dermacentor taiwanensis	3/5[a], 1/1[b]	-	-
Haemaphysalis flava	6/36[a]	3/10[a]	1-3/90[c]
Haemaphysalis formosensis	-	10/16[a]	-
Haemaphysalis hystricis	+[d]	5/9[a]	-
Haemaphysalis longicornis	-	1/1[a], 6/9[e], 5-33/33[f]	-
Ixodes ovatus	1/13[a]	-	1/16[c]

[a]Hemolymph test by immunoperoxidase stain using *R. japonica*-specific monoclonal antibody (22).
[b]Isolation (28).
[c]Polymerase chain reaction (PCR) using the primers designed for amplifying the genomic DNA from only *R. japonica* (25).
[d]PCR method same as the above (24). Number was not shown.
[e]Hemolymph test by immunoflurescein stain using *R. japonica*-specific monoclonal antibody (23).
[f]Restriction fragment length polymorphism of PCR product (23).

(ATCC VR-1363), later named *R. japonica,* a new SFG rickettsia; the latter strain (Katayama) was the first isolate from JSF-endemic areas outside Kochi (3,32). The Katayama strain type and *R. japonica* were demonstrated by serologic analysis using monoclonal antibodies (33). Sero-typing by use of the reciprocal cross-reactions of mouse antisera to six human isolates from Toku-shima and the type strain YH or *R. japonica* also indicated that these are the same species (34). In 1988, another strain was isolated from a patient in Awaji Island, Hyogo Prefecture, which is considered a new area of JSF-endemic disease (35).

Recently, an isolate from a febrile patient in Wakayama Prefecture was also reported as *R. japonica* (36). In an electron microscopy study, *R. japonica* were generally recognized as short rods or pleomorphic coccobacillary forms less than 2 mm in length and 0.5 mm in diameter and could be found not only in the cytoplasms but also in the nuclei of the host cells (37). A multilayered mesosome-like structure was observed in the rickettsiae multiplying in a host cell (38). This unique structure has not been reported in other species except in *Rickettsia prowazekii* (39). After the initial isolation, at least 20 rickettsial strains have been isolated from JSF patients by cell culture techniques or nude mouse passage in Tokushima, Kochi, Hyogo, Chiba, and Wakayama Prefectures. However, it has not been determined if their strains differ in virulence.

Ten diseases caused by SFG rickettsiae have been reported in humans (40): Rocky Mountain spotted fever, Mediterranean spotted fever, Siberian tick typhus, African tick bite fever, Queensland tick typhus, Japanese spotted fever, Israeli spotted fever, Astrakhan spotted fever, Flinders Island spotted fever, and rickettsialpox. The clinical symptoms of JSF—a triad of high fever, skin eruptions, and tick bite eschar—are similar to those of typical SFG rickettsioses. With regard to skin eruptions, eschar, and severity of the disease, JSF is more akin to Mediterranean spotted fever and Siberian tick typhus than to Rocky Mountain spotted fever. Recent tick surveys indicated that the most probable vectors of JSF are *H. flava, H. longicornis,* and *I. ovatus.*

In Japan, the clinical features of JSF are similar to those of Tsutsugamushi disease; however, close clinical observation exposes the differences between the two diseases. Widespread outbreaks of Tsutsugamushi disease have been reported repeatedly in recent years (21). No fatal cases of JSF have been reported. However, death rates from other SFG rickettsioses (approximately 2.5% for Mediterranean spotted fever [41] and 3% to 7% from Rocky Mountain spotted fever [42]) suggest that unless JSF is treated appropriately, it can pose the same risk. If JSF is suspected, empiric treatment should begin without delay during the early stages of disease.

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Polycystic Kidney Disease: An Unrecognized Emerging Infectious Disease?

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Polycystic kidney disease (PKD) is one of the most common genetic diseases in humans. We contend that it may be an emerging infectious disease and/or microbial toxicosis in a vulnerable human subpopulation. Use of a differential activation protocol for the Limulus amebocyte lysate (LAL) assay showed bacterial endotoxin and fungal (1→3)-β-D-glucans in cyst fluids from human kidneys with PKD. Fatty acid analysis of cyst fluid confirmed the presence of 3-hydroxy fatty acids characteristic of endotoxin. Tissue and cyst fluid from three PKD patients were examined for fungal components. Serologic tests showed *Fusarium*, *Aspergillus,* and *Candida* antigens. IgE, but not IgG, reactive with *Fusarium* and *Candida* were also detected in cyst fluid. Fungal DNA was detected in kidney tissue and cyst fluid from these three PKD patients, but not in healthy human kidney tissue. We examine the intertwined nature of the actions of endotoxin and fungal components, sphingolipid biology in PKD, the structure of PKD gene products, infections, and integrity of gut function to establish a mechanistic hypothesis for microbial provocation of human cystic disease. Proof of this hypothesis will require identification of the microbes and microbial components involved and multifaceted studies of PKD cell biology.

Examining the hypothesis that polycystic kidney disease (PKD) is an emerging infectious disease and/or microbial toxicosis in a vulnerable population of humans must begin with a review of the conceptual tools that relate disease etiology and progression to the identification of microbes, their cellular components, and shed toxins in affected persons (1). An emerging infectious disease can be defined, in part, as an existing disease for which microbes are newly recognized as a causative factor and/or as a factor contributing to disease progression. The microbe may be 1) present at the site of a lesion, 2) disseminated throughout the body, or 3) localized to an anatomic site separate from the primary lesions. In this third case, one or more toxins released by the microbe into distribution fluids, usually blood, produce the pathologic effect(s); this process resembles microbial toxicosis, where the patient is exposed only to the toxin(s) present in the diet, environment, and gut microflora. Distinguishing between an infectious disease and microbial toxicosis is essential if the source of the toxin is to be removed or reduced. Concurrent infection and microbial toxicosis (e.g., endotoxicosis, mycotoxicosis) can also occur. Although mycotoxicosis is commonly understood to refer to the absorption of small organic fungal toxins, such as aflatoxin, fumonisin, or trichothecene, detection of toxin in body fluids and tissues is often difficult. Exposure to toxin may be episodic. For many known and newly discovered microbial toxins, analytical methods pertinent to the levels of toxins involved in acute and chronic disease, especially in vulnerable subpopulations, need to be developed and verified in human tissues and fluid. We have taken the view that the presence of

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signature components of microbial genera/species that produce toxins may indicate that all components of the microbe, including its shed toxins, are also present. As a corollary, absorption of the detectable microbial components, which are often larger in molecular size than the classical mycotoxins and endotoxins, should presume the absorption of other components and toxins from this organism.

On its face, PKD would not appear to be an emerging infectious disease. Classically viewed as an inherited disease that follows Mendelian genetics, PKD in its autosomal dominant form affects 600,000 people in the United States at a rate of 1 in 400-1,000 persons; the autosomal recessive form occurs in 1 per 44,000 births. An acquired form of PKD occurs in patients on renal dialysis. Animal models of inherited and chemically induced PKD have been described (2-4). The current consensus is that fundamental anomalies in cell differentiation or maturation explain the array of anomalies in cystic renal epithelium. Although cysts can sometimes be detected in utero, loss of kidney function to the point of end-stage renal disease occurs by the sixth decade in 50% of autosomal-dominant PKD patients. Despite substantial progress in molecular genetics studies of PKD, the role of defective gene products in its causation and progression to end-stage renal disease has remained elusive (3,5,6). Indeed, additional factors have been proposed as being necessary for progression of PKD to end-stage renal disease. PKD patients have higher rates of renal and urinary tract infections and higher rates of illness and death from infection in general than healthy persons (7,8). Since these infections in otherwise-healthy-persons do not lead to PKD, PKD patients could be exhibiting heightened vulnerability to infection and sensitivity to microbial cell components and toxins. How such sensitivity and vulnerability might occur is unknown. The disease does not appear to involve an immunocompromised state. Thus, alternative possibilities need to be considered, such as altered colonization of the uroepithelium and altered bowel structure and colonization.

Microbes and Microbial Components

Werder et al. (9) performed perhaps the critical experiment in establishing a pathogenic role for microbes in PKD. Using genetically cystic mice, they found that mice grown under germ-free conditions survived substantially longer than cystic littermates grown under ambient conditions. Gardner et al. (10,11) extended these observations when they reported that rats fed a chemical cystogen also had lower rates of renal cystogenesis when raised under germ-free conditions; moreover, coadministering the ubiquitous cell wall component of gram-negative bacteria, lipopolysaccharides (LPS), and the chemical cystogen produced higher rates of cyst formation than using cystogen alone. Thus, LPS was considered a provocateur of the underlying genetic (or chemically induced) susceptibility to renal cystogenesis. From this line of research has emerged the possibility that LPS, either alone or in combination with other microbial or environmental factors, promote disease progression, and removal of such provocateurs would slow the progression of PKD to end-state renal disease.

Endotoxin is the mixture of LPS and other bacterial cell wall proteins and constituents that is found in bacterial infections (12). LPS is isolated by solvent extraction of bacterial cell walls; its chemical purity may vary across preparations. The portion of LPS responsible for most of its known biological activities is Lipid A; the carbohydrate side chains also elicit responses in humans (13). The chemical composition of LPS depends on the genera and species of bacteria from which it is isolated (13,14). As knowledge of the structure and biological activities of LPS has increased and merged with the infectious disease vocabulary based on endotoxin, these terms are sometimes used interchangeably. For example, the highly purified LPS used as positive reference material in the Limulus amebocyte lysate (LAL) assay is called Control Standard Endotoxin. Even though interchanging the terms LPS and endotoxin may promote appreciation of microbial involvement in disease, we prefer the term endotoxin in discussing disease because it respects the structural and pharmacologic heterogeneity of LPS and mixtures of other microbial components present in vivo.

When Miller et al. (15) measured putative endotoxin levels by the classic LAL assay in culture-negative urine samples of male PKD patients with no clinically apparent infection, 80% contained detectable LAL-positive material, while the urine samples of healthy male volunteers did not. In cyst fluid from PKD patients, Gardner et al. (16) reported LAL-positive material and cytokines typically induced by LPS. The origins of the renal and urinary endotoxin were not known; possible sources include the gut

microflora, occult urogenital infections, cryptic colonizations, and abnormal handling by the liver of the normal daily load of absorbed endotoxin. Enhanced exposure to endotoxin in the PKD patient is important since endotoxin plays a fundamental role in human health and disease. In low doses, endotoxin enhances the immune system, but in larger amounts it is lethal. In combination with drugs and other substances, it can cause tissue damage and severe disease; examples of endotoxin synergy with other substances resulting in disease include alcohol in cirrhosis, aspirin in Reyes syndrome, and trichothecene T-2 toxin (a mycotoxin from *Fusarium*) in gastrointestinal tract damage (17). Endotoxin can also promote the translocation of bacteria from the gut into the blood (18). In susceptible persons, chronic exposure to endotoxin has been linked to rheumatoid arthritis (19); tentative links with atherosclerosis have also been reported (20). Although suggestive of endotoxin in PKD, the LAL methods used above were not specific for endotoxin, since they were susceptible to false positive and/or negative results.

Bacterial Endotoxin in Human PKD Renal Cysts

In this report, we describe our recent findings concerning 1) endotoxin levels recorded after recent improvements in LAL methods, 2) analysis of fatty acids specific for bacterial endotoxin, and 3) the presence of other microbial components in human cyst fluid. The LAL assay (Figure 1) is the classic method used to detect the low levels of endotoxin in biologic fluids and pharmaceuticals.

Both endotoxin (in eight of eight patients) and (1→3)-β-D-glucans (in two of eight patients) were detected when methods based on differential activation of the two Limulus pathways were applied to cyst fluids from six autosomal-dominant PKD patients, one autosomal-recessive PKD patient, and a patient with simple cysts, where 200 ml from a single simple cyst were recovered (Table 1). None of these patients exhibited clinical evidence of infection, had been on hemodialysis, or received human immunologic products before nephrectomy, which could have accounted for ß-D-glucan reactivity (26). For six patients (four autosomal dominant, one autosomal recessive, and one with simple cysts), only endotoxin was detected in cyst fluids; gel clot end points for both the LAL and LAL+laminarin assays were equal

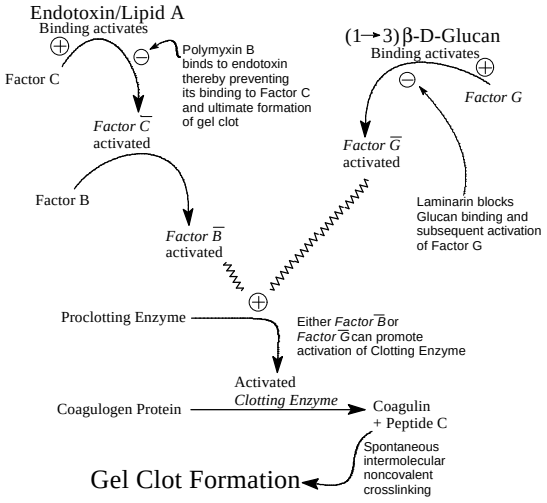

Figure 1. Cascade of biochemical interactions and reactions leading to gel clot formation in the Limulus amebocyte lysate assay. Endotoxin binding to Factor C via the Lipid A moiety results in its activation and in turn the sequential activation of Factor B, which results in the subsequent activation of the proclotting enzyme. Binding of polymyxin B to endotoxin blocks Limulus reactivity in those samples where endotoxin is the initiating molecule. In contrast, (1→3)-β-D-glucans bind to a different component, Factor G, in the Limulus assay reagent, thereby leading to its activation and subsequent cascade of reactions to gel formation. The addition of laminarin, an inhibitor of glucan binding to Factor G, to Limulus assay reagent blocks reactivity of samples containing glucan (21).

Lipid A binds to and activates Factor C resulting in a cascade of enzymatic reactions leading to gel clot formation, the positive endpoint (22). Also present in the classic LAL reagents is Factor G, which is responsible for a side cascade (i.e., the alternative pathway). Factor G is stimulated by (1→3)-ß-D-glucans, associated primarily with fungal cell walls (23). Addition of laminarin, an inhibitor of glucan binding to Factor G (24), permits a more specific measurement of endotoxin-induced gel clot formation and provides a means of screening for (1→3)-β-D-glucans in biological samples, when used in a differential activation protocol with other inhibitors and activators. As two internal controls, polymyxin B can block the LAL reactivity of endotoxin (25), and known quantities of Control Standard Endotoxin can be added to duplicate samples to detect inhibition of the assay.

for each fluid tested, indicating that the β-D-glucan stimulated pathway was not activated. When exposed to polymyxin B, LAL-positive cyst fluids became negative. This is consistent with the conformational changes that occur when this antibiotic binds to Lipid A to form a stoichiometric

Table 1. Limulus assay results from human kidney cyst fluids

Donor No.	Patient data	No. Cysts Tested	Cysts ET positive (%): Endotoxin units/ml[a]	Cysts ß-D-glucan positive (%): pg/ml
1	AD[b] 45 yr F	5	3 (60%): 0.48 EU/ml	nd[c]
2	AD 52 yr F	6	4 (67%): 1.71 EU/ml	nd
3	AD 48 yr M	L[d] 9	5 (56%): 1.45 EU/ml	nd
		R[e] 11	6 (55%): 2.27 EU/ml	nd
4	AD 41 yr F	16	3 (19%): 0.88 EU/ml	3 (19%): 120 pg/ml
5	AD 37 yr M	11	2 (18%): 3.84 EU/ml	6 (55%): 106 pg/ml
6	PD[f] AD 43 yr F	13	4 (31%): 0.26 EU/ml	nd
7	AR[g] 5 wk M	pool(8)	3.84 EU/ml	nd
8	SC[h] 48 yr F	1(200ml)	3.84 EU/ml	nd

[a]Mean EU/ml or pg (1→3)-β-D-glucan/ml
[b]Autosomal-dominant polycystic kidney disease (PKD)
[c]Not detected
[d]Left kidney
[e]Right kidney
[f]Peritoneal dialysis
[g]Autosomal recessive PKD
[h]Simple kidney cyst (not PKD)

Kidneys were from seven patients who had received or were awaiting renal allografts. Cyst fluid was obtained ex vivo from both surface (cortex) and sub-surface (medullary) cysts by aseptic aspiration from seven excised kidneys (post-nephrectomy) of PKD patients; simple cyst fluid was recovered by radiologically guided needle aspiration. All kidneys and cyst fluids were collected under an approved Institutional Review Board protocol. The mean number of cysts aspirated per kidney was 10 (range 1-21); two kidneys were obtained from one of the patients with both being used for studies. Fluid from individual cysts in each kidney were collected separately, except for the autosomal-recessive PKD patient in whom cyst volumes were too small (~ 0.1 ml) for individual testing. Therefore, fluid from eight cysts in close proximity were pooled. All procedures used pyrogen-free materials in the collection, handling, and processing of kidneys and cyst fluids and preparation of commodities and reagents for the Limulus assay. Depyrogenation was carried out at 250°C for 4 hours, which eliminates both endotoxin and (1→3)-ß-D glucans (27). Cyst fluids were stored at -70°C. Assessment parameters of cysts included color and size; for fluids, color, turbidity, pH, mg protein/ml, specific gravity, and blood. A commercial dipstick was used to detect and semiquantitate cyst fluid pH, specific gravity, and blood (Ames 10 SG Multistix, Miles, Inc., Elkhart, IN, USA). Blood sensitivity was 0.015-0.062 mg/dL hemoglobin, equivalent to 5-20 intact red blood cells per microliter. Results were recorded as 1-4+. For specific gravity, determinations permitted ranged from 1.000-1.030; for pH, 5.0-9.0. Protein was by the method of Lowry et al. (28).

The LAL gel clot end point assay (22) was performed according to the manufacturer's instructions (Charles River ENDOSAFE, Charleston, SC). The mechanism of the LAL assay is illustrated in Figure 1. To quantify endotoxin, undiluted samples and serial twofold dilutions through 1:64 were tested. To detect false positives due to activation of the alternate (1→3)-β-DG pathway, LAL assay reagent fortified with the glucan inhibitor laminarin was used (23). Laminarin was courtesy of Dr. J. Cooper, Charles River ENDOSAFE, Charleston, SC. Laminarin had been rendered endotoxin free by treatment with 0.2 N NaOH at 50°-60°C for 6 hours; treatment did not affect activity of laminarin in the LAL assay (23). The standard gel clot end point method was used, except laminarin was added to the LAL assay reagent (23). Minimum sensitivity of both assays was 0.03-0.06 Endotoxin Units (EU)/ml (3-6 pg control standard endotoxin, CSE; 10 EU/ng of EC-5 (*E.coli* O133) U.S. Standard endotoxin; for β-DG, ³10 pg/ml (29).

Known endotoxin-positive (tap water) and -negative (pyrogen-free water) samples were run as controls along with a CSE curve. The endotoxin concentration was estimated by multiplying the maximum dilution producing a positive test against the sensitivity of lysate that was verified daily. The content of β-DG in cyst fluid was estimated by the difference in titers between the endotoxin specific assay (LAL+laminarin) and the conventional LAL assay (30). Portions of all samples were additionally spiked with 0.25 EU CSE and tested for inhibitory substances. If inhibition was observed in the LAL assays, samples were boiled for 5 minutes, cooled to room temperature, and retested. Samples were incubated, in some cases after boiling, for 30 min at room temperature with polymyxin B (5 mg/assay) before LAL testing. Polymyxin B inhibits the LPS-activated pathway via Factor C by its direct binding to LPS, which yields a nonreactive complex (25). An alternative approach was to use 20% dimethyl sulfoxide (DMSO) to inhibit Factor C directly (23); DMSO was not used to permit detection of nonendotoxin, but LAL-reactive materials that were not inactivated by binding to polymyxin B. However, in our system DMSO did completely block the LAL reaction in the presence of CSE.

After centrifugation of the cyst fluid, the LAL-positive material was located only in the supernate for autosomal-recessive- and simple cyst-derived fluids. For autosomal dominant-derived fluids, the reactive material was located in the pellet. Thus, centrifugation of fluids to remove particulate material before the LAL assay may influence results.

LAL assay inhibition was detected in nearly two-thirds of 73 cyst fluids tested, including autosomal recessive and simple cyst fluids. Dilution of cyst fluid through 1:16 was often required to overcome the inhibitory effect in unboiled specimens. Boiling of entire fluids or supernates and pellets for 5 minutes eliminated LAL assay inhibition in virtually all specimens at all dilutions. Exceptions were some undiluted and 1:2 dilutions of chocolate-colored cyst fluids, where inhibition was not eliminated in both test and spiked specimens of autosomal dominant fluids.

complex that is inactive in the assay (25). In contrast, kidney tissue from one male patient (donor 4) and one female patient (donor 5) yielded cyst fluids that contained separately endotoxin or β-D-glucan; however, no single fluid contained both substances (Table 1). Fluids that were LAL positive in the presence or absence of polymyxin B, but negative in the presence of laminarin are consistent with the detection of β-D-glucan. Donor 6 had been on peritoneal dialysis for slightly more than 1 year before nephrectomy. Although cyst fluids were all initially LAL negative, when the incubation time of the LAL assay was increased from 1 to 2 hours, 4 of 13 cyst fluids were endotoxin positive, albeit at much lower concentrations than the fluids from patients not on dialysis. None of the cyst fluids from donor 6 were positive for β-D-glucan after longer incubations. Longer incubation times might also have produced LAL-positive material in LAL-negative cyst fluids from the other donors; therefore, we view the percentage of LAL-positive cyst fluids for each patient as a minimum value.

The concentrations of endotoxin-specific material in autosomal dominant cyst fluids was 0.12 to 3.84 EU/ml, with substantial levels of endotoxin (3.84 EU/ml) also found in a pool of eight cysts from one autosomal recessive kidney and simple cyst fluid from one donor. The mean concentration of endotoxin across all of the autosomal dominant fluids tested (including those that were LAL negative) was 0.65 EU/ml. Using 10 EU/ng as a conversion factor yields 65 pg endotoxin/ml cyst fluid; normal plasma levels of endotoxin are <4-5 pg/ml (31). Intravenous injection of 5 EU/kg body weight (350 EU/70 kg) can induce shock in the average adult male (21). We estimate that a single cystic kidney in an adult male (3-5 kg kidney weight of which 33% is cyst fluid) contains 648 to 1,080 EU/kidney or about two lethal doses of endotoxin per kidney. Unexplained fever and flank pain in PKD patients have been attributed to release of IL-1 from rupture or hemorrhage of cysts (27). On the bases of the high levels of endotoxin observed in this study and the high sensitivity of humans to endotoxin, we propose that the release of endotoxin from the cyst into the peritoneum or blood may be an important initiator of a cascade of biologic events after leakage or rupture of renal cysts.

For one donor (donor 3) both kidneys were available for cyst fluid analysis (nine left and 11 right kidney cysts, respectively). When the frequency of LAL-positive fluids (endotoxin) was compared, no significant difference was found (Table 1). However, a threefold difference in the frequency of inhibitor detection was observed in left kidney cyst fluids compared with the right kidney (90% vs. 31%, respectively). Thus, LAL testing of fluids for endotoxin requires vigilance for both false negative and positive materials.

Signature Bacterial Fatty Acids

If endotoxin is present in renal cysts, fatty acids unique to endotoxin can be used to confirm its presence. The Lipid A region of LPS contains on average four 3-hydroxy (3-OH) acyl groups of various chain lengths (13,14). Acid hydrolysis of LPS releases the 3-OH fatty acids and other fatty acids. Diverse bacterial genera exhibit characteristic ratios of fatty acids of various chain lengths and patterns of hydroxylation; compilations of such signature fatty acid profiles are available for many genera (14). Initially, aliquots of five cyst fluids were analyzed in a single blind manner by gas chromatography-mass spectrometry as described by Mayberry and Lane (32). 3-Hydroxy (3-OH) fatty acids of *n*C:12:0 and *n*C:14:0 carbon length were detected but not quantified in the three cyst fluids that were positive in the endotoxin specific LAL assay; they were not detected in the two cyst fluids that were LAL negative. Thus, complete concordance of LAL reactivity and signature fatty acid analysis was observed. 3-OH fatty acids (C:14, C:16) were also reported in LAL-positive cyst fluids by a separate reference laboratory (Microbiological Insights, Inc., Knoxville, TN). Additional chemical analyses of cyst fluid are required before a classic signature fatty acid profile and linkage to one or more bacterial genera are possible. Also, we cannot discount the possibility that 3-OH fatty acids released during degradation of endotoxin were incorporated into mammalian sphingolipids that were positive in the LAL assay, thereby accounting for both the presence of such fatty acids and LAL reactivity in cyst fluids.

(1→3)-β-D-Glucans (β-DG)

In addition to endotoxin, (1→3)-β-D-glucans were identified in cyst fluids from two patients by differential activation of the LAL assay (Table 1). The range of β-DG reactive material in cyst fluids was estimated at 40 to 160 pg/ml. In plasma of healthy persons, levels of β-DG are lower than 6.9 pg/ml (26). β-DG, a ubiquitous constituent

of filamentous fungal and yeast cell walls (33), is exceeded only by endotoxin in its reactivity in the LAL assay; glucans lacking a 1→3 linkage are not LAL reactive (34), nor are mannans, dextrans, and cellulose (24,26). Although diverse forms of glucans are components of fungal cell walls, those with (1→3)-β linkages are the most potent in causing hypersensitivity pneumonitis and other severe pulmonary illnesses (35). Although β-DGs are indicative of fungal cell walls, their occurrence alone does not identify the genera and species of the fungus that produced it. To confirm the presence of fungal components and determine the source of β-DG, serologic tests were performed on three β-DG positive and two β-DG negative cyst fluids from three autosomal-dominant PKD patients (Table 2). Cyst fluid from donor 6 that was negative for LAL-reactive material was also free of detectable fungal antigens and anti- from both kidneys containing β-DG-positive material (donors 4 and 5; Table 1) were also positive for fungal antigens. Fluid from donor 5 also exhibited fungal antibodies; fluid from donor 4 was not tested for antibodies to fungi.

Fungal serologic tests provided insights into potential sources of β-DG. Initial measurements showed *Fusarium solani* antigen. These findings suggest the antigen to be from *F. solani* or a shared antigen present in other *Fusarium* species (L. Kaufman, pers. comm.). IgE (but not IgG) antibody to *Fusarium moniliforme* was also detected. *Aspergillus* galactomannan antigen and *Candida albicans* serotype *A. mannan* were present in selected cyst fluids. Cross-reactivity of *Fusarium* with *Aspergillus* and *Penicillium* is unlikely as 1) the *Fusarium* antibody (prepared against the mycelium) was cross-absorbed with *Aspergillus*, and 2) the immunodominant group of *Aspergillus* and *Penicillium* species, galactomannan with a galactofuranosyl moiety (38), is absent in *Fusarium* species (39). As the serologic tests for *Fusarium* and *Aspergillus* in cyst fluids are experimental and have not been evaluated clinically, these data are indirect presumptive indication of the presence of these fungal antigens. The paucity of fungal serologic and chemical methods for detection and identification of emerging fungal pathogens in human tissues and body fluids and fungal components in the human diet has been noted by others.

Also detected were IgE antibodies to *Candida*. IgE antibodies to both *Fusarium* and *Candida* in cyst fluid suggest recruitment of immunologic defenses against fungi. The site of production of these IgEs and route of entry into

Table 2. Serologic results of five cyst fluids from three autosomal-dominant PKD patients tested for fungal antigens or antibodies to fungal components

Donor;Cyst No.	LAL[a] Findings	Serologic Findings
D6;1	Neg. Et[b];Neg. ß-DG[c]	Antigens not detected
D6;2	Neg. ET;Neg. ß-DG	IgE and IgG reactive with fungi were not detected
D4;3	Neg. ET;Pos. ß-DG	Antigens: *Fusarium solani* at 1:2 dilution; *Aspergillus* galactomannan at >50 ng/ml
D5;4	Neg. ET;Pos. ß-DG	Antigens: *Aspergillus* galactomannan at >50 ng/ml; *Candida albicans* serotype A mannan at 10 ng/ml
D5;5	Neg. ET;Pos. ß-DG	IgE reactive with *Fusarium moniliforme*; *C. albicans* (weak positive)

[a]Limulus amebocyte lysate assay
[b]Endotoxin
[c]ß-D-glucan

Fungal serology was performed on three cyst fluids from donors 4 and 5; (Table 1) with ß-DG positivity and two cyst fluids from donor 6, which were negative for both endotoxin and ß-DG after extended incubation in the LAL assay. Fluids were tested for antibody to *Penicillium notatum* (*Penicillium chrysogenum*) and *Penicillium frequentans*, *Aspergillus fumigatus*, *Candida albicans* (yeast), and *Fusarium moniliforme*. The Pharmacia CAP systems RAST FEIA (IgE) and IgG FEIA were employed (Pharmacia & Upjohn Diagnostics, Kalamazoo, MI, USA). The tests were modified by substituting cyst fluid for plasma; interference of fluid with the test was not observed. Tests to detect fungal antigens were performed by the Centers for Disease Control and Prevention, Atlanta, GA. To detect *Fusarium* sp. antigen, a modified aspergillosis microimmunodiffusion test (36) was used with experimental rabbit anti-*Fusarium solani* antibody; kidney cyst fluid was substituted for plasma. A double-antibody sandwich enzyme immunoassay was used to detect *C. albicans* serotype A mannan concentrations in cyst fluids; specificity of this test in sera is reported as 100% (37). An experimental ELISA inhibition assay was used to detect *Aspergillus* galactomannan (Steven Hurst, unpub. protocol) Standard serum reference curves were used for extrapolating quantities of *C. albicans* mannan and *Aspergillus* galactomannan in cyst fluids. However, these tests were not designed for cyst fluids, but for serum antigen detection, and must, therefore, be viewed as estimated quantities.

the cyst are unknown; a hypersensitivity reaction cannot be discounted. In human and experimental PKD, the severity of cystic disease correlates with the numbers of circulating neutrophils; neutrophilia is related to exposure to endotoxin and cytokines, both of which have been reported in cyst fluids (16). Our finding of fungal antigens and antibodies raises the additional possibility of leukocyte activation in PKD by ß-DG and mannan released from fungi, as ß-DG and mannan have been reported to stimulate cytokine production (40).

Serologic testing is frequently used to detect fungal infection. Cyst fluids collected from PKD patients at sequential times during progressive stages of the disease were not feasible. Therefore, quantitative and/or qualitative changes in antigen or antibody titers could not be used to determine active fungal infections in these patients. Although antigens of *Aspergillus* or *Fusarium* species are generally interpreted as apparent or covert infection, another possibility is the absorption and distribution of fungal components independent of infection (i.e., mycotoxicosis versus infection). While *Aspergillus* enters primarily by inhalation, this route does not appear to be the predominant source of *Fusarium*; fewer than 1% of air samples contained *Fusarium* species (41). Fumonisin from *Fusarium* is present in grains, rice, and corn consumed by humans (42), with regional variations in levels and the type and processing of dietary product. Because of their occurrence in the food supply and their effect on experimental animals, it has been proposed that fumonisins may contribute to kidney disease in humans (43).

ß-D-Glucans and mannans are shed by fungi during growth (26,39) and thus, like bacterial endotoxin, can potentially be distributed through blood and lymph. Diverticular disease and other anomalies in the structural and functional integrity of the gut may occur in up to 80% of PKD patients (2,44). In addition to diminished barriers to the absorption of gut-derived endotoxin, the outpouchings (diverticula) become overgrown by minor subpopulations of gut microflora (45), thereby generating increased quantities of unusual mixtures of potentially absorbable microbial components. Diverticular disease (44) and acquired renal cystic disease (46) occur in patients on hemo- and peritoneal dialysis. To complete the symmetry of this line of reasoning, outpouchings of the renal tubule in PKD are described as both cysts and diverticula (2). Direct measurement of intracyst pressure and elasticity of the basal lamina have led to the rejection of the obstruction hypothesis (i.e., a physical balloon inflation) of cystogenesis in favor of a cell-mediated restructuring of the basal lamina coupled with electrolyte transport into the cyst to expand intracyst volume (47). Although diverticular disease is thought to be a pressure-driven lesion, gut diverticula in PKD and dialysis patients may also involve a cellular lesion further promoted by pressure. Thus, diverticula in kidney and gut are associated with microbial components. It is not known if the processes of renal cystogenesis, formation of gut diverticula, and the walling off of infecting material are all facets of the same defense response in humans. Endotoxin in relatively high concentrations was found in fluid from a simple cystic kidney (Table 1); intestinal or biliary obstruction and urinary tract infections have been associated with simple kidney cysts (2). Although endotoxin has been proposed as being associated with "high sodium cyst fluids" (47), we observed endotoxin in cyst fluids of low and high sodium content (data not shown).

Relevant to our study is the report of the capacity of ß-DG to prime cell systems, which results in sensitization to bacterial endotoxin and infection (48), an example of ß-DG-endotoxin enhancement; ß-DG and endotoxin also demonstrate a strong synergistic effect on macrophages (49). In addition, there have been reports of synergy between mycotoxin and endotoxin (50).

Detection of Fungal DNA

To identify fungal DNA as evidence of past or current fungal infection and/or the absorption and distribution of fungal components to kidney cysts, polymerase chain reaction (PCR) methods were used to amplify and thus detect fungal DNA in PKD cyst fluids and kidney tissue. Six cyst fluids from three patients and two samples of kidney tissue from two autosomal-dominant PKD patients were tested; all were positive for fungal DNA. A sample of normal human kidney was negative for fungal DNA (Figure 2 A-C). Because culture confirmation was not achieved from the cyst fluids, expanded studies with species-specific probes are warranted.

Despite substantial effort, we have not been able to culture either bacteria or fungi from PKD cyst fluids by axenic methods, but fungi were detected in cultured epithelial cells from PKD kidney tissue (see below); methods appropriate to culture L-forms or other cell-wall-defective microbes

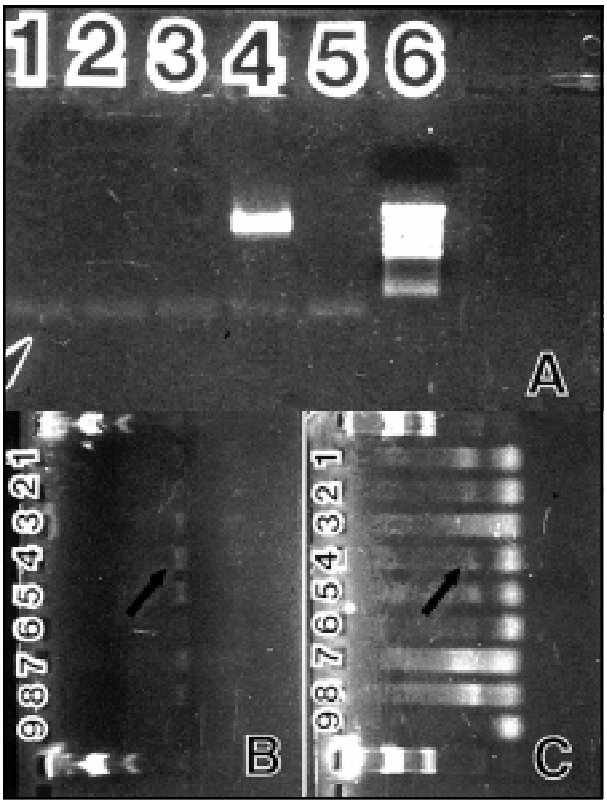

Figure 2. Amplification results of normal and PKD kidney tissue and cyst fluids with universal fungal primers ITS 1 and NL 4. 2A: DNA from healthy human kidney tissue diluted 1:10, 1:100 and 1:1,000 (lanes 1-3); control fungal DNA, *A. tamarii* (lane 4); negative control (lane 5); 1 kb ladder (lane 6); arrow indicates migration front. 2B (NL 4) and 2C (ITS 1): two cyst fluids, donor 6, negative for detectable endotoxin and b-DG (lanes 1 and 5); two cyst fluids, donor 4, positive for b-DG (lanes 2 and 3) and positive for *Fusarium solani* antigen (lane 3); two cyst fluids, donor 5, positive for b-DG (lanes 4 and 6); two PKD kidney tissues, donor 5 (lane 7) and donor 4 (lane 8); negative control (lane 9). Large arrows in 2B and 2C point to 560 bp molecular weight marker; small arrows point to product bands.

Polymerase Chain Reaction

Specimens for PCR included 1) normal human kidney tissue (0.46 g) from a 7-year-old girl who died of a head injury; the kidney was not transplantable because of the presence of a hematoma but was otherwise normal; the section used for PCR excluded the area of hematoma. 2) 0.5 g of cystic tissue, devoid of cyst fluid, from each of two autosomal-dominant PKD patients (donors 4 and 5, Table 1). 3) multiple cyst fluids (0.5-1.0 ml each; donors 4, 5, and 6). The positive control consisted of DNA extracted from *A. tamarii* (NRRL 26010) plus primer. The CTAB extraction method for fungal genomic DNA was employed. The universal oligonucleotide primer pair ITS 1 and NL4 for fungi, as previously described (51), was used; NL 4 is the reverse primer. PCR master mix (37) with 1.5 mM $MgCl_2$ and primers in a final reaction volume of 100 ml was subjected to the following thermal cycler parameters (Perkin Elmer Thermo Cycler 1): 96°C—30 sec; 53°C—30 sec and 72°C—30 sec for 40 cycles, followed by a final 7 min extension at 72°C and soak at 4°C. The presence of the expected molecular weight PCR products (600 bp) was confirmed by ethidium bromide staining after separation on a 1.0% agarose gel. The amplified products were compared visually for similarity on the basis of the presence or absence of bands; variations in band intensity were not considered.

A-C represent the banding patterns obtained by agarose gel electrophoresis of healthy kidney tissue, cystic kidney tissues, and cyst fluids following PCR amplification with the universal fungal primer pair, ITS 1 and NL 4. Amplified fungal products were not detected in the negative PCR controls (2A, lane 5; 2B and C, lanes 9) or normal human kidney tissue (2A, lanes 1-3). In marked contrast, distinct bands of the predicted size of each fungal primer used (~ 600 bp; 45) were detected in all cystic kidney tissues and fluids examined from the three patients (2B-C); minor fragments were not detected. The migration patterns were consistent with the positive fungal DNA control (2A, lane 4).

Although fungal DNA might have been anticipated in tissues and fluids from PKD kidneys showing b-DG or positive serologic findings, fungal DNA was also found in PKD cyst fluid from donor 6 (2B and C: Lanes 1 and 5), which lacked these components. The b-DG positive cyst fluids tested from donors 4 and 5 are in Lanes 2 and 3 and 4 and 6, respectively. The fluid depicted in Lane 3 also had positive serologic results for *F. solani* antigen; serologic testing was not done on the other fluids assayed by PCR. Each cyst fluid must be considered an independent sample, requiring direct measurement; assumption of cyst content on the basis of other fluids within the same kidney is not valid (16). Fungal DNA was also detected in kidney tissue from donors 4 and 5 (Figure 2B and C; Lanes 8 and 7). Differences were noted between the two primers used; ITS 1 yielded amplified products in all PKD samples while NL 4 amplified less well, being more concordant with the detection of b-DG. The amplicons are presumptive evidence of fungal DNA.

were not performed. Gram stains of cyst fluid supernates and pellets did not show intact bacteria. Electron microscopy also did not show intact bacteria, but occasionally, a field consistent with fungal cell walls was observed (not shown); intact fungi were not observed. Nonetheless, it was only from PKD kidneys where b-DG was identified in cyst fluid that fungal organisms were frequently isolated from PKD epithelial cell cultures. Other human and animal cells isolated and propagated in the laboratory by the same reagents and work spaces were free of fungal growth. The fungi were identified as belonging to the genera *Paecilomyces* and a new species of *Penicillium* (manuscript in preparation). Fungi have been reported, albeit infrequently, as etiologic agents in renal infections in PKD (8). Given all of the above, it is possible that viable fungi were present in the PKD renal tissue, but only

their remnants were in the cyst fluid. Emerging human awareness of infectious disease may well describe our experience in this study of PKD.

Infectious Disease, Microbial Toxicosis, and Sphingolipid Biology

Emerging knowledge of sphingolipid biology and PKD's vulnerability to microbial toxins offer an opportunity for a fresh look at PKD. As Spiegel and Merrill (52) have noted, sphingolipids fall into two broad categories, both of which are altered in PKD (53,54). Complex sphingolipids (i.e., ceramide with a carbohydrate or phosphocholine head group on carbon-1) interact with growth factor receptors and the extracellular matrix and adjacent cells and act as binding sites for gram-positive and -negative bacteria and microbial toxins. In the second category of sphingolipids, ceramide sphingoid bases (i.e., sphingosine, sphinganine, phytosphingosine) and their 1-phosphates modify the activity of protein kinases and phosphatases, ion transporters, and a growing number of signal transduction processes. However, the dynamics of human sphingolipid biology occur within the context of a microbe-dominated environment.

Thus, we propose a third category called "microsphingoids," sphingolipid-like molecules of microbial origin that mimic or antagonize the actions or metabolism of human sphingolipids. Examples include, but are not limited to, various mycotoxins (42,55-57), bacterial sphingolipids (58), and endotoxin (59,60). In this report, we have shown the presence of endotoxin and fungal antigens or antibodies to at least *Fusarium*, *Aspergillus*, and *Candida* in human PKD tissues and fluids; from human PKD cells in vitro we have encountered *Paecilomyces* and *Penicillium*. These fungi produce mycotoxins that inhibit multiple enzymes required for sphingolipid biosynthesis (55; Figure 3) and alter the activity of various elements of signal transduction cascades, such as protein phosphatases, calmodulin, and GTP-binding proteins (56,61). Such mycotoxins are also found in the human diet, which is itself an emerging concern in food safety (42,43,55). Rietschel et al. (13) noted the similarity of LPS to glycosphingolipids. Wright and Kolesnick (59) have reviewed the structural similarity of the Lipid A region of bacterial LPS with ceramide and the ability of LPS to mimic the ceramide-induced alterations in protein kinase and phosphatase activities. Endotoxin is also reported to alter the structure of mammalian sphingolipids (62) and to initiate cytokine-mediated cascades that generate ceramide, sphingosine-1-phosphate, and lysosphingolipids, all of which exhibit biologic activity (13,63). Not to be ignored are bacterial sphingolipids formed by genera such as *Bacteriodes*, which represent approximately 30% of the gut microflora (58). The role of bacterial sphingolipids and their metabolites in human biology and their bioavailability in disease are poorly understood. The term "microsphingoid" is intended to highlight the contribution of microbes to sphingolipid biology in human health and disease.

Could pertubations in sphingolipid biology caused by genetic anomalies and/or "microsphingoids" account for both infantile autosomal recessive and adult onset forms of kidney cystic disease? Hannun (64) has reviewed the role of sphingolipids as "biostats" that regulate diverse cellular programs executed in response to various stimuli. Such biostatic regulation could account for both acute and chronic changes in cellular behavior and differentiation in the kidney (65). Calvet et al. (2,66) have proposed two models to explain the immaturity or dedifferentiated state of epithelial cells found in hereditary and acquired cystic kidneys: In infantile cystic disease, the epithelial cells never reach terminal differentiation and are trapped in an immature state, while in adult forms of cystic disease, toxin-induced injury to an initially mature renal epithelium is followed by inability of the epithelium to recover to a fully differentiated state. In genetically cystic *cpk/cpk* mice (a model for autosomal recessive PKD), Deshmukh et al. (53) reported altered levels of ceramide and complex glycosphingolipids in kidney tissue from cystic mice, but not in their phenotypically normal littermates. Lower levels of ceramide and sulfatide, but higher levels of glucosyl- and lactosylceramide and ganglioside GM3, were measured. Our research in infantile and adult human PKD kidney tissue and cyst fluids showed no detectable free sphinganine, the primary sphingoid base formed during de novo biosynthesis of mammalian sphingolipids (54). Thus, anomalies in sphingolipid biology exist in cystic kidney tissue.

It is difficult to separate concepts of sphingolipid biology from considerations of infectious disease, microbial components, and "microsphingoids" in PKD. Potential consequences of alterations in glycosphingolipids on the surface of PKD cells include enhanced microbial

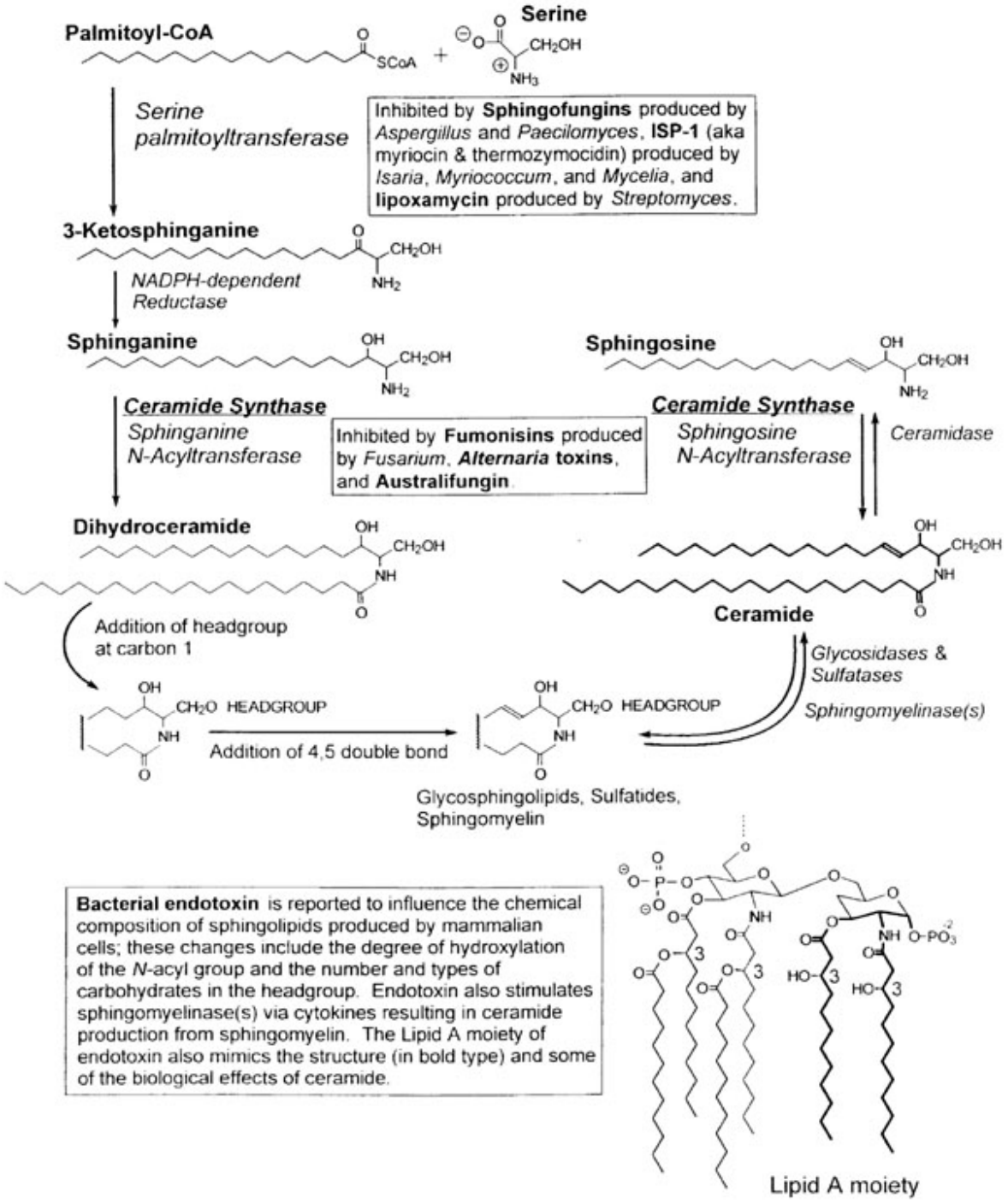

Figure 3. Sites of impact of endotoxin and mycotoxins on sphingolipid metabolism. In PKD, renal sphingolipid formation is altered. Such compromised sphingolipid pathways would be expected to be vulnerable to these highly potent microbial toxins, especially during chronic exposure within renal cysts.

colonization due to the availability of complex sphingolipids as binding sites. Binding of microbes or their components to such sphingolipids may even promote cystogenesis, as binding to complex sphingolipid has been reported to cause changes in differentiation and morphology of cells in vitro (67). Altered biosynthesis of sphingoid bases affects the ratios of sphinganine to sphingosine with consequences to signal transduction (61) and may even alter the antimicrobial environment, as sphingoid bases are reported to have direct antifungal and antibacterial activity (68). The levels of sphingosine in human cyst fluid (Hjelle, unpub.) are comparable to the levels that induce a state of cytoresistance (cellular dedifferentiation) in kidney cells in vitro (69). In addition to products of mammalian sphingolipid metabolism, endotoxin in relatively high amounts was found in cyst fluids from infantile and adult forms of PKD and in simple cysts (Table 1). Could "microsphingoids" or microbial toxins injure and then prevent repair of renal epithelial cells? Data from diverse scientific disciplines support this possibility.

LPS influences nephron formation (70) and renal cystogenesis (11). Fumonisins are potent nephrotoxins (43,55), alter repair mechanisms in kidney cells in vitro (71), and induce apoptosis (72,73). Renal cysts are occasionally reported after chronic exposure of rodents to fumonisins (74). Rates of programmed cell death are abnormal in PKD kidney tissue (75,76); ceramide is a pivotal messenger in apoptosis (64). In PKD, altered processing and sorting of cell membrane proteins and secretory material occurs (2-4); sphingolipids influence processing, sorting, and movement of membranes and ion transporters (64,65), as does fumonisin in kidney cells in vitro (77). In PKD, various electrolyte transport systems are altered (2). cAMP-mediated electrolyte fluxes were proposed as important in cyst formation (3). Fumonisins are reported to activate cAMP response elements (78). Cyst fluid contains uncharacterized substances that promote cystogenesis in vitro (3). Although LPS isolated from *Escherichia coli* did not induce the full array of anticipated cystogenic responses in kidney cells in vitro, the structure, potency, and array of elicited biologic responses of endotoxin depend on the genera and species of bacteria from which it was isolated (13). Coupled with the presence of fungal components, cyst fluid likely represents a complex mixture of "microsphingoids" and toxins that may change over time with dynamic consequences to cyst formation. The heterogeneity of cyst volume and content of growth factors and cytokines in adult PKD has been noted (3,16).

Concepts of infection and "microsphingoids" appear to converge with the expanding knowledge of PKD gene products. The structures of the PKD1 (polycystin) and PKD2 gene products share homology with α1E subtype of voltage-activated, calcium channels (3,6) and a sea urchin protein likely involved in calcium fluxes during fertilization (79). LPS alters voltage-activated, calcium channels (80) and osmo-regulation (81) in mammalian cells. The PKD1 gene product also contains regions of homology with ligand and receptor domains putatively involved in binding to adjacent cells and extra-cellular matrix (3); one such domain is similar to C type lectins that can bind microbes (82). Tissues, including kidney and gut, expressing polycystin in adult PKD, may exhibit a relatively greater leakiness to normal molecular and par-ticulate traffic, as seen in kidney cysts (3) and susceptibility to diverticulosis (3,44). Polycystin also shows homology with apoprotein from low density lipoprotein. Because LPS and sphingo-lipids bind to and are transported by serum lipoproteins, the low-density lipoprotein binding site in polycystin may also enable accumulation and/or transport of "microsphingoids."

Secondary mutations in PKD genes appear to be required for clonal cyst formation (3,5), as is a loss of renal tissue through dysregulation of apoptosis (76). Alterations in the expression of the *bcl*-2 gene product in mice results in polycystic kidneys and dysregulation of apoptosis (83). Although infection increases translocation of the *bcl*-2 gene in human lymphoid tissue (84), it is not known if infection in PKD patients causes such a dysregulation of *bcl*-2 in kidney tissue.

Regarding infection and sphingolipids, ceramide is emerging as a pivotal molecule in the immune system (63) and fumonisins are reported to alter immune function (85). It is ironic that our findings potentially link *Fusarium* to PKD, as fumonisin is used as a molecular tool to study sphingolipid metabolism and signal trandsduction in PKD. Not to be overlooked are the classic bacterial sphingomyelinases encountered during infection and released from gut microflora (e.g., *Staphylococcus aureus* and *Clostridium perfringens*) that can stimulate ceramide formation by hydrolysis of sphingomyelin. Thus, the vulnerability of PKD patients to infection may be related, in part, to anomalies in sphingolipid biology that influence

1) "biostatic" mechanisms of cell regulation, 2) the structure of the plasma membrane and the function of PKD gene products, 3) the availability of glycosphingolipids as binding sites for microbes and their components, 4) the bioavailability of microbial components present in the gut, and 5) antimicrobial defenses in general. Such vulnerability is likely influenced by repeated courses of antimicrobial therapy that provide selection pressure for colonization with a modified microflora. The extent to which sphingolipid biology in PKD is influenced by genetic defects rather than microbial factors is yet to be defined.

Anomalies caused by the PKD gene defect(s) alone cannot explain cystogenesis. As shown by Werder et al. (9), raising genetically cystic mice in a germ-free environment essentially eliminated cystogenesis and increased survivorship to nearly 100% over that of littermates raised in ambient environment for 18 months. Even in cyst fluid from infantile PKD, relatively high levels of endotoxin were found (Table 1). This suggests that microbial toxins are available early in this disease. Prenatal exposures to toxins and "microsphingoids" are unknown. Although our finding of fungal DNA in eight of eight samples of autosomal-dominant PKD tissue and cyst fluids examined suggests an intimate association of fungal exposure to renal cysts, a contributing multifaceted microbial toxicosis involving diet and gut microflora cannot be excluded. By itself, the finding of microbial components at the site of lesion does not prove a causal role for the microbe(s) in disease progression (1). However, a working hypothesis can be formed on the basis of evidence that microbes promote progression of the primary disease and components of the microbes act on mammalian biology to cause effects plausibly related to the known pathophysiology of the disease.

Although there is an established body of knowledge that PKD is a genetic disorder, our data indicate that bacterial endotoxin, ß-DG, and likely other microbial components are available within the kidney to provoke cystogenesis. We have provided chemical and advanced LAL assay-based evidence of bacterial endotoxin and ß-DG in human PKD cyst fluids. From cyst fluids and PKD kidney tissue we have provided evidence of fungal DNA and cell components by serologic testing and PCR with universal fungal primers. We have integrated these findings into a working hypothesis based on emerging knowledge of PKD gene products, altered sphingolipid metabolism in PKD, the effects of LPS on renal cystogenesis, the mimicry of ceramide by LPS and the effects of mycotoxins on mammalian sphingolipid biology and signal transduction, the occurrence of infection in PKD, and the impact of altered gut permeability and microbial colonization on progression of PKD. Is PKD a genetic disease promoted by microbial influences? Tests of this multifaceted hypothesis require awareness of a breadth of issues drawn from numerous scientific disciplines. Identification of the microbes and microbial components involved will require a concerted analysis using highly sensitive and specific methods. As awareness of the importance of sphingolipid biology in health and disease grows, so will the appreciation that microbial influences will need to be considered in pharmacologic studies that seek to manipulate ceramide and complex sphingolipid biochemistry in disease. The ubiquitous and highly potent bacterial endotoxin is again one of the usual suspects examined as provocateur of disease; in this case, endotoxin's modus operandi of coopting the signal transduction machinery of ceramide to cause chronic disease may ultimately be revealed.

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Borna Disease

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Borna disease virus, a newly classified nonsegmented negative-strand RNA virus with international distribution, infects a broad range of warm-blooded animals from birds to primates. Infection causes movement and behavioral disturbances reminiscent of some neuropsychiatric syndromes. The virus has not been clearly linked to any human disease; however, an association between infection with the virus and selected neuropsychiatric disorders has been suggested. We reviewed recent advances in Borna disease virus research, focusing on evidence of infection in humans.

Although Borna disease was first recognized in the early 1800s as a neurologic syndrome with an infectious basis, Borna disease virus (BDV) has only recently been characterized as the causative agent. BDV is the prototype of a newly recognized virus family, *Bornaviridae*, within the nonsegmented negative-strand RNA viruses (order *Mononegavirales*) (1,2).

The molecular biology of the virus has several unusual aspects, including nuclear localization for replication and transcription (3), overlap of open reading frames and transcription units (4,5), posttranscriptional modification of subgenomic RNAs (6,7), and marked conservation of coding sequence across various animal species and tissue culture systems (8,9). BDV replicates at lower levels than most known viruses (10,11), is not lytic, and persists in the nervous system despite a vigorous immune response. In the classic syndrome, infected animals exhibit movement and behavior disorders (10,12,13); however, clinical signs may be dramatic, subtle, or inapparent depending on the integrity and intensity of the host immune response to viral gene products (14).

Natural Infection and Transmission

Originally described as a disease of horses, Borna disease has also been found in sheep, llamas, ostriches, cats, and cattle (15). Because an even larger variety of species has been infected experimentally, the host range is likely to include all warm-blooded animals; no data exist concerning infection of species other than warm-blooded hosts.

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The geographic distribution of BDV is unknown. Natural infection has been reported only in Central Europe, North America, and parts of Asia (Japan and Israel). However, this apparent geographic restriction may reflect lack of reliable methods and reagents for diagnosis of infection or failure to consider the possibility of BDV infection. Recent reports of asymptomatic naturally infected animals suggest that the virus may be even more widespread than previously thought (16-18).

Neither the reservoir nor the mode of transmission of natural infection is known. An olfactory route for transmission has been proposed because intranasal infection is efficient, and the olfactory bulbs of naturally infected horses show inflammation and edema early in the course of disease (19,20). BDV nucleic acid and proteins in peripheral blood mononuclear cells (PBMC) also indicate a potential for hematogenous transmission (21-28). Experimental infection of rodents results in virus persistence and is associated with the presence of viral gene products in saliva, urine, and feces (28); such secreta/excreta are important in the transmission of other pathogenic viruses (e.g., lymphocytic choriomeningitis virus and hantaviruses). Thus the rodent provides the potential for both a natural reservoir and vector; however, because natural BDV infection has not been reported in rodents, their role in BDV transmission to other domesticated animals and humans remains speculative. No data concerning vertical transmission of BDV in natural or experimental hosts have been published.

Animal Models for BDV Pathogenesis

Borna disease in naturally infected horses and sheep is characterized by agitated aggressive

behavior that progresses over weeks to paralysis and inanition (29). Because its immune-mediated disease most closely resembles that in naturally infected horses and sheep, the Lewis rat has been selected as a rodent model. Rats infected as adults exhibit hyperactivity and exaggerated startle responses coincident with viral gene products in limbic system neurons and infiltration of mononuclear cells into the brain (13,30). The inflammation recedes over several weeks, but the virus persists and animals show stereotyped motor behavior, dyskinesias, and dystonias associated with distinct changes in the central nervous system (CNS) dopamine system (12,31), as well as decreased activity and cachexia (13). In contrast, rats infected as neonates have a disease characterized by stunted growth, hyperactivity, subtle learning disturbances, and altered taste preferences and do not mount a cellular immune response to the virus (32,33).

Behavioral disturbances have been reported in experimentally infected primates: tree shrews and rhesus monkeys. Infected tree shrews have altered social and sexual behavior, manifested as abnormal dominance relationships and failure to mate (34). Infected rhesus monkeys are initially hyperactive and subsequently become apathetic and hypokinetic (35).

BDV and Neuropsychiatric Disease

Serology (Table 1)

Its broad host and geographic range suggests that BDV might cause human neuropsychiatric disease. Because the behavioral disturbances in animals resembled those of affective disorders, particularly bipolar depression, initial studies investigated these disorders. The earliest work to suggest a link between BDV and human mental illness came from a serologic survey in 1985 of 285 patients with affective disorders in the United States, 694 psychiatric patients in Germany, and 200 healthy controls (36). An indirect immunofluorescence assay (IFA) was used to detect antibodies reactive with a BDV-infected cell line; sera from 12 (4.3%) patients from the United States and four (< 1%) patients from Germany were immunoreactive; no sera from controls were immunoreactive. Sera from many of these patients were subsequently analyzed by a Western immunoblot assay based on BDV nucleoprotein (N) and phosphoprotein (P) purified by affinity chromatography from infected rabbit kidney cells (37). In this study of 138 patients with affective disorders and 117 healthy controls, antibodies to N were found in 53 (38%) patients versus 19 (16%) controls; antibodies to P were found in 16 (12%) patients versus five (4%) controls; antibodies to both proteins were found in nine (6.5%) patients versus one (< 1%) control.

Table 1. Serum immunoreactivity to Borna disease virus in various diseases

Disease	Prevalence				Assay	Ref.
	Disease	(%)	Controls	(%)		
Psychiatric (various)	(4/694)	0.6	(0/200)	0	IFA	(36)
	(13/642)	2	(11/540)	2	IFA	(60)
	(200-350/5000)	4-7	(10/1000)	1	WB/IFA	(61)
	(18/60)	30	(1/100)	1	WB	(24, 25)
	(18/132)	13.6	(3/203)	1.5	WB	(27)
	(13/55)	23.6	(4/36)	11.1	IFA	(51)
	(6/49)	12.2			IFA	(38)
Affective	(12/265)	4.5	(0/105)	0	IFA	(62)
	(12/285)	4.2	(0/200)	0	IFA	(36)
	(53/138)	38	(19/117)	16	WB	(37)
	(6/52)	11.5	(3/203)	1.4	WB	(27)
	(10/27)	37			IFA	(38)
Schizophrenia	(29/90)	32	(4/20)	20	WB	(43)
	(16/114)	14	(3/203)	1.5	WB	(27)
	(1/4)	25			IFA	(38)
CFS	(6/25)	24			WB	(26)
MS	(15/114)	13	(10/483)	2.1	IP/IFA	(63)
HIV-positive	(36/460)	7.8	(11/540)	2	IFA	(60)
HIV-early	(61/751)	8.1	(10/483)	2	IP/IFA	(61)
HIV-LAP	(34/244)	14	(10/483)	2	IP/IFA	(63)
Schisto/malaria	(19/193)	9.8	(10/483)	2	IP/IFA	(63)

Abbreviations: IFA, immunofluorescence assay; WB, Western immunoblot; IP, immunoprecipitation; CFS, chronic fatigue syndrome; MS, multiple sclerosis; HIV, human immunodeficiency virus; LAP, lymphadenopathy; Schisto/malaria, schistosomiasis or malaria.

To establish a correlation between immunoreactivity to BDV and the duration and severity of psychiatric disease, Bode et al. performed IFA on multiple serum samples taken at different times from 71 patients with various diagnoses including minor or major depression, paranoid psychosis, schizophrenia, anxiety disorder, and personality disorder (38). Overall, the prevalence of immunoreactivity to BDV was

greater than 20%, a marked increase from the 2% to 4% found in an earlier study that assayed specimens from each patient at one time. Thirty-seven percent of patients with major depression, 25% with paranoid psychosis, and only 6% or fewer with reactive depression and other neurotic conditions were seropositive by day 17 of illness. Because BDV gene products have been identified in PBMC of infected rats (28), PBMC from patients with neuropsychiatric diseases were examined for viral antigens by fluorescence activated cell sorting analysis (39). Of the 70 patients tested, more than 40% were antigen carriers, twice the number predicted by the previous serologic survey.

Some similarities between particular animal models of BDV infection and schizophrenia have been reported. The subtle signs of disease caused by infection in neonatal rats (e.g., learning deficiencies, hyperactivity with CNS abnormalities including cerebellar disorganization and loss of dentate gyrus granule cells [32,33,40,41]) are consistent with a longstanding hypothesis that schizophrenia reflects an early brain insult (e.g., infection) resulting in abnormal brain development (42) and prompted investigation of the role of BDV infection in the pathogenesis of schizophrenia (43). A Western immunoblot assay based on BDV N, P, and matrix protein (M) purified from infected human neuroblastoma cells was used to examine sera from 90 schizophrenic patients and 20 healthy controls. Antibodies to one BDV protein (N, P, or M) were detected in 29 (32%) patients and four (20%) controls. Antibodies to two or more BDV proteins were detected in 13 (14.4%) patients and zero controls. Antibodies to M protein were found in 12 (13.3%) patients and zero controls. Immunoreactivity to two or more BDV proteins or M protein was significantly associated with abnormal brain morphology in magnetic resonance image analysis (MRI) and the clinical diagnosis of deficit syndrome (a schizophrenia subgroup characterized by social withdrawal, neurologic dysfunction, and neuroanatomic abnormalities). Similar findings indicated an association between antibodies reactive with BDV proteins and MRI evidence of cerebral atrophy in schizophrenic patients (44).

Molecular Epidemiology (Table 2)

Interest in the potential role of BDV as a human pathogen and in BDV-infected animals as models for human neuropsychiatric diseases guided efforts to identify the infectious agent. Because of low viral productivity and tight association of BDV with plasma membranes, classic methods for virus isolation have been unsuccessful; however, BDV nucleic acids have been independently cloned from horse isolates by using a purely molecular subtractive cloning approach (45,46). The viral genome was subsequently cloned from viral particles (4) and nuclear extracts of infected cells (47). With the advent of viral sequence information, new diagnostic reagents

Table 2. Borna disease virus nucleic acid in patients with various diseases

Disease	Tissue	Prevalence				Divergence*	Ref.
		Disease	(%)	Controls	(%)		
Psychiatric	PBMC	(4/6)	66.7	(0/10)	0	0-3.6	(22)
(various)	PBMC	(5/12)	41.7	(0/23)	0	0-4	(27)
	PBMC	(22/60)	37	(8/172)	4.7		(24, 25)
	PBMC-coculture	(3/32)	9.4	(0/5)	0	0.07-0.83	(23, 53)
Affective	PBMC	(1/3)	33.3	(0/23)	0		(27)
	PBMC	(1/6)	16.7	(0/36)	0		(51)
	PBMC	(0/9)	0				(56)
Schizophrenia	PBMC	(7/11)	63.6	(0/23)	0		(27)
	PBMC	(5/49)	10.2	(0/36)	0		(51)
	PBMC					4.2-9.3	(54)
	Brain	(0/3)	0	(0/3)	0		(55)
	CSF	(0/48)	0	(0/9)	0		(55)
	PBMC	(0/9)	0	(0/9)	0		(55)
	PBMC	(0/26)	0				(56)
CFS	PBMC	(3/25)	12			6.0-14	(26)
Hippocampal sclerosis	Brain	(4/5)	80				(53)

Abbreviations: PBMC, peripheral blood mononuclear cells; CSF, cerebrospinal fluid; CFS, chronic fatigue syndrome.
*Divergence of P-gene nucleotide sequence from common BDV isolates (strain V [4] and He/80 [9]).

for BDV infection have been introduced, including recombinant proteins for serology as well as oligonucleotide primers and probes for molecular epidemiology.

The earliest experiments in BDV molecular epidemiology examined whether conserved virus sequences could be identified across several host species. Reverse transcription polymerase chain reaction (RT-PCR), as well as extensive experimental passage through rabbit and rat brains and cell lines from various species, was used to amplify and clone coding and noncoding sequences from virus strains divergent by over 50 years' growth in nature. Because of inaccuracies in viral RNA-dependent RNA polymerases, most single-stranded RNA viruses have sequence divergence of 10^3 to 10^4 per site per round of replication (48-50). Sequence analysis of BDV isolates showed a much lower rate of divergence. For N and P sequences, maximum variability was 4.1% at the nucleotide level and 1.5% at the predicted amino acid level (8). Similar sequence conservation was later found for sequences from naturally infected donkeys, sheep, and cats (9, H. Ludwig, pers. comm.). The extent to which sequence conservation in BDV represents enhanced polymerase fidelity, or more likely, selective environmental pressures is unknown.

Extending molecular analysis to human materials is both complex and controversial. If BDV infects neuropsychiatric disease patients, viral nucleic acids are present at lower concentrations in human brain and PBMC than in previously studied naturally or experimentally infected hosts because investigators who report isolation of BDV nucleic acid from human PBMC must use the highly sensitive technique of nested RT-PCR. Groups in Germany and Japan have detected viral nucleic acid in PBMC of patients with neuropsychiatric diseases: Bode and colleagues found BDV nucleic acids in four (66.7%) of six patients (22); Sauder and colleagues found BDV sequences in 13 (50%) of 26 neuropsychiatric patients (seven patients with schizophrenia, one with affective disorder, and five with other psychiatric disorders) (27); Kishi and co-workers found BDV nucleic acids in PBMC of 22 (37%) out of 60 neuropsychiatric patients (24) and eight (4.7%) out of 172 blood donor controls (25); Igata-Yi et al. detected BDV nucleic acids in six (10.9%) of 55 neuropsychiatric patients (five patients with schizophrenia, one with depression) versus zero of 36 blood donor controls (51); and Nakaya et al. reported BDV nucleic acid in three (12%) of 25 chronic fatigue syndrome patients (26). Additionally, BDV nucleic acids and immunoreactivity have been detected in the hippocampus of four of five North American patients with postmortem diagnosis of hippocampal sclerosis (52).

Genomic analyses of BDV isolates from human patients have yielded differing estimates of nucleotide sequence conservation (Table 2). Extensive conservation of the N and P open reading frames, consistent with previous findings in field and tissue culture isolates (8), has been reported (22,27). Infectious BDV has been isolated after cocultivation of PBMC from neuropsychiatric patients with a human oligodendroglial cell line (23). Sequences of these isolates were highly conserved with previously identified sequences (53); other groups have described greater sequence divergence (26,54). However, differences in levels of sequence conservation may reflect variations in methods for RT-PCR amplification rather than true strain differences (27).

In two reports RT-PCR did not yield evidence of BDV nucleic acid in neuropsychiatric disease patients (Table 2): No BDV nucleic acid was found by RT-PCR analysis of brain tissue, cerebrospinal fluid, or PBMC from patients with schizophrenia (55); similarly, no BDV nucleic acid was found in RT-PCR studies of PBMC from 26 patients with schizophrenia and nine with affective disorders (56).

Special Considerations for BDV Diagnostics

Although a wide variety of assays (IFA, Western immunoblot, radioimmunoprecipitation, and enzyme-linked immunosorbent assay) and antigen preparations (infected cells, infected cell extracts, and recombinant proteins produced in prokaryotic or baculovirus systems) have been used for BDV serology, generally accepted standards for diagnosis of human BDV infection have not been established. Because only limited data concerning interassay comparability for serology within individual laboratories (and none between different laboratories) have been established, discrepancies between investigators may reflect differences in clinical populations, assay sensitivity, or other factors.

Similarly, agreement concerning methods for molecular diagnosis of BDV infection is limited. Most investigators use RT-PCR; some use a method sensitive to 100 to 300 copies of RNA template (nested RT-PCR, 80 to 100 cycles),

while others use less sensitive methods (no nesting, 30 cycles). Only investigators using the more sensitive method have reported BDV gene products in human materials (PBMC, brain).

RT-PCR, particularly nested RT-PCR, is prone to artifacts because of inadvertent introduction of template from laboratory isolates or cross-contamination of samples. Because putative human isolates detected by nested RT-PCR are similar in sequence to known animal and tissue culture isolates, it has been argued that they represent low-level contaminants. However, the finding of sequence conservation is consistent with previous analyses of well-characterized isolates disparate by host species and geography (8,9) and cannot be used to discount the validity of positive nested RT-PCR results.

Future Directions

The broad potential host range of BDV suggests that humans are targets for infection. Rodents with persistent BDV infection and minimal overt signs of disease and domestic animals and livestock could serve as vectors. Serum antibodies reactive with BDV have been detected in asymptomatic farmworkers exposed to ostriches with a Borna disease-like syndrome (57). Immunoreactivity to BDV and neurologic disturbances have been reported in a farm worker exposed to seropositive asymptomatic horses and sheep (58). Finally, although no human cases of disease have been linked to feline infection, there is evidence of BDV infection in house cats in Europe (59) and Japan (18). However, even though BDV could infect humans and is likely to do so, a number of questions remain unanswered: The sources and routes of potential human infection are not clear, no detailed epidemiology has been done in animal populations, and transmission from domestic animals to humans has not been demonstrated.

Viral gene products are readily detected in CNS of natural and experimental hosts without such sensitive methods as nested RT-PCR. The need to use sensitive methods to detect BDV nucleic acid in humans indicates that the virus is present only at low levels. Thus, if BDV can be implicated as a factor in human neuropsychiatric disease, mechanisms for pathogenesis may be different from those found in other natural and experimental hosts. Although a higher prevalence of markers for BDV infection has been reported in neuropsychiatric patients than in controls, no single neuropsychiatric disease has been correlated with BDV infection. Efforts to link BDV with neuropsychiatric disease have not used accepted epidemiology standards.

To rigorously address the issues of BDV epidemiology and pathogenesis, multicenter groups in Europe and the United States are collaborating to collect and perform blinded analysis of human clinical materials by standardized methods and reagents. The objectives of these projects will be to determine the prevalence of serum antibodies to BDV in patients and controls and the extent to which various assays for antibodies are in accord, the prevalence of BDV nucleic acids in brains and PBMC of patients and controls, and the correlation between antibodies to BDV or viral nucleic acids and a particular neuropsychiatric disease.

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The Rickettsia: an Emerging Group of Pathogens in Fish

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Piscirickettsia salmonis is the first of the previously unrecognized rickettsial pathogens of fish to be fully characterized. Since the recognition of *P. salmonis* in 1989, the impact of rickettsial pathogens in fish has become increasingly apparent. Growing awareness of the emergence of these fastidious intracellular organisms has led to the discovery of rickettsial diseases among diverse species of fish from different geographic locations and aquatic environments. The source, reservoir, and mode of transmission of these agents as well as appropriate methods of disease prevention and control remain to be established.

The Emergence of Rickettsial Diseases in Fish

The earliest report of a rickettsialike organism in fish occurred in 1939 during examination of diseased *Tetrodon fahaka* from the Nile River in Egypt. The microorganisms were observed within stained tissue cells, but no attempts were made to culture them (1). In 1975 Ozel and Schwanz-Pfitzner (2) first cultured rickettsialike organisms from fish while examining rainbow trout (*Oncorhynchus mykiss*) for Egtved virus. While cultivating the virus in RTG-2 cells (3), they observed that cells also contained an intracellular rickettsialike organism. However, research on the organism was limited, and its taxonomic position and relevance to disease were not determined. The microorganism was not maintained and is no longer available for evaluation. No further reports of rickettsia in fish appeared until 1986 when Davies observed rickettsialike organisms in the tissue of dragonets (*Callionymus lyra L.*) collected in waters off the coast of Wales (4) (Table 1).

The role of rickettsiae as emerging pathogens of fish became apparent in 1989 (5), when a previously unrecognized bacterium (6) was isolated in the chinook salmon (*Oncorhynchus tshawytscha*) embryo cell line, CHSE-214 (7) and was demonstrated to be the cause of epizootics in marine-netpen-reared coho salmon species and (*Oncorhynchus kisutch*) in Region X, Chile (8).

Table 1. Unidentified rickettsialike organisms observed in and/or isolated from fish

Fish host species	Geo. location	Salt/Fresh Water	Obs./ Iso.	Ref.
Fokaka (*Tetrodon fahaka*)	Egypt	F[a]	O[c]	1
Rainbow trout (*Oncorhynchus mykiss)*	Europe	F	I[d]	2
Dragonet (*Callionymus lyra*)	Wales	S[b]	O	5
Masu salmon (*Oncorhynchus masou*)[e]	Chile	S	O	
Mozambique tilapia (*Oreochromis mossambicus*)	Taiwan	S & F	I	15
Nile tilapia (*O. niloticus*)				
Blue tilapia (*O. aureus*)				
Redbelly tilapia (*Tilapia zillii*)				
Wami tilapia (*T. hornorum*)				
Blue-eyed plecostomus (*Panaque suttoni*)	Colombia	F	O	16
Atlantic salmon (*Salmo salar*)	Chile	F	I	19
Sea bass (*Dicentrarchus labrax*)	France	S	O	17

[a]F = Host collected from fresh water
[b]S = Host collected from salt water
[c]O = Observed only
[d]I = Isolated in cell culture
[e]Sandra Bravo, Puerto Montt, Chile

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Piscirickettsia salmonis, was the first rickettsialike organism isolated from fish, characterized, and demonstrated to cause disease in experimentally infected hosts (6). Initially, rickettsial infections of fish were thought to be confined to coho salmon in southern Chile, but the agent and the disease it causes have since been observed in other locations (Table 2). In British Columbia, Brocklebank et al. (9,10) observed that sea-farmed Atlantic salmon (*Salmo salar*) also manifested pathologic features indistinguishable from those of salmonids in Chile. The causation of the disease was established by experimental infection followed by isolation in CHSE-214 cells. The organism was morphologically similar to *P. salmonis*, but comparatively less virulent for salmonids. These researchers also provided anecdotal reports that the same condition occurred as early as 1970 in pink salmon (*Oncorhynchus gorbuscha*) held in sea water tanks in British Columbia; beginning in the 1980s, the condition was also seen in coho and chinook salmon from local sea farms rearing salmonids. Rickettsiae morphologically similar to those implicated in the diseases in Chile and Canada also caused low level mortality in Atlantic salmon held in sea water cages in Norway (11) and Ireland (12). We have identified all of these agents as *P. salmonis* by direct fluorescence antibody tests using the polyclonal antiserum (unpub. data; 13).

Until 1994, all recognized rickettsial diseases of fish had been observed in species of salmonids cultured in sea water. However, it is now apparent that these pathogens affect fish over a broad host and geographic range and in both fresh water and marine environments. Chern and Chao (14) reported an epizootic rickettsial disease in several species of tilapia in both marine and fresh water ponds in Taiwan (Table 1). Death rates associated with this disease reached 95% at certain sites. Khoo et al. (15) reported a rickettsialike organism in moribund specimens of a fresh water tropical fish, the blue-eyed plecostomus (*Panaque suttoni*), shipped to the United States from Colombia. In France, Comps et al. (16) found rickettsialike organisms in the brain of moribund juvenile seabass (*Dicentrarchu labrax*) exhibiting abnormal swimming behavior and in Chile, Gaggero et al. (17) isolated *P. salmonis* from diseased coho salmon and rainbow trout held only in fresh water. A different, and as yet unidentified, bacterium has been isolated in fish cell cultures from diseased Atlantic salmon in Chile that had been reared only in fresh water.

Table 2. *Piscirickettsia salmonis* LF-89[T] observed in and/or isolated from fish

Fish Host Species	Geo. Location	Salt Fresh Water	Obs./ Iso.	Ref.
Coho salmon (*Oncorhynchus kisutch*)	Chile	S[a]	I[c]	6
Rainbow trout (*Oncorhynchus mykiss*)	Chile	S	O/I[d]	9
Chinook salmon (*Oncorhynchus tshawytscha*)	British Columbia Canada	S	O/I	11
Atlantic salmon (*Salmo salar*)				
Coho salmon				
Pink salmon (*Oncorhynchus gorbuscha*)				
Atlantic salmon	Ireland	S	O[e]	13
Atlantic salmon	Norway	S	O/I	12
Coho salmon	Chile	F[b]	I	18
Rainbow trout				

[a] S = Host fish collected from salt water
[b] F = Host fish collected from fresh water
[c] I = Isolated in cell culture

The organism is smaller than *P. salmonis*, ca. 0.2-0.8 vs. ca. 0.5-1.5 in diameter, and both are usually coccoidal in shape. The unidentified bacterium was intracellular in spleen and kidney, did not grow on several types of bacteriologic media, and reportedly did not react with polyclonal antibodies against *P. salmonis* (18). The rickettsialike organisms observed in and/or isolated from nonsalmonid fish have not been characterized sufficiently to determine their relationship to *P. salmonis*. Taxonomic placement of these agents requires additional study.

Neorickettsia helminthoeca, the cause of the "salmon poisoning" disease of canids, is associated with fish but is not a fish pathogen. It is carried by the digenetic trematode, *Nanophyetus salmincola*, a parasite of salmonid fish in the Pacific Northwest (19). *N. helminthoeca* has been cultured in canine and murine cells but does not grow in cells of salmonid fish (20). Phylogenetically, *N. helminthoeca* is more closely related to the ehrlichiae and the rickettsia than it is to *P. salmonis* based on an analysis using the April 3, 1997, 16S rRNA gene sequence database containing 6,000 sequences. In addition, when *P.*

salmonis was tested with *N. helminthoeca* antiserum by indirect immunofluorescence, no reaction was observed (6).

There is no indication that *P. salmonis* or other rickettsial pathogens of fish cause disease in humans or other warm blooded animals. We speculate that the optimum temperature of 15°C to 18°C with no growth at 25°C and above prevents *P. salmonis* from becoming established in warm-blooded animals.

P. salmonis

Taxonomic Position

P. salmonis is the best characterized of the rickettsiae observed in and/or isolated from fish. The type species LF-89^T was isolated from a diseased coho salmon from a sea water netpen site in southern Chile during an epizootic. The organism has been placed in a new genus in the order *Rickettsiales*, family *Rickettsiaceae* based primarily on their similar morphology and obligate intracellular nature (6). It has been deposited with the American Type Culture Collection as ATCC VR 1361.

Many rickettsiae observed in or isolated from fish since 1989 have been identified serologically as *P. salmonis* (Table 2). We tested isolates from coho salmon and rainbow trout from Chile and from Atlantic salmon reared in Chile; British Columbia, Canada; and Norway. All five isolates are morphologically similar to LF-89^T and react with polyclonal antibodies against the type strain. Certain monoclonal antibodies developed in our laboratory can differentiate between these isolates (unpub. data). The 16S ribosomal DNA of these five isolates from three geographic locations were amplified by PCR to assess the genetic variability in this species or species-complex. The PCR products were sequenced and compared with other bacterial small subunit rRNA sequences. The genus Piscirickettsia belongs within the gamma subdivision of the proteobacteria and is not closely related to species of Erlichiae, Rickettsia, or Neorickettsia from the subdivision of alpha proteobacteria (Figure 1). The similarities

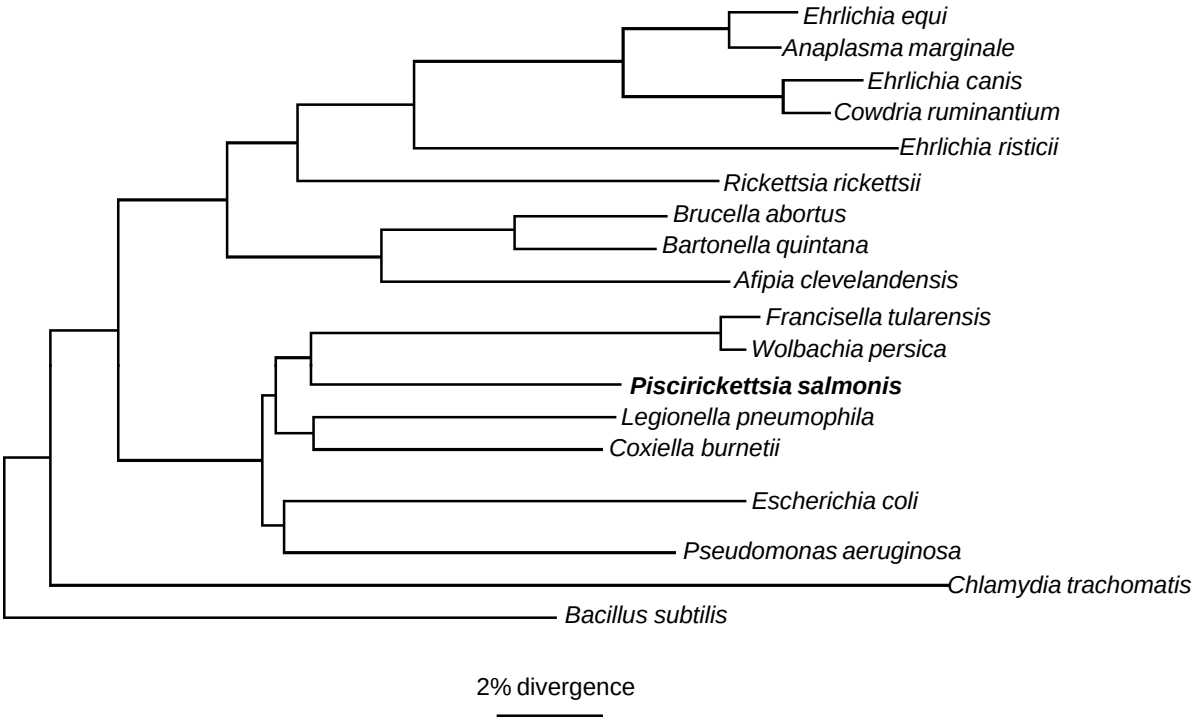

Figure 1. Phylogenetic relationships of *Piscirickettsia salmonis*, selected rickettsiae and bacteria. Evolutionary distances were calculated by the method of Jukes and Cantor (34). After eliminating regions of ambiguity and uncertain homology 1,313 positions of the 16S rDNA gene were compared.

between Piscirickettsia, Ehrlichiae, and Rickettsia species are approximately 80%. Similarities between Piscirickettsia, Coxiella, and Wolbachia are approximately 86% to 88%. The five *P. salmonis* isolates form a tight monophyletic cluster with relatedness of 99.7% to 98.5%. Two of the isolates from Chile and those from Norwegian and Canadian fish are closely related (>99.4% similarity). One isolate from Chile, EM-90, differed from the other four isolates (21). Similarity values for EM-90 were 98.5% to 98.9%.

To further clarify the genetic variability in this genus, the internal transcribed spacer (ITS) and 23S rDNA of six isolates have been analyzed. One spacer sequence was identified per isolate, and the region did not contain a tRNA gene. The ITS sequences were 311-bp in length and varied among the isolates (95.2% to 99.7% similarity). Only one ITS sequence was obtained for each of the *P. salmonis* isolates, suggesting the presence of one rRNA operon, which agrees with reports for other slow growing organisms (22).

Approximately 1,900-bp of the 23S rDNA gene have been analyzed for the six isolates, and similarities ranged from 97.9% to 99.8%. Three Chilean isolates and the Norwegian and Canadian isolates are closely related (99.1% to 99.7% ITS and 99.3% to 99.8% 23S rDNA similarities). The sequence of isolate EM-90 differed from those of the other five isolates (similarities ranged from 95.2% to 96.9% ITS and 97.6% to 98.5% 23S rDNA).

Phylogenetic trees constructed with the 16S, ITS, and 23S rDNA data showed similar topography, further reinforcing the relationships indicated between the isolates. Comparison of the *P. salmonis* ITS with the 16S rDNA region shows that it has diverged on average 3.15 times faster than the 16S rDNA gene, while the 23S rDNA gene has diverged 1.6 times faster than the 16S rDNA gene. This is similar to findings with other obligate intracellular bacteria (23).

Characteristics of the Type Strain LF-89^T

P. salmonis, type strain LF-89^T is a nonmotile, gram-negative, obligatedly intracellular bacterium. It is predominately coccoid (ca. 0.5-1.5 µm in diameter) and also occurs as rings or pairs of curved rods (Figure 2). It replicates within membrane-bound cytoplasmic vacuoles (Figure 3) in selected fish cells lines and in the cells of tissues throughout infected fish. Thin sections of the bacterium examined by electron microscopy display typical gram-negative cell walls and the protoplasmic structure of a prokaryote. Giemsa stains the rickettsiae cells dark blue. LF-89^T does not react with the monoclonal antibody made against the group-specific chlamydial LPS antigen (5).

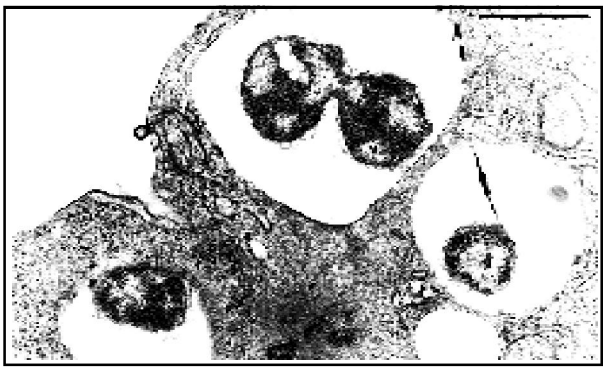

Figure 2. *Piscirickettsia salmonis* within a cytoplasmic vacuole in CHSE-214 cell line 4 days post inoculation. Note organisms dividing within vacuole. May Greenwald-Giemsa stain. Bar = 1 µm.

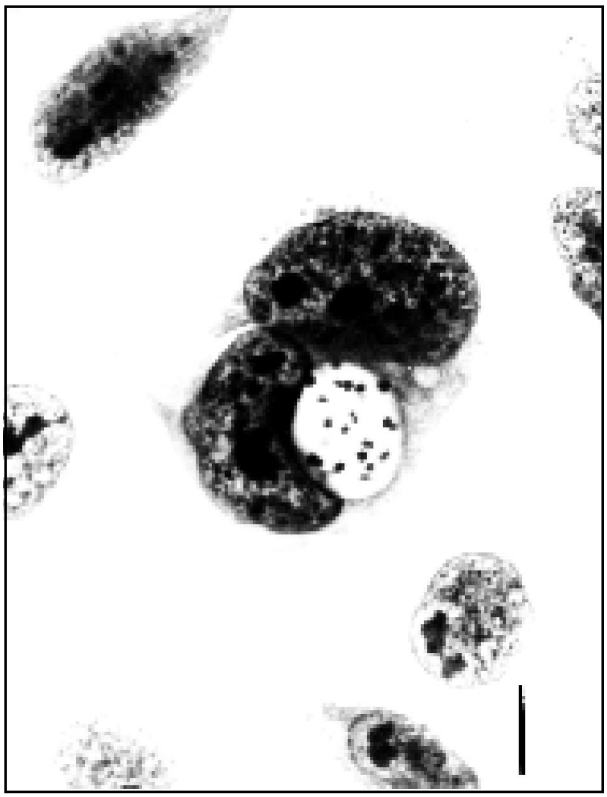

Figure 3. *Piscirickettsia salmonis* undergoing apparent binary fission within a vacuole in the cytoplasm of infected CHSE-214 cells. Bar = 10 µm.

P. salmonis can be cultivated in certain fish cell lines where it produces a cytopathic effect. It does not replicate on any known cell-free media, and its in vitro growth characteristics have been described (6). Replication is optimal at 15°C to 18°C, retarded above 20°C and below 10°C, and does not occur above 25°C. It replicates to titers of 10^6 to 10^7 $TCID_{50}$ ml^{-1} in fish cell cultures. Titers are decreased 99% or more by a single cycle of freeze-thaw at -70°C. The addition of 10% DMSO to the freezing medium acts as a cryopreservative. In vitro, it is sensitive to a broad range of antibiotics.

Tenfold dilutions of spent medium from an LF-89^Tinfected cell culture injected into groups of 40 juvenile coho salmon and groups of 30 juvenile Atlantic salmon resulted in death rates of 88% to 100% during a 42-day experiment (8). Typical signs of the disease were present in the inoculated experimental coho, whereas in Atlantic salmon, the only gross sign of disease was death. The LD_{50} was not obtained, but death in both species followed a dose-response pattern, and LF-89^T was reisolated from each group inoculated.

Extracellular survival of LF-89^T was tested under selected environmental conditions (24); infectivity remained for at least 14 days in preparations of semipurified LF-89^T suspended in high salinity sea water at 5°C, 10°C, and 15°C. Infectivity was rapidly reduced in preparations suspended in fresh water. Titers dropped below the level of detection (10^2 $TCID_{50}$ ml^{-1}) immediately after suspension in fresh water, and no infectious material could be recovered from these preparations.

Piscirickettsiosis

P. salmonis produces an epizootic disease of fish called piscirickettsiosis. Death rates associated with piscirickettsiosis in salmonids range from a high of 90% among coho in Chile (25) to a low of 0.06% in Canada and Norway (9,11). All species of salmonids cultured in Chile are affected by this disease, but the highest death rates occur in coho salmon cultured in salt water. Fish in sea water netpens begin to die 6 to 12 weeks after their transfer from fresh water (5). Deaths peak in the fall and rise again the following spring (26).

A variety of clinical signs are associated with *P. salmonis* infection, but few are specific to piscirickettsiosis. Moribund fish collect at the water surface along the edges of the sea cages; they are lethargic, dark, and show loss of appetite (9); the gills are pale, and hematocrits are frequently 25% or less. The first signs observed are often hemorrhages and lesions of the skin. The lesions range from small areas to shallow ulcers up to 2 cm in diameter (25). Internally, the kidney is swollen and the spleen enlarged. Petechial hemorrhages are found on the swim bladder and viscera. Diagnostic ringshaped, cream-colored lesions are present on the livers of chronically infected fish (Figure 4). In acute cases, death may be the only gross sign of disease (27).

The histopathologic symptoms associated with *P. salmonis* infection and the disease it causes have been described, but a great deal of work remains to be done (25-27). Piscirickettsiosis produces marked pathologic changes in most internal organs of infected fish, where severe changes occur in the intestine, kidney, liver, and spleen. Necrosis and inflammation may occur throughout the body, especially in cells adjacent to blood vessels. Epithelial hyperplasia results in lamellar fusion of the gills. The bacterium is commonly observed within macrophages and in the cytoplasm of infected host cells.

Mode of Transmission

The source, reservoir, and means of transmission of *P. salmonis* continue to be important

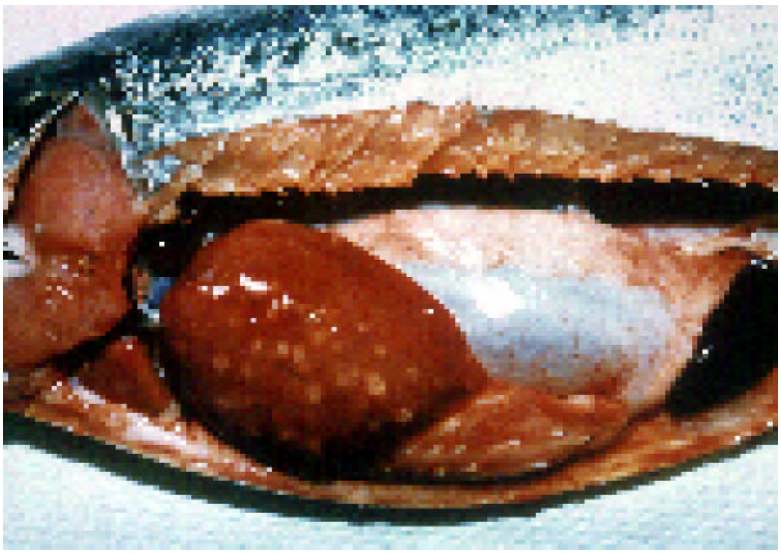

Figure 4. Coho salmon infected with *Piscirickettsia salmonis*. Note cream-colored lesions on liver, enlarged spleen, pale gills, and hemorrhaged areas within the peritoneal cavity.

areas of study. Although piscirickettsiosis has been observed in salmonid fish over a widespread geographic area (Table 2), with the exception of the predictable annual epizootics in Chile, the infections seem sporadic and of limited virulence in other parts of the world. No association other than the marine environment and the host species is apparent between locations in Canada, Norway, and Ireland. One or more naturally occurring aquatic animals, perhaps only transiently present, may provide the source and reservoir of these rickettsiae in the widely separated diverse areas. No alternate host has been identified.

P. salmonis has been experimentally passed by injection (8,26), but the normal mode of transmission for this agent (horizontal, vertical, or through a vector) has not been demonstrated. Except in *Coxiella burnetii*, an intermediate host or vector is required for transmission of rickettsiae in the terrestrial environment (28). *C. burnetii* not only has an intermediate host but appears to develop a sporogenic phase that protects it from drying when it is host or vector free. No vector or sporogenic phase has been observed for *P. salmonis*. *P. salmonis* may not require an intermediate host or sporogenic stage for survival and transmission in the aquatic environment. The extended extracellular survival time of this organism in salt water (24) may be of sufficient duration to permit horizontal transmission without a vector. The almost immediate deactivation of the rickettsia in fresh water makes direct horizontal transmission unlikely unless *P. salmonis* was protected by host(s) cell membranes or tissue exudates.

Limited research designed to demonstrate the role of horizontal transmission has provided mixed results. Garcés et al. (8) saw no evidence of horizontal transmission between injected coho salmon dying of piscirickettsiosis and uninjected salmon held in a cage in the same tank of flowing fresh water. Cvitanich et al. (26) reported horizontal transmission between injected and sham-injected coho salmon held in static fresh and sea water aquaria. Environmental conditions differed between experiments (e.g., flowing water at a mean temperature of 10.5°C vs. static water at 15°C), therefore, meaningful comparisons could not be made. Nevertheless, this research suggests that under certain circumstances, horizontal transmission is possible.

Although rickettsiae are found in the gonads of infected fish, vertical transmission of *P. salmonis* has not been demonstrated. The limited number of piscirickettsiosis cases reported in fresh water indicate that vertical transmission is, at best, rare. Until definitive studies are conducted, the questions of source, reservoir, and normal mode of transmission of *P. salmonis* remain unanswered.

Detection and Identification

Inoculation of susceptible fish cell lines is the most sensitive method for detecting *P. salmonis* (29). However, isolation of an infectious agent sensitive to low levels of antibiotics routinely used in cell culture presents a problem. All cultures must be maintained in antibiotic-free medium.

Diagnostic specimens collected aseptically in the field may become contaminated by other bacteria. For this reason, preliminary diagnosis of piscirickettsiosis is normally made by examining Gram, Giemsa, methylene blue, or acridine orange-stained kidney imprints or smears, and confirming their identity by serologic methods, e.g., immunofluorescence (13) or immunohistochemistry (30).

A nested polymerase chain reaction (PCR) using universal 16S rDNA bacterial outer primers and *P. salmonis*-specific internal primers was developed to detect the genomic DNA. The nested PCR assay allowed detection of less than one *P. salmonis* tissue culture infectious dose 50 ($TCID_{50}$). Using the *P. salmonis*-specific primers in a single amplification allowed detection of 60 $TCID_{50}$. The specificity of PCR was assessed with a panel of four salmonid and 15 bacterial genomic DNA preparations. Products derived from amplification were observed only from *P. salmonis* DNA (31).

Restriction fragment length polymorphism analysis of the 16S rDNA products from six isolates of *P. salmonis* demonstrated that one isolate, EM-90, was different. Two additional primers were developed that differentiate EM-90 from the other five *P. salmonis* isolates (31).

PCR using DNA extracted from spleens of tilapia or paraffin-embedded tissue of the blue-eyed plecostomus did not produce amplification products. These tissues were collected and examined from fish during an epizootic caused by rickettsialike organism (unpub. data). Amplification using 16S rDNA universal primers was successful, which suggests that the rickettsialike organism infecting these fish were not *P. salmonis*.

Control of the Disease

Although *P. salmonis* is sensitive in vitro to many of the antibiotics commonly used to control other infectious diseases of fish, e.g., tetracycline, erythromycin, and oxolinic acid (6,26), these preparations have not been useful in treating fish with piscirickettsiosis. Antibiotic levels may not reach sufficient concentrations within the host cells in vivo, to terminate replication of the pathogen.

The lack of effective methods for treating piscirickettsiosis has encouraged the development of disease prevention techniques. Vaccines have been successfully used for control of certain gram-negative bacterial diseases of fish; however, at present no efficacious preparations have been developed to protect fish against *P. salmonis*.

Questions remain concerning *P. salmonis* and the disease it causes in fish. The reservoir(s) of the infectious agent should be determined if the spread of the disease is to be controlled (32). There is a need to determine the mode(s) of transmission of *P. salmonis* to fully understand the pathogenesis of piscirickettsiosis. The infectivity of *P. salmonis* for native, nonsalmonid fish has not been investigated. Difference in virulence between *P. salmonis* in Chile and salmonids in the Northern Hemisphere is an important consideration. It must be determined if intrinsic differences in the isolates, the host fish, the environment, or some combination of factors is responsible for these differences. The rickettsiae from salmonid fish in the fresh water and marine environments should be compared, and the relationships between the rickettsiae from salmonid fish and those from other fish species should be determined.

A new group of fish pathogens was recognized in 1989 with the isolation and identification of *P. salmonis* LF-89^T from cultured salmonids in Chile (5,6). The rickettsial etiology of an infectious disease of fish in a variety of locations and host species suggests that they are an important group of emerging fish pathogens (33). These pathogens have caused severe mortality among cultured salmonids in Chile and are not present among stocks of important food fish cultured in British Columbia, Canada, Norway, and Ireland. Taiwan has experienced serious losses amon talapia, another important food fish. At least one species imported for aquaria are also infected with a rickettsialike agent. The lack of antimicrobial drugs and vaccines for prevention and control emphasize the need for additional research. The method of transmission of *P salmonis* is not understood and needs to be researched. The movement of fish and spread of the agent make the develoment of improved diagnostic techniques important.

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Rhodococcus equi and *Arcanobacterium haemolyticum*: Two "Coryneform" Bacteria Increasingly Recognized as Agents of Human Infection

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Rhodococcus equi and *Arcanobacterium haemolyticum,* formerly classified in the genus *Corynebacterium,* are members of the loosely defined taxon "coryneform" bacteria. Although they are the etiologic agents of distinct human infections, both organisms are frequently overlooked, which results in missed or delayed diagnoses. *R. equi,* long known as an important pathogen of immature horses, has become in the past three decades an opportunistic pathogen of severely immunosuppressed humans. Most cases are secondary to HIV infection. When specifically sought in throat swab cultures, *A. haemolyticum* is found responsible for 0.5% to 2.5% of bacterial pharyngitis, especially among adolescents. These two microorganisms represent a spectrum of disease in humans: from a mild, common illness to a rare life-threatening infection. Each organism elaborates lipid hydrolyzing enzymes (cholesterol oxidase by *R. equi* and sphingomyelinase D by *A. haemolyticum*) that are toxic to animals and humans and damaging to mammalian cell membranes. The participation of the cytotoxins in pathogenicity is discussed. Greater awareness of the properties of these two bacteria may promote faster, more accurate diagnoses and better clinical management.

A variety of factors contribute to the underreporting of human infections caused by bacteria in the genus *Corynebacterium* and closely related genera. The group, often referred to as "coryneform," comprises taxonomically diverse gram-positive rods resembling *Corynebacterium diphtheriae* and displaying pleomorphism and irregular cellular arrangements (1,2). The group includes human and animal pathogens, as well as commensal bacteria. The control of diphtheria in industrialized countries and the subsequent deemphasis of the genus *Corynebacterium* have contributed to discounting isolates characteristic of the genus as contaminants. Even reference laboratories report difficulty in the speciation of gram-positive pleomorphic rods that resemble corynebacteria (1). Because of the emergence of a number of coryneform bacteria as important human pathogens, rigorous biochemical and molecular tools have increasingly been applied to isolates. The resulting improved epidemiology and taxonomy have led, for example, to the definition of CDC groups JK and D-2 in the genus *Corynebacterium*, now recognized as important opportunistic pathogens (1). Similarly, more accurate characterization of some species caused them to be removed from the genus *Corynebacterium*. Excellent reviews of the pathogenicity and epidemiology of these diverse genera have been published (1,2). This article explores two pathogenic coryneform bacteria: *Rhodococcus equi,* a rare often fatal human pathogen, in which virtually all human infections occur among compromised hosts; and *Arcanobacterium haemolyticum*, which is responsible for many respiratory infections in healthy people. This article aims to bring about improved recognition of these two easily overlooked pathogens and considers mechanisms underlying the diseases, the immune response of the hosts, and treatment protocols.

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Epidemiology and Clinical Presentation

R. equi

Originally isolated by Magnusson in 1923 from granulomatous lung infections in young horses (3), *Corynebacterium* (now *Rhodococcus equi*) remains an important pathogen of foals. Much of the considerable body of knowledge about *R. equi*, including its pathogenicity and immune response to infection, derives from veterinary studies and has been recently updated (4).

R. equi is readily found in soil, especially where domesticated livestock graze (5). The stool of horses and other animals is the source of soil contamination. Infection in humans derives from environmental exposure (2,5), and the organism may be ubiquitous in soil (6). While early cases occurred mostly in persons with a history of contact with horses, only 20% to 30% of recent cases can be traced to such contact (7). A review of cases in the three decades since the first reported human infection in 1967 is presented in Table 1.

R. equi is a rare opportunistic pathogen found in severely compromised patients, and most commonly in recent years, in human immunodeficiency virus (HIV)-infected persons. Early cases, most in patients receiving immunosuppressant therapy, were more likely to be successfully treated with antimicrobial agents than cases in AIDS patients (8). Most often, patients have a slowly progressive granulomatous pneumonia, with lobar infiltrates, frequently developing to cavitating lesions visible on chest x-ray. Other sites of infection include abscesses of the central nervous system, pelvis, and subcutaneous tissue, and lymphadenitis (7,8-10). Cases of lung infection caused by inhalation and cutaneous lesions caused by wound contamination have been documented; the latter are almost the only *R. equi* infections reported in healthy persons, frequently children (11). Delays in accurate diagnosis of *R. equi* are still common (2,7), despite increased awareness of this organism as an opportunistic pathogen in humans. Factors for delayed diagnosis include the insidious onset of disease, clinical resemblance of the infection to mycobacterial, fungal, and actinomycotic infections, and the relatively non-descript bacteriologic profile of *R. equi*. Morphology, partial acid fastness, and a distinctive histopathologic profile in bronchial specimens (Figure 1 A and B) contribute to accurate diagnosis. Numerous polymorphonuclear leukocytes with intracellular pleomorphic gram-positive bacteria, microabscesses, pseudotumors, and malakoplakia are noted on tissue (7,11). Malakoplakia is a relatively rare granulomatous inflammation not

Table 1. *Rhodococcus equi* case reports in humans: 1967–1996

Years	Cases	Predisposing condition (number)	Primary site of infection (number)	Deaths	References[a]
1967–76	7	Corticosteroid (1) Cancer/immunosuppressant (3) Renal transplant (2) None[b] (1)	Lung (6) Lymphatic (1)	0	8–10
1977–86	15	Corticosteroid (1) Cancer/immunosuppressant (4) Renal transplant (2) HIV (7) Alcoholism (1)	Lung (14) Blood (1)	8	8–10,22
1987–96	93	Cancer/immunosuppressant (8) Renal transplant (3) HIV (67) Other[c] (8) (1[b]) None (7) (4[b])	Lung (72) Lymphatic (2) Blood (8) Wound (6) Other[d] (5)	34	8–13,22

[a]In the interest of space, case compilations are cited in lieu of individual case reports.
[b]Child
[c]Includes intravenous drug use, lab infection, emphysema, kidney disease.
[d]Central nervous system, gastrointestinal

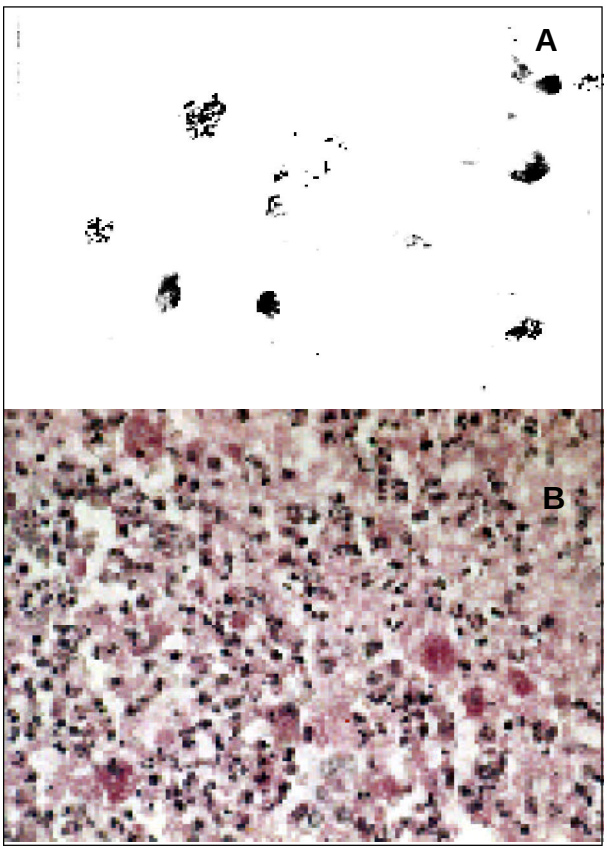

Figure 1. A. Bronchial tissue Gram stain showing intrahistiocytic coccobacillary forms of *Rhodococcus equi*. Original magnification, x 1,000. B. Open lung biopsy showing coalescent microabscesses with numerous histiocytes containing *Rhodococcus equi* organisms. PAS stain. Original magnification x 250. Figure provided by Dr. Margie Scott, Vanderbilt University Medical Center.

typically associated with histology of lung infection and can be of help in forming a differential diagnosis (11,12). Firm diagnosis and differentiation from similar pathogens require the isolation and identification of *R. equi* from sputum, bronchial washings, open-lung biopsy, or other specimens reflective of pathology. Blood cultures from severely immunosuppressed patients with focal *R. equi* infection often contain the organism. In sixty-five percent of cases secondary to HIV infection, the organism is found in patients' blood cultures (11). Deaths exceed 50% among AIDS patients with documented *R. equi* pneumonia and are almost always preceded by multiple relapses, which are common even when successful treatment is ultimately achieved (Table 1; 13).

Corynebacterium haemolyticum

A. haemolyticum was first described and named by MacClean et al. (14), who isolated it from pharyngeal infections in U.S. soldiers and natives in the South Pacific. Classification of the organism generated controversy until the definition in 1982 of a new genus, *Arcanobacterium* (secretive bacterium), in which it remains the only species (15).

Unlike *R.equi* infection, where invasive clinical disease underscores the need to detect and identify the causative agent of infection, *A. haemolyticum* infection is often reported from deliberate screening for the organism of a large number of patients with sore throats. After it was identified during World War II from patients with pharyngitis (14), it was occasionally reported from Europe, the United States, and in 1981, Sri Lanka (16 cases) (16). Most cases involve pharyngitis and/or tonsillitis, and approximately 50% are exudative. Throat infections are often accompanied by cervical lymphadenopathy (17,18). Diagnosis of cases (distinct from screening studies) often occurs only after recurrent infections, which are thought to be related to incorrect initial diagnosis, resulting in less-than-optimum treatment (19). Infection is most common in 15- to 25-year-old persons, and is thought to result from droplet transfer from infected persons (20). Symptoms resemble those of ß-hemolytic streptococci or viral infection. The spectrum of disease ranges from sore throat to, in rare cases, a life-threatening membranous pharyngitis resembling diphtheria (18,20). An erythematous morbilliform or scarlatinal rash of the trunk, neck, or extremities is associated with 20% to 25% of cases (19), enhancing the possibility of misdiagnosis as streptococcal infection or penicillin allergy, because ß-lactam therapy is frequently initiated without accurate diagnosis. A recent report des-cribes in detail the dermatologic manifestations of *A. haemolyticum* infection (20).

The demonstration that *A. haemolyticum* is not a component of the human commensal flora was essential to establishing its role in human infection. Studies of more than 2,000 cases each found the organism only in association with clinical symptoms (17,19). Table 2 summarizes several case compilations, including the incidence of infection among culture-positive bacterial sore throats, as well as data on coinfection. Some 0.5% to 3% of cases of pharyngitis can be traced to *A. haemolyticum* depending on the population studied, with the highest numbers among 15- to

Table 2. Representative pharyngitis screenings for *Arcanobacterium haemolyticum*

Period of study (reference)	Number of isolations	Rash	Coinfection	Incidence[a] (%)	Clinical features (population)
1978–80 (16)	16	0	13 (*C. diphtheriae, S. pyogenes, E. coli, P. aeruginosa*)	NR	Symptomatic throat swabs and pyoderma (Sri Lanka)
1981–85 (19)	81[b]	37	NR	2.0	Symptomatic throat swabs (Sweden)
1990–92 (17)	42	17	NR	0.36	Symptomatic throat swabs; 5 cases monospot positive (Ottawa, Canada)
1991–92 (42)	19	5	11 (streptococci, groups A, B, G)	0.49	Symptomatic throat swabs, 3922[c]; (Finland)
1992–95 (43)	16	12	5 (streptococci, groups A, B, ß-hemolytic)	0.75	Symptomatic throat swabs, 2121[c]; (Czech Republic)

NR-not reported
[a] Incidence refers to the proportion of sore throat cases in each study cited yielding *A. haemolyticum*.
[b] 1 of 550 asymptomatic specimens yielded *A. haemolyticum*.
[c] Figures refer to the total specimens screened.

30-year-old patients (19). Clearly, accurate diagnosis depends on differentiating *A. haemolyticum* from more common pathogens. *A. haemolyticum* occurs relatively often in polymicrobic infections together with typical respiratory pathogens such as streptococci. The isolation of classical pathogens from specimens that also contain *A. haemolyticum* exacerbates the tendency to overlook the organism.

Taxonomy, Bacteriology, and Differential Identification

R. equi

On the basis of the chain length of mycolic acids and other properties of its lipids, *R. equi* was reclassified in the suprageneric taxon nocardioform actinomycetes (1,2,5). *R. equi* is a strictly aerobic gram-positive bacterium displaying rod-to-coccus pleomorphism, with fragmenting and occasionally palisading forms. It is nonfastidious. Colonies on blood agar from clinical specimens can be mucoid and coalescing. Typical salmon pink pigmentation develops on blood agar, but often only after 2 to 3 days incubation. Growth on Lowenstein-Jensen medium allows earlier detection of pigment (M. Scott, pers. comm.). Positive routine biochemical tests include catalase and urease, but *R. equi* is generally nonreactive. Acid-fast staining of direct smears and fresh isolates is helpful in identification but is rarely observed on subculture (5,7). Some diagnostic laboratories use a commercial kit (API Coryne strip (bioMerieux-Vitek, Hazelwood, MO) for identification.

Also helpful in identifying *R. equi* is synergistic hemolysis (resembling the CAMP test), displayed by cross-streaking on sheep blood agar with any of a number of other bacteria, including *A. haemolyticum*, *Staphylococcus aureus*, and *Corynebacterium pseudotuberculosis* (21; Figure 2). Synergistic hemolysis is discussed among mechanisms of pathogenesis (below). In addition, antagonism between imipenem and other ß-lactam antibiotics used against strains of *R. equi* provides the opportunity of differentiating the organism from taxonomically related species (22).

A. haemolyticum

Organisms are gram-positive rods—slender at first, sometimes clubbed, or in angular arrangements. Coccal forms predominate as the organism grows. The organism is facultatively anerobic. Growth is enhanced in blood and in the presence of CO_2. Some sugars are fermented, and the organism is catalase negative. Hemolysis is best observed on human blood, and Gaston et al. (20) suggest routine plating of specimens suspected of containing *A. haemolyticum* on human blood agar to distinguish *Streptococcus pyogenes*. Pitting beneath colonies on human blood agar is helpful in identification. Synergistic hemolysis with *R. equi* (or inhibition of the hemolytic zone of *S. aureus*, (Figure 2) is useful in

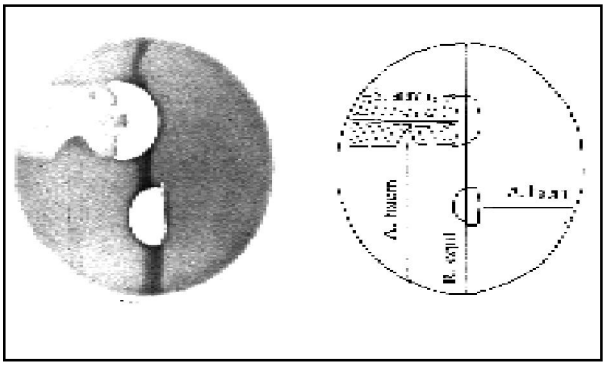

Figure 2. Cooperative (and antagonistic) hemolytic reactions on sheep blood agar, demonstrating cooperative hemolysis between *Rhodococcus equi, Arcanobacterium haemolyticum*, and *Staphylococcus aureus*. Partial hemolysis by *S. aureus* (cross-hatched on diagram) is inhibited in the proximity of *A. haemolyticum*.

identification, especially as it may rule out group B streptococci. Poor growth on tellurite assists in differentiation from *Corynebacterium diphtheriae.*

Pathogenesis and the Immune Response

R. equi

Because *R. equi* is a rare and recently emergent cause of human infection, mechanisms of its pathogenicity are not well defined. However, the much-studied infection in foals and an experimental model in mice provide data which, together with the available human data, give insight into the workings of the pathogen. Its nature as a facultatively intracellular bacterium, able to persist, grow, and ultimately destroy macrophages (23-25), is the property of *R. equi* most closely associated with virulence in each host. Foal alveolar macrophages having ingested *R. equi* did not undergo phagosome-lysosome fusion and were irreversibly damaged in electron micrographs (26). Results of pathology tests (microscopy and roentgenography) reflect significant inflammation, consistent with that found in such other intracellular pathogens as *Mycobacterium tuberculosis,* which elude pulmonary clearance. Open lung biopsies in humans show numerous polymorphonuclear leukocytes, foam cells, and cavitating lesions with intracellular bacteria (7,11; Figure 1). Also as in mycobacteria, cell wall mycolic acids are present. These acids may contribute to the ability of *R. equi* to grow in macrophages; virulence of strains for mice was found related to the carbon chain length of the molecules (27). Granuloma formation was observed when killed *R. equi* strains, regardless of virulence, were introduced into inbred mice, supporting a role for mycolic acids or other cell wall glycolipids in pulmonary inflammation (28).

A key contributor to virulence in the foal and the mouse model is a group of large (85-90 kb) plasmids, encoding 15-17 kDa antigens among strains isolated from almost all natural infections in foals (29). Strains cured of the plasmid are cleared in experimental infections, and intracellular replication in murine macrophages is greatly diminished in its absence (25,28). In contrast, of 39 strains isolated from humans (29 with AIDS), 31 strains did not express the virulence associated plasmid and were nonvirulent in mice (6). The investigators suggest that intracellular growth and, therefore, virulence among human strains may not be explained by the same determinants as foals, while mycolic acids may play a role.

Nordmann (23) found that intracellular growth in mouse and human macrophages of strains isolated from AIDS patients was related to ß-lactam resistance, the production of a bacteriophage, and virulence in inbred mice. Virulence was not attributable to 17kDa virulence antigens, but soluble cytotoxic substances were associated with the virulent phenotype. The cytotoxic activity remains to be characterized, and its relationship to known cytotoxic activities of *R. equi* remains to be elucidated. In addition to numerous hydrolytic enzymes typical of the genus *Rhodococcus* (5), strains of *R. equi*, irrespective of virulence, produce cholesterol oxidase, which is responsible for the organism's participation in synergistic hemolytic reactions with other bacteria (30; Figure 2). Experiments using cultured mouse macrophages with phagocytosed *R. equi* suggest a role for cholesterol oxidase in macrophage destruction in infections. Macrophages undergo oxidation of membrane cholesterol, and the accumulation of oxidized cholesterol is significantly enhanced by the cophagocytosis of *C. pseudotuberculosis,* a related coryneform bacterium producing sphinomyelinase D (31). Toxicity to vertebrates as a result of enzymatic oxidation of membrane cholesterol is documented in diverse systems, most dramatically by lethality to hypercholesterolemic rabbits (31).

Because *R. equi* is uniquely an opportunistic pathogen in humans, it is of interest to consider the precise nature of the immune deficiency that

underlies susceptibility to this organism. Recent investigations in immunodeficient mice are especially instructive. T-cell subsets, specifically functional CD4+ lymphocytes, are necessary to effect complete clearance of *R. equi* challenge (23,32). Specifically, Kanaly et al. showed that CD4+ Th1 cells (expressing interferon-gamma) are sufficient to achieve clearance from the lungs of mice (32,33). Consistent with these data, peripheral mononuclear cells, from patients with AIDS, challenged in vitro with *R. equi* failed to secrete high levels of interferon-gamma in comparison with cells from healthy donors (34). Investigations implicating a specific defect in the Th1 phenotype in the pathogenicity of AIDS make these studies especially provocative and suggest a role for immunotherapy in *R. equi* infection (33).

While cell-mediated immunity appears to have a primary role in protecting against *R. equi* infection, the participation of humoral antibody has been established in foals. Passive immunization with hyperimmune serum is efficacious in prophylaxis, and severity of disease is inversely related to circulating antibody (1,5). Mastroianni et al. (35) demonstrated antibody to the major antigens of *R. equi* in four AIDS patients with cavitating pneumonia. The role of such antibody in the natural history of the infection remains to be elucidated.

A. haemolyticum

Little is known about the mechanisms by which *A. haemolyticum* produces infection or brings about the skin manifestations frequently associated with it. The organism is known to produce uncharacterized hemolytic agent(s) (20) and two biochemically defined extracellular products: a neuraminidase and a phospholipase D (PLD) acting preferentially on sphingomyelin and generating ceramide phosphate in the target membrane (36). Of these, PLD is known to bring about tissue damage, as elaborated by this organism as well as the closely related bacterium, *C. pseudotuberculosis,* an important pathogen of sheep. Soucek et al. (36) found that the enzyme was responsible for the dermonecrotic, as well as the synergistic hemolytic, activity of the organisms that elaborate it. The PLD gene from *A. haemolyticum* has been cloned and shown to have a high degree of homology with that of *C. pseudotuberculosis,* where it is thought to participate in vascular permeability and dissemination of the pathogen (37). Evidence relates PLD with toxicity of *C. pseudotuberculosis*. Targeted mutagenesis of the PLD gene of *C. pseudotuberculosis* confirmed the role of the enzyme in virulence and specifically in dissemination in the host (38). Mutant bacteria had a reduced ability to establish infection in goats and were unable to disseminate by the lymphatics to secondary sites. A PLD sharing many properties with corynebacterial PLDs, including biochemical and biological activities, is responsible for the toxicity of the venom of the brown recluse spider (39). The role of potentiated cytotoxicity caused by the combined activity of PLD and cooperative agents such as cholesterol oxidase in disease is not established, but suggested by in vitro data involving cophagocytosis as described above (31).

Treatment

R. equi

Increased recognition of *R. equi* as a cause of life-threatening infection in severely immunocompromised persons has promoted a number of studies of in vitro antimicrobial susceptibility of clinical isolates (11,13,40,41). While variations exist, most strains were susceptible to inhibition by glycopeptide antibiotics (including vancomycin and teicoplanin) and rifampin. Macrolide antibiotics, such as erythromycin and clarithromycin, were also inhibitory to many strains. Resistance to ß-lactam antibiotics (with the exception of carbapenems, specifically imipenem) was generally reported, and is not related to the production of a ß-lactamase.

Because of relapse in spite of treatment in a majority of cases (11) and high mortality rate, especially among AIDS patients (Table 1), there is no standard treatment protocol for pulmonary and/or systemic *R. equi* infections. However, several principles reflect the accumulated experience of investigators. Careful and repeated culture and susceptibility testing during treatment is required to discover acquired resistance, in a manner similar to the treatment of mycobacterial infection (11,22). Tolerance to the cidal effects of some drugs and the need for long-term therapy (generally 2 months to life-long treatment; 40,41) make bactericidal testing a useful addition to laboratory studies. In consideration of the severe immunosuppression of patients and proclivity to relapse, investigators generally promote a combination of at least two drugs parenterally (usually including a

glycopeptide or rifampin) followed by oral maintenance therapy (11,41). Recommendation of lipophilic antimicrobials that penetrate macrophages is controversial (11,41). A proposed regimen involves parenteral glycopeptide plus imipenem for at least 3 weeks, followed by an oral combination of rifampin, plus either macrolides or tetracycline (41). Examples of efficacious protocols for parenteral treatment are available (Table 1).

Surgical lung resection has been reported occasionally since the emergence of human cases, especially where large focal lesions develop (9,10), and has sometimes been efficacious in combination with antimicrobial therapy. As cases of *R. equi* continue to be recognized among AIDS patients, antimicrobial prophylaxis against this opportunistic pathogen may prove a benefit.

A. haemolyticum

In vitro testing of *A. haemolyticum* isolated from human infections shows susceptibility to erythromycin, gentamicin, clindamycin, and cephalosporins (42). Reports of treatment failure with penicillin in spite of low minimum inhibitory concentrations have been attributed to tolerance and to failure to penetrate the intracellular location of the pathogen. Erythromycin has been proposed as the drug of choice, with parenteral antimicrobial drugs used for serious infections (20). The general similarity of the susceptibility pattern of *A. haemolyticum* to more commonly encountered pharyngeal pathogens, including *S. pyogenes*, makes culture and accurate diagnosis essential if cases are to be recognized for their true etiology. Its participation in polymicrobic infections (Table 2) requires that *A. haemolyticum* be specifically sought in appropriate specimens to obtain accurate diagnosis and to allow epidemiologic analysis.

R. equi and *A. haemolyticum* represent distinct poles of infectious disease: one a ubiquitous soil organism producing life-threatening opportunistic infections and the other a readily treatable respiratory infection of healthy young persons. In both instances, a high degree of suspicion is required to make accurate and timely diagnoses of infections. Diagnostic failure may result in a graver clinical profile including deaths for *R. equi* and, in many undiagnosed or misdiagnosed cases, for *A. haemolyticum*. As members of the morphologically defined taxon "coryneform" bacteria, these organisms exemplify properties of the group that require further elucidation. Weakly pathogenic and noninvasive, the group includes environmental bacteria; animal pathogens "crossing-over" to become human opportunistic pathogens; commensals similarly infecting compromised hosts; and producers of a wide variety of hydrolytic enzymes bearing a poorly defined relation to virulence (1). Both *R. equi* and *A. haemolyticum* elaborate a cytotoxic protein (cholesterol oxidase or sphingomyelinase D) known to be responsible for systemic harm to animals. Coincidentally, these products potentiate each other's cytotoxic action. The participation of the agents in harm to a host, alone or in combination with other substances, is consistent with available data, but yet unproven. Of particular interest is the role of cholesterol oxidase in destruction of alveolar macrophages in *R. equi* pneumonia.

Clarifying the role of synergistic or cooperative cytotoxins in one or more infectious diseases will surely improve our understanding of others because of the common occurrence of these agents among bacterial pathogens (21). It is difficult to envision the potentiated hemolytic combination of *R. equi* and *A. haemolyticum* at the site of an infectious lesion. However, cooperatively hemolytic combinations have been shown to result from the partnership of hydrolytic enzymes (e.g., phospholipases, which are ubiquitous in tissue) with the cytotoxins of pathogenic bacteria (10). Similarly, the hydrolytic enzymes of commensal bacteria or copathogens that occur, for example, in *A. haemolyticum* pharyngitis, can readily be envisioned to participate in potentiated cytotoxicity in host tissue. Together with improved recognition of these two pathogens, greater understanding of their toxic products should prove beneficial.

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Is Creutzfeldt-Jakob Disease Transmitted in Blood?

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Creutzfeldt-Jakob disease (CJD) has been considered infectious since the mid-1960s, but its transmissibility through the transfusion of blood or blood products is controversial. The causative agent's novel undefined nature and resistance to standard decontamination, the absence of a screening test, and the recognition that even rare cases of transmission may be unacceptable have led to the revision of policies and procedures worldwide affecting all facets of blood product manufacturing from blood collection to transfusion. We reviewed current evidence that CJD is transmitted through blood.

Creutzfeldt-Jakob disease (CJD), a rare neurodegenerative disorder, affects 0.5 to 1 persons per million population worldwide each year (1-8). CJD is a human spongiform encephalopathy; others are kuru, which is associated with ritualistic cannibalism in the Fore tribe of Papua New Guinea; Gerstmann-Sträussler-Scheinker syndrome, an inherited disorder; and fatal familial insomnia, inherited as an autosomal-dominant trait. Animal spongiform encephalopathies include scrapie, bovine spongiform encephalopathy (BSE), transmissible mink encephalopathy, and wasting disease of elk most frequently referred to as transmissible spongiform encephalopathies; other names such as prion dementias, transmissible degenerative encephalopathies, and infectious cerebral amyloidoses are also used.

The classic clinical symptoms of CJD are rapidly progressive presenile dementia, myoclonus, and progressive motor dysfunction. No treatment is available, and survival averages less than 1 year (most often 2 to 6 months) (9). Diagnosis is based on symptoms, electroencephalograms, and neuropathologic tests (10,11).

The pathophysiology of CJD is incompletely understood, although it is known that in persons with the disease, the normal soluble prion protein (PrP^{C}) is conformationally shifted into a more stable, less soluble ß-pleated protein. The term PrP^{CJD} indicates the abnormal isoform found, with a variety of distribution patterns in the brain of persons with CJD. Limited proteolysis of PrP^{CJD} by Western blot analysis shows four patterns of protease-resistant prion protein (12,13).

No screening assay is available to detect PrP in asymptomatic persons. Cerebrospinal fluid protein markers can distinguish CJD from other neurodegenerative disorders in certain settings (14,15). However, they are not CJD-specific and are not markers of PrP. The etiologic agent is believed to be a prion (proteinaceous infectious particle), although viral etiologies have been proposed (16-18). Sporadic CJD may also result from the spontaneous conversion of PrP^{C} into PrP^{CJD} or from somatic mutation in the prion protein gene.

Three epidemiologic forms of CJD are well recognized: sporadic (the most common), familial, and infectious/iatrogenic. The familial form (5% to 10% of cases) results from mutations in the coding sequence of the PrP gene located on chromosome 20. Polymorphisms at codon 129 have been correlated with genetic susceptibility to human prion diseases (19). Fewer than 1% of human cases of CJD are iatrogenic (20). A new variant (nv-CJD), which occurred temporally in association with BSE in cattle in the United Kingdom, was recently reported (21); a direct association with food consumption remains

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uncertain. Possible transmission of CJD through receipt of blood or blood products is a concern. Routes of iatrogenic transmission are summarized below, followed by a discussion of possible bloodborne transmission.

Iatrogenic CJD

Although first described in the 1920s, CJD was not considered a transmissible disease until 1966 when kuru was shown to be transmissible (22). In 1968, CJD was confirmed to be a transmissible spongiform encephalopathy when it was shown to be transmitted to chimpanzees (23). Virtually every case of CJD attributed to infection is iatrogenic; transmission between humans has been clearly demonstrated during neurosurgical procedures with contaminated instruments and through central nervous system tissue and extract transfer. Worldwide, more than 100 cases of transmissible CJD have been detected, and new cases continue to appear (Table 1; 27).

The first report of suspected iatrogenic CJD was published in 1974 (24). Animal experiments showed that corneas of infected animals could transmit CJD, and the causative agent spreads along visual pathways (25,26). A second case of CJD associated with a corneal transplant was reported without details (27). In 1977, CJD transmission caused by silver electrodes previously used in the brain of a person with CJD was first reported (28). Transmission occurred despite decontamination of the electrodes (later shown to transmit disease to experimental primates [29]) with ethanol and formaldehyde. Retrospective studies identified four other cases likely of similar cause (30-32). The rate of transmission from a single contaminated instrument is unknown, although it is not 100% (31). In some cases the exposure occurred weeks after the instruments were used on a person with CJD.

CJD was first reported in a recipient of a dura mater transplant in 1987 (33); a second case was identified in 1989 in a 25-year-old man from New Zealand, who also received dura mater (34). Because the same company produced dura mater for both patients, the dura mater was suspected as the source of iatrogenic CJD. Approximately 15 case reports have been published, providing information on 20 cases associated with dura mater transplant; a recent review article indicates that 25 such cases have occurred throughout the world (Australia, Canada, Germany, Italy, Japan, New Zealand, Spain, the United Kingdom, and the United States) (27). However, Japan has very recently reported more than 40 cases of CJD in dura mater recipients (J. Tateishi, Pers. Comm.). Most dura mater cases have been associated with a single manufacturer whose manufacturing processes were inadequate to inactivate the prion agent. This, combined with pooling of the dura mater has led to the relatively large number of cases. The earliest reported transmission occurred in a patient who received a dura mater transplant in 1969 (35). Although recommendations to reduce the risk were made in 1987, cases have continued to appear because of the long incubation period. Notably, one of the most important epidemiologic characteristics of dura mater transmissions is the young age of the first reported cases.

By 1985, a series of case reports in the United States showed that when injected, cadaver-extracted pituitary human growth hormone could transmit CJD to humans (36). Shortly thereafter, it was recognized that human gonadotropin administered by injection could also transmit CJD from person to person (37).

Strain typing based on molecular PrP analysis and a polymorphism (methionine/methionine, valine/valine or methionine/valine) found at codon 129 of the PrP gene has been proposed as a tool for distinguishing between iatrogenic and sporadic CJD (12,13). Homozygosity at this site may predispose a person to acquired forms of

Table 1. Transmissible cases of Creutzfeldt-Jakob Disease

Mode of infection	No. of patients	Agent entry into brain	Mean incubation period (range)
INSTRUMENTATION			
Neurosurgery	4	Intracerebral	20 months (15-28)
Stereotactic EEG	2	Intracerebral	18 months (16-20)
TISSURE TRANSFER			
Corneal transplant	2	Optic nerve	17 months (16,18)
Dura mater implant	25	Cerebral surface	5.5 years (1.5-12))
TISSURE EXTRACT TRANSFER			
Growth hormone	76	Hematogenous	12 years (5-30)
Gonadotrophin	4	Hematogenous	13 years (12-16)

EEG = electroencephalogram
Table taken from (27)

CJD, may lead to shorter incubation periods, and has been associated with various phenotypes of the disease (11,38). The proportion of polymorphism among persons with sporadic CJD is more similar to the proportion of polymorphism among persons with iatrogenic CJD than to that in the general population (Table 2), which suggests that simple stochastic events do not fully explain sporadic CJD; were that so, the distribution of homozygosity would be the same in both healthy controls and persons with sporadic cases. The clinical symptoms in patients with iatrogenic and with sporadic CJD have been compared and are indistinguishable (18).

Table 2. Amino acid phenotypes for codon 129 in patients with iatrogenic Creutzfeldt-JakobDisease

Tested groups	Met/Met (%)	Met/Val (%)	Val/Val (%)	Homo-zygous (%)	Total No. tested (n)
Sporadic CJD (38)	78	12	10	88	73
All Iatrogenic	60	11	29	89	63
Cases (27)					
CNS route of infection	80	10	10	98	20
Peripheral route of infection	51	12	37	88	43
Healthy controls (27)	37	51	11	49	261
Healthy controls (38)	48	42	10	58	1397

Met = methionine; Val = valine

Is CJD Transmitted by Blood Transfusions?

Animal Experimentation Data

Human CJD has been reported to be transmitted to mice by injecting blood from human patients directly into mouse brain (39,40). However, this evidence has not been widely duplicated by other laboratories, and a comprehensive review of three decades of research with nonhuman primates by the National Institutes of Health indicated that the blood of humans with CJD injected either peripherally or centrally into primates did not transmit CJD (41).

Some evidence indicates that blood of experimentally infected animals contains an infective agent. PrP infectivity resides predominantly or exclusively in lymphocytes and monocytes rather than in granulocytes (42,43); there has been no evidence of infectivity in erythrocytes, platelets, or plasma, but low infectivity has not been completely excluded. Animal studies have demonstrated that the agent causing scrapie replicates first in the spleen and other lymphoid tissues but reaches highest titer in the brain, where it results in the clinical appearance of disease (44). Hence, peripheral tissues in contact with blood also harbor PrP infectivity. Animal transmission data indicate that human spleen, lymph nodes, serum, and cord blood are irregularly infective for animals, although few cord blood samples have been tested (27). Brown has recently reported transmission of mouse scrapie using fractionated blood administered by intracerebral inoculation (P. Brown, Pers. Comm.). Studies of experimental CJD in guinea pigs and mice have shown that the infectious agent is present in the brain, viscera, and blood before clinical disease develops (43,45).

Several factors must be considered in reviewing animal evidence regarding transmission of CJD in blood: the level of PrP infectivity of the study tissue, the species barrier, and the route of administration. Human CJD can be readily transmitted to nonhuman primates (low species barrier) by intracranial injection (high efficiency of the infective route) of contaminated brain (high titer of infectivity). Human CJD is not readily transmitted to dissimilar species (such as rodents) and is even less readily transmitted when low-titer tissues (e.g., blood cells) and/or a low efficiency route of inoculation is used. In animals, peripheral inoculation of brain tissue transmitted CJD only irregularly, but the incubation periods were comparable to central administration (41). Barriers to transmission also include the difficulties in infecting peripheral cells and crossing the blood brain barrier and possibly the lack of structural similarity between peripheral PrP^{CJD} and brain PrP^{C}. Strain variation may also contribute to the difficulties of transmission to animals. Deslys et al. suggest that the route of inoculation may induce strain differentiation, which then facilitates subsequent transmission by the same route (46). Others disagree (47). The evidence from animal studies is inconclusive regarding transmission of CJD between humans by transfusion, and study design and laboratory techniques are controversial.

Evidence from Studies of Humans

Human Case Series and Case Reports

Case series and case reports provided information linking CJD to receipt of dura mater and human growth hormone. However, no human cases have yet been causatively linked to blood transfusion. A transplant recipient developed CJD (48) after receiving a liver from a donor who died of a cerebrovascular accident and had no symptoms of CJD; however, an autopsy was not performed, and brain tissue was not available for further investigation. The liver recipient did not have any of the known PrP gene coding sequences pathognomonic of familial transmission. The donor also provided a heart (to a recipient who died shortly after surgery) and a kidney (to yet another recipient; it was removed after 2 months); the latter recipient remains well. The liver recipient also received transfusions of blood, plasma, albumin, and anticytomegalovirus immunoglobulin from other donors. None of the blood donors is known to have had CJD, but one of the albumin donors died of a rapidly developing undiagnosed dementia.

Four Australians have been reported with CJD following transfusion (49). The patients had cerebellar signs; however, no other evidence of iatrogenic cause was described (50). The source of blood transfusions was undocumented. Genetic testing results were not provided; it is uncertain if cases were of the familial type, and no other information on alternative iatrogenic sources was provided.

In Canada, an albumin recipient died of neuropathologically confirmed CJD after receiving albumin from a pool containing blood from a person who died of neuropathologically confirmed CJD (D.G. Patry, pers. comm.). Eight months separated the receipt of albumin and development of symptoms, a much shorter period by a factor of three than seen in any other putative iatrogenic case, which makes iatrogenic transmission unlikely. A complete investigation is under way.

Without an experimental, diagnostic, or epidemiologic tool that can distinguish sporadic from iatrogenic disease or link the agent from a source with a recipient, it is difficult to draw any conclusions from the case reports described above. Statistically, a certain number of persons exposed to pooled blood would be expected to develop CJD. In addition, bias is introduced into case reports because of strong suspicion regarding iatrogenic sources. Verifying the statistical probability of cases by calculating the expected number of cases is difficult, if not impossible, because CJD is rare.

Surveillance

Population surveys or surveillance systems of the worldwide epidemiology of CJD indicate that CJD occurs in the population at a rate of 0.05 and 1.5 cases per million per year. After 1979, the rates are 0.3 to 1.5 cases per million per year (1-8). The age distribution patterns are consistent and show that cases in persons under 30 years of age are extremely rare; cases in persons under 50 years of age are rare; and the peak age of onset is 60 to 70 years. In age-specific data, rates decline in older age groups (2,6-8,51).

If CJD is transmissible in blood, cases should occur in young persons, particularly if the incubation period is short. Even if the incubation period is decades long, one would expect cases in young persons because of transfusions given to infants and children. However, CJD is rare in young persons and remains rare over time. Alternatively, the rare diagnosis of CJD in younger age groups may be due to preferences for neurologic disease diagnoses in these groups. Recent attention to nv-CJD in 14 persons under the age of 40 years in the United Kingdom, which has an intensive active surveillance system, will certainly affect investigations of unexplained mental deterioration among younger populations (22). The British Paediatric Surveillance System has initiated investigations into undiagnosed progressive neurologic disease among children (C. Verity, A. Nicoll and R. Will, pers. comm.), and Canada will initiate a similar system. In the United States, the Centers for Disease Control and Prevention has enhanced surveillance for CJD in persons under 55 years of age (52).

If CJD is transmitted in blood, the last three to four decades might show a detectable increase in cases reflecting the increasing use of blood transfusions. In fact, surveillance data demonstrate that CJD rates are increasing in some countries. Interpretation of this finding is difficult: most surveillance systems were initiated in the last two decades; in countries with intensive surveillance, "catch-up" from previous underreporting led to initial increases in case numbers and rates; few countries have sufficiently intensive surveillance systems to conclude that there is no risk from blood; and the death

certificates used for surveillance in some countries are sometimes incomplete, which may introduce a bias toward easily ascertained cases (8,51).

If CJD is transmitted in pooled blood products, clusters would be detected; indeed, surveillance systems have expected such clusters. However, observation of one or two cases often leads to more careful search for cases. Most such clusters have been attributed to familial disease (1,53). Cluster investigations in surveillance systems have not systematically searched for blood-related transmission.

Surveillance systems have found cases of CJD among persons who have received blood transfusions, but none have been linked to blood transmission. In the United Kingdom, the well-established surveillance system identified nv-CJD, despite its rarity, demonstrating the capability of surveillance to find rare diseases; nv-CJD was recognized quickly because of the young age of the patients and the novel neuropathologic findings. However, the absence of evidence is not evidence of the absence of transmission of CJD through blood, for two reasons: 1) surveillance systems designed to identify rare diseases need intensive resource allocation to detect sentinel events and 2) blood-borne transmission may go unnoticed when examining population data if unaccompanied unique epidemiologic features (such as extreme youth or novel neuropathologic features).

If CJD is transmitted in blood, cases could increase in industrialized countries, where access to blood transfusion is greater. The number of reported cases is larger; however, cases have been found in every country in which they have been sought, although some countries have limited surveillance capacity. While some countries have higher rates of CJD, there is no evidence that this is due to transmissible forms of the disease. Rather, the higher rates are most likely due to older age distributions in industrialized countries' populations, surveillance biases following intensified surveillance for CJD, and familial clusters.

Finally, in many surveillance systems, the age distribution pattern for CJD is uniform (2,7,8,51). The pattern is more consistent with early exposure to an agent with long incubation periods, a common population exposure that peaked long ago and is now declining, or a heritable disorder that causes shortened lifespan than with a risk which increases with age (e.g., spontaneous genetic mutation or stochastic change of the normal prion to the ß-pleated form). However, CJD may be uniformly underdiagnosed in older age groups; because of nv-CJD there will likely be increased attention to differential diagnoses among elderly persons dying of rapidly progressing dementing illnesses. We do not suggest that all sporadic cases are due to external exposure such as blood, but rather we draw attention to an important epidemiologic characteristic of CJD that is not consistent with an entirely stochastic or age-related event.

Case Control Studies

In three countries, case-control studies of CJD have included questions regarding exposure to blood: Japan (54), the United Kingdom (55-58), and the United States (59). In the Japanese study, only one case-patient and three controls received blood transfusions. Two of the United Kingdom studies indicate no difference in exposure to blood between the case-patients and controls (55,58). A comparison of the frequency of receipt of blood among persons with CJD and controls matched for age and sex using data collected in the United Kingdom CJD surveillance system between 1980 and 1984 (56) showed no difference in the history of blood receipt between those with and without CJD. Although an odds ratio (OR) is not calculated for the data, our calculations indicate the OR is 0.78 (95% confidence interval 0.38, 1.58) with a power of only 30% to detect an OR different from one (60). Davinipour (mid-Atlantic United States) also found that blood exposure posed no risk, with an OR for blood transfusion of 0.6 (59). However, with only 26 cases, the confidence intervals or number of patients exposed was not mentioned, although the OR is described as significant. Wientjens et al. analyzed pooled data for 178 cases and 333 controls from three case-control studies (Japan, United States, United Kingdom) and did not find an OR different from one for blood transfusion (61).

Although not using a formal case-control study, Operskalski et al, compared rates of human immunodeficiency virus (HIV) dementia among three HIV-positive groups (persons with hemophilia, "other blood recipients," and "blood donors") using data from the Transfusion Safety Study (62). They hypothesized that if CJD was misdiagnosed as HIV dementia but was the true cause of dementia, there would be an excess rate

of HIV dementia in persons with hemophilia due to their high rates of exposure to blood and blood products (persons with hemophilia were exposed to the blood or product made from pools of hundreds of thousands if not millions of donations). In addition, since the study contained data from as early as the 1950s, long periods of observation were available. Rates of HIV dementia in the study populations were compared, and CJD in pooled plasma derivatives did not pose a risk. However, it cannot be concluded that rare diseases such as CJD would be detectable in increased rates of misdiagnosed HIV dementia or that diseases with long incubation periods may have sufficient time to develop in persons with hemophilia. Any disease with an incubation period longer than that of HIV would be in competition with AIDS and hepatitis B or C as a cause of death, and persons with hemophilia and HIV infection die young. In addition, the data included the cause of death of 1,000 HIV-infected persons but did not provide information on duration of observation or an estimate of the rate of disease that would be necessary before even one additional case would be observed.

The primary weakness found in these published case-control studies is the use of the categories "exposure to blood" or "blood transfusion" as a surrogate for exposure to blood containing the agent of CJD. If the agent of CJD is frequently present in blood used for transfusions, the case-control study design is sufficiently robust to detect a risk. However, transmission of CJD through transfusion of blood contaminated with the agent of CJD appears to be rare. Consequently, a negative finding of risk attributable to blood in these studies may simply reflect an absence of exposure to the disease. Study design will have to account not only for a rare disease, but possibly for a rare exposure as well.

In addition, when hospitalized persons are used as controls, the design must consider Berkson's bias, a selection bias that occurs when the selection method for controls affects the rate of exposure to the agent studied (63). The study design must then show that hospitalized controls do not have a different rate of potential exposure to blood transfusion from the general population. The use of population controls may be more appropriate for studying blood exposure.

Another weakness in the existing studies was the use of a reporter, most often a relative, to collect information about exposure to blood or blood products. Oral history of blood exposure tends to underestimate the rate of exposure to blood. In one Canadian hospital conducting a retrospective study, approximately 40% of patients did not know if they had received a blood transfusion (C. Kennealy, Pers. Comm.). In a central Ontario hospital, 25% of recipients did not know if they had received a blood transfusion (S. King, Pers. Comm.). Given that CJD and transfusion-transmitted CJD (if it occurs) are rare, the published case-control studies lack the power to detect very low ORs.

The 126 hemophilia centers in the United States have been asked to report deaths of persons with neurologic symptoms for neurologic confirmation of cause of death; no CJD cases have been reported in persons with hemophilia (B. Evatt and L. Schonberger, Pers. Comm.). The Canadian Haemophilia Society has requested assistance from the Health Canada, Laboratory Centre for Disease Control, to initiate a similar study in Canada (D. Wong-Reiger, Pers. Comm.). In Canada, a combined active surveillance system and case control study will begin in 1997 to identify the risk for CJD as a result of transfusion with blood from a person with CJD. Many of the methodologic problems in other studies have been addressed in the design of this study.

Cohort Studies

The investigation of a patient with CJD who donated 35 units of blood in 20 years identified 27 persons who definitely received his blood and eight who probably received blood; for 20 units, the recipients could not be identified (64). Eighteen (33%) of the identified recipients had died. None of the recipients had exhibited neurologic disease, although some were observed only briefly; only eight were observed for longer than 5 years.

In the United States, the American Association of Blood Banks has initiated a long-term cohort investigation. By linking the names of persons who received blood from CJD patients with the national death records, the cause of death for each person will be determined. Of the 147 recipients identified, 80 have died; for 65, the cause of death is known but no cases of CJD have been found (M. Sullivan, Pers. Comm.).

Cohort studies are unlikely to accumulate enough cases of exposure to allow a reasonably precise estimate of risk. However, if pooled product confers uniform risk to each recipient, data from a large number of exposed recipients

can be collected. The cohort studies under way may be able to identify higher than expected rates of disease, thus indicating transmission through blood transfusion without quantifying the rate of transmission.

Conclusions

Many health professionals are concerned that CJD may be transmissible through blood. As noted by Brown, "iatrogenic disease from this source would dwarf in importance all other sources by virtue of the sheer number of people who theoretically have been or could be at risk" (27).

Animal studies indicate that the infective agent of CJD is present in blood but in low titer, and sufficient evidence of animal transmission suggests that the disease has the potential to be transmitted through blood (65). However, human epidemiologic evidence only indicates that if blood transmission occurs, it is likely rare. Some researchers suspect that the agent of CJD is ubiquitous, therefore, we are commonly exposed to it; perhaps, another, as yet unidentified factor, "turns on" the disease. If so, is there any part of the human population that is not exposed? Most people are exposed to some components of blood products through vaccines. Some people may be protected by strain variation through exposure to nonvirulent forms, so only subgroups are susceptible to a virulent form (66). Polymorphism at codon 129 may restrict susceptibility to a small portion of the population, or most transfusions may not contain a dose large enough to cause infection.

Can experimental studies answer these questions? Sufficiently large numbers of study animals could overcome problems such as the species barrier and low transmission rates due to a low dose or peripheral route of inoculation. The studies would require strict adherence to proper laboratory technique and replication in at least one other laboratory. Unanswered questions include the following: Can the transmissible spongiform encephalopathies of animals be transmitted, by transfusion, within the species when spiked blood is used? Within species from "naturally infected" blood? From humans to animals with spiked blood? From humans to animals with "naturally infected" blood? Does transmission by blood become more efficient after passage? Can human blood cells carry the agent? What are the routes for infection of the brain if infection is peripheral?

Can epidemiologic methods detect blood transmission of CJD if it is rare? Epidemiologic tools such as outbreak investigations, case-control studies, and cohort studies are limited in their ability to detect rare events. In addition, during the long incubation period, patients often move away from the location where they received the transfusion; forget their exposure; and essentially lose their membership in a recognizable cohort. The absence of a test for exposure, such as an antibody test or gene sequencing of an agent as used in investigating HIV-related outbreaks, makes the investigation of iatrogenic CJD extremely difficult, hence the importance of vigilance, in the form of case reports from alert and informed clinicians, followed by critical field investigation. Surveillance systems provide the core resources for identifying and investigating unique or unexplained events. Followup case-control studies allow the observation and recording of the actual chain of exposure to blood.

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A New Tick-borne Encephalitis-like Virus Infecting New England Deer Ticks, *Ixodes dammini*[1]

To determine if eastern North American *Ixodes dammini,* like related ticks in Eurasia, maintain tick-borne encephalitis group viruses, we analyzed ticks collected from sites where the agent of Lyme disease is zoonotic. Two viral isolates were obtained by inoculating mice with homogenates from tick salivary glands. The virus, which was described by reverse transcriptase polymerase chain reaction and direct sequencing of the amplification products, was similar to, but distinct from, Powassan virus and is provisionally named "deer tick virus." Enzootic tick-borne encephalitis group viruses accompany the agents of Lyme disease, babesiosis, and granulocytic ehrlichiosis in a Holarctic assemblage of emergent deer tick pathogens.

American zoonotic foci of the agents of Lyme disease (*Borrelia burgdorferi*, a spirochete) and human babesiosis (*Babesia microti*, a protozoon) were first recognized in coastal New England and the northern Great Plains during the 1960s and 1970s (1). Human granulocytic ehrlichiosis (caused by rickettsia, *Ehrlichia microti* or *Ehrlichia phagocytophila/equi* [2]) joined this guild (3) of deer tick-transmitted pathogens (*Ixodes dammini* [4,5]) during the 1990s. *B. burgdorferi* has infected *I. dammini* and their rodent hosts at least from the beginning of the 20th century (6). In Eurasia, closely related and ecologically equivalent ticks (*Ixodes ricinus* and *Ixodes persulcatus*) transmit analogs of each of these agents (7), including several zoonotic variants of the Lyme disease spirochete, babesiosis (caused by *Babesia divergens*), tick-borne fever (caused by *E. phagocytophila*), and certain flaviviruses (tick-borne encephalitis [TBE]). Although analogs of three of the four members of the Eurasian guild of *I. ricinus/persulcatus*-transmitted pathogens are well established in North America, no arbovirus has been reported from *I. dammini* or other American members of the *I. persulcatus* species complex. Accordingly, we determined whether arboviral infection is present in adult *I. dammini* from deer or vegetation in coastal New England, where Lyme disease is frequent.

Partially fed female ticks were removed from carcasses of deer shot by hunters during November 1995 or were derived from host-seeking ticks swept from vegetation and permitted to partially engorge on rabbits in the laboratory. All ticks were held in vials designated by site, at 4°C, and in a humidified chamber until dissection, no more than 1 month after they were removed from hosts.

Each field-derived tick was dissected into a drop of sterile Hanks Balanced Salt Solution with 15% fetal bovine serum (HBSS/FBS), and one of its salivary glands was stained by the Feulgen reaction (8). The corresponding gland was pooled in 0.5 mL HBSS/FBS with that from four other ticks and was homogenized; 0.4 mL of each pool was subcutaneously injected into an adult C3H/HeJ mouse. Blood smears were made 1 week after injection to detect ehrlichiae or piroplasms. All mice were observed for 1 month.

Neonatal (2 to 5 days after birth; "suckling") or subadult (<18 g) outbred CD-1 mice (Charles River Laboratories, Wilmington, MA) were used to propagate putative viral pathogens. Suckling mice were intracerebrally inoculated with 0.03 mL of a clarified and 0.22 μm filtered 20% suspension of whole mouse brain in HBSS/FBS. Alternatively, aseptically drawn plasma from putatively infected mice was directly injected. Siblings of the suckling or subadult mice received 0.03 mL of sterile HBSS/FBS.

Suspensions of brain tissue from mice that had died were centrifuged, the pellet was resuspended in Trizol Reagent (BRL Life Technologies, Bethesda, MD), and RNA was extracted according to the manufacturer's protocol. RNA was reverse transcribed by using POW-1, POW-2, ENV-A and POW-6 primers. Primer design was based upon the published cDNA sequences for TBE group viruses (9). Reverse transcriptase polymerase chain reaction (RT-PCR) was performed with

[1]The taxonomy continues to be controversial (5).

dedicated pipettors in an area physically separated from where amplification products were manipulated.

Amplification products were excised from 3% agarose gels, purified by using the GeneClean kit (Bio 101 Inc.), and cycle sequenced at the University of Maine Biotechnology Center. Each sequence was verified by sequencing amplification products from two independent PCR reactions. These sequences (Ips1, Ips2, CT 390) have been deposited in GenBank (accession numbers U93287, U93288, U93289).

To determine whether ticks that (as larvae) had fed on putatively infected mice became infected with a viral pathogen, living nymphs were directly placed in Trizol Reagent within individual screwcapped microcentrifuge tubes and allowed to remain overnight. A heat-sealed micropipet tip was used to crush each tick, and the resulting homogenate was extracted as described. RNA was analyzed for evidence of TBE group-specific env using primers POW1/POW2 in the described RT-PCR assay.

Distinctive inclusions were detected by light microscopy in the Feulgen-stained salivary glands of one tick in each of two pools (Figure 1). The staining properties and shape of these inclusions were distinct from those of an ehrlichia or a piroplasm. Of the mice that were inoculated with pooled salivary tissues from these ticks with the unidentified salivary inclusions, two died approximately 14 days later after a prodrome that included signs of neurologic involvement. Ehrlichiae or babesiae were not detectable in their peripheral blood. One had received an inoculum derived from ticks sampled in a site in Massachusetts and the

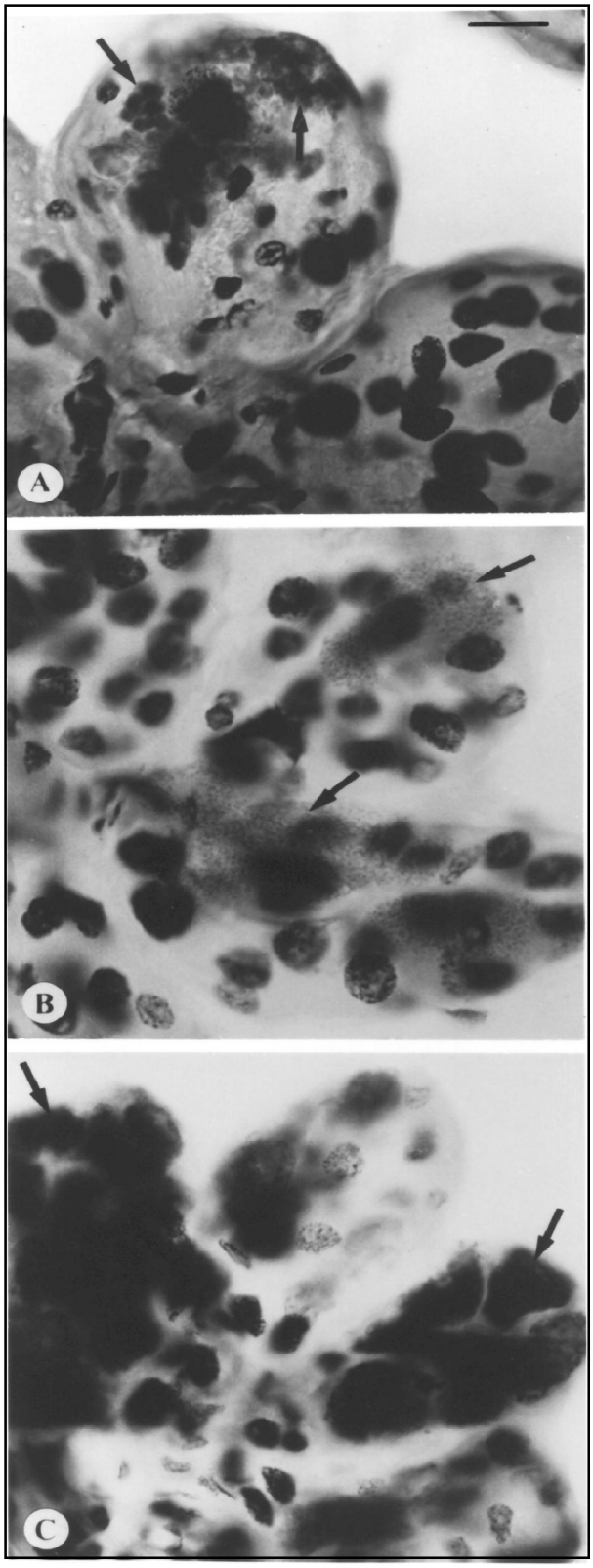

Figure 1. Comparison of morphology and tinctorial properties of Feulgen stained, intact tick salivary glands infected by three of the deer tick pathogen guild. Spirochetes only transiently migrate through salivary tissues, and thus would not be visualized by this technique. (A) *Ehrlichia microti*. Polyhedral clusters of rickettsiae (arrows) within hypertrophied salivary acinus. (B) *Babesia microti*, dense stippling of sporoblasts (arrows). Each minute dot represents the nucleus of a sporozoite. (C) Deer tick virus. Hypotrophied salivary acinus filled with amorphous masses of pinkstaining (=Feulgen positive) material (arrows). Scale bar = 10 μm.

other from ticks in Connecticut (Table). Mice that had received salivary gland material that did not contain similar inclusions remained healthy. A neurotropic microbial pathogen was suspected.

Table. Prevalence of deer-tick virus infection in adult *Ixodes dammini* sampled from deer or vegetation during November 1995 from Massachusetts and Connecticut

Sampling area*	No. examined	No. infected
W MA	17	0
C MA	15	0
NE MA	157	1
SE MA	175	0
NW CT	7	1
NE CT	6	0
SW CT	26	0
SE CT	23	0
Total	465	2

*The deer management zones, or study sites, belong to one of four geographic quadrants for Connecticut (NE, northeastern; NW, northwestern; SE, southeastern; SW, southwestern) or of four regions in Massachusetts (W, western; C, central; NE, northeastern; SE, southeastern).

To determine whether an arbovirus may have infected the two mice that died, we injected homogenized samples of brain tissue into suckling mice. Although we initially anticipated that an atypical rickettsial agent might have caused the death of these mice, we found that the putative agent could be passaged by serially inoculating clarified and filtered suspensions of brain tissue of affected mice into either suckling or adult mice. Blood or plasma from moribund mice was poorly infectious. We concluded that a neurotropic infectious agent was present in at least two of the 465 ticks that were sampled originally.

To identify this microbe, RNA was extracted from an aliquant of each of the infected salivary gland homogenates and from a suspension of the brain tissue of each of the two mice that died. This preparation was used as template for an RT-PCR employing primers designed to amplify a portion of the TBE group virus nonstructural protein or envelope genes. Amplification products were sequenced and compared to viral sequences accessioned in GenBank. Parsimony analysis of the derived sequences for both genes indicated that the two viral isolates from different sites were identical, grouped within the TBE complex, but were distinct from known viruses (Figure 2). Powassan virus was the most closely related organism, with 84% pairwise similarity in the portion of the envelope gene that was analyzed. This apparently novel member of the TBE group of flavivirus is provisionally named deer tick virus (DTV).

Suckling mice became paretic and died by 5 days after DTV was intracranially injected. During the third serial passage, the adult mouse LD50 for subcutaneous injection of a 20% brain suspension, performed in adult CD-mice, was estimated at 6.3 x 10^4/mL. Although adult laboratory mice died within 14 days of infection, all eight adult *P. leucopus* survived without apparent ill effect. White-footed mice *(Peromyscus leucopus*) were from a laboratory colony at the Harvard School of Public Health and represent mice originally collected from Nantucket Island in 1992. Their ability to survive infection suggests that white-footed mice may serve as reservoirs of this virus in nature.

A xenodiagnosis (10) was performed to detect cryptic ehrlichial infection. We permitted noninfected, laboratory-reared larvae of *I. dammini* to engorge on one of the original C3H mice that showed signs of disease. After molting, the derived nymphs were permitted to engorge on outbred Swiss mice. Both mice died 2 weeks later, suggesting that DTV had been transmitted by these ticks. Indeed, two of eight nonengorged ticks from the same feeding contained DTV RNA by an RT-PCR assay. Nymphs from the same batch of larvae that had been fed on noninfected mice did not contain DTV RNA. We concluded that subadult *I. dammini* efficiently transmit DTV.

The presence of this new TBE-like virus in New England deer ticks confirms that this guild of *Ixodes*-transmitted pathogens is as diverse in the Nearctic as in the Palearctic and is consistent with an original ancient Holarctic distribution of these ticks and their pathogens. We hypothesize that the guild survived the Pleistocene period in refugia located near the terminal moraine sites where the deer tick-transmitted zoonoses were first described (5). The alternative, that the American guild may have evolved in parallel with that in Eurasia, seems less persuasive.

DTV may derive from Powassan virus, which mainly cycles between North American woodchucks (*Marmota monax*) and *I. (Pholeoixodes) cookei* (11), a one-host tick that is distantly related to *I. dammini* and only rarely feeds on humans or mice. Because deer ticks occasionally feed on woodchucks, they may carry this virus to the mouse population. Indeed, *I. dammini* are competent laboratory vectors of *I. cookei*-derived Powassan virus (12). Although the extent of

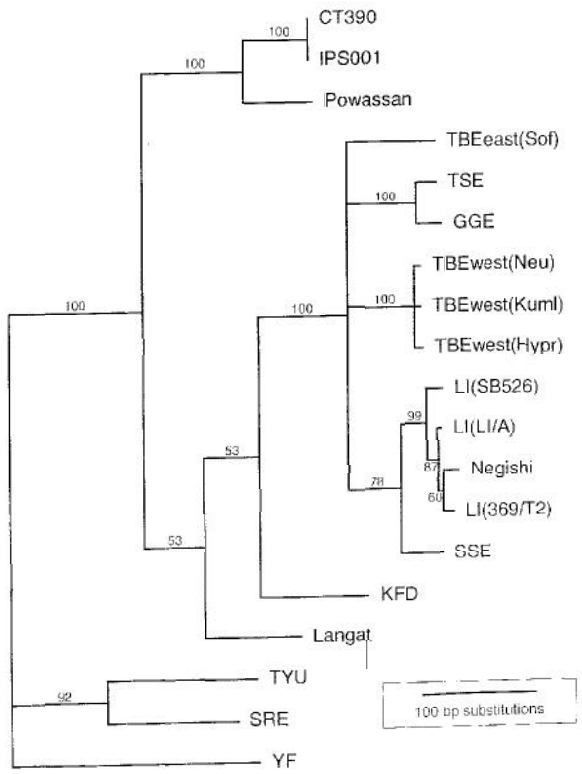

Figure 2. Maximum parsimony phylogram indicating the relationship of deer tick virus (DTV) to other tick-borne encephalitis group viruses. Sequences in GenBank for representative tick-borne flavivirus envelope genes were aligned and compared by using yellow fever virus as the outgroup. Values above branches indicate bootstrapped confidence values. Branch lengths are proportional to percent similarity in sequence. IPS 001, mouse brain derived DTV (Massachusetts isolate); CT390, mouse brain derived DTV (Connecticut isolate). POW, Powassan virus; TBE, tick borne encephalitis; TSE, Turkish sheep encephalitis; GGE, Greek goat encephalitis; LI, louping ill virus; SSE, Spanish sheep encephalitis; KFD, Kyasanur Forest disease virus; TYU, Tyuleniy virus; SRE, Saumarez Reef virus. The topology of the phylogram that was independently derived from *NS-5* does not differ from that of *env* and is not presented.

geographic variation in its genes remains unknown, Powassan is as molecularly distinct from DTV as Central European encephalitis virus (also known as Western subtype of TBE) is from Russian spring summer encephalitis (also known as Eastern subtype of TBE) or Louping Ill viruses (Figure 2). DTV, therefore, may repre-ent a new subtype of Powassan virus.

Many tick-borne agents may be detected more readily if ticks are allowed to partially feed, thereby initiating reactivation (13). We regarded as unlikely the possibility that any infections detected in partially fed ticks simply represented infections that had been ingested. In that case, other ticks in the same collections would also tend to be infected. Our focus on the analysis of the salivary glands also reduces the possibility that an ingested pathogen rather than one derived from a previous bloodmeal would be detected. Indeed, the developmental cycle of these agents would not readily allow rapid invasion and residence in the salivary glands during the infecting bloodmeal (but see 14). Accordingly, the two infections detected had been transstadially passaged as opposed to periprandially acquired.

Approximately 100 ng of extracted RNA was added to a 20 µL reverse transcriptase (RT) reaction containing 5mM MgCl2, 50mM KCl, 100mM tris-HCl (pH 8.3), 5mM each of the four deoxynucleotides, 20U RNase Inhibitor, 50U RT, and 25 pmols of the appropriate downstream primer (POW-2 [5' GCTCTCTAGCTTGAGCTCCCA 3'] or POW-6 [5'TTGTGTTTCCAGGGCAGCGCCA 3']). To a 50 µL PCR reaction using the Elongase system (BRL Life Technologies) and a final Mg^{++} concentration of 1.5mM was added 1/20th of the RT reaction and 25pmol of primers POW-1 [5'TGGATGACAACAGAAGACATGC 3'] and POW-2, which amplify a 291 bp region of the TBE virus complex nonstructural protein gene (*NS-5*). Alternatively, primers POW-6 and ENV-A [5'GTCGACGACGAGGTGCACGCATCTTGA 3'] were added to amplify a 689 bp region of the TBE virus envelope gene (*env*).

Sequences derived from RT-PCR were compared with those accessioned in GenBank. Sequence alignments were generated with the PILEUP program of the Wisconsin Genetics Computer Group, and these prealigned sequences were analyzed by parsimony using PAUP 3.1 (D. Swofford, Illinois Natural History Survey, Champaign, IL) with yellow fever virus as the outgroup. Characters were equally weighted and treated as unordered. Phylogenies were constructed by using the heuristic search option with the tree bisection reconnection branch swapping method. The robustness of the maximum parsimony tree was estimated by performing 1,000 bootstrapped replicates. Separate comparisons were done for *env* and *NS-5* because fewer *NS-5* sequences were available for many of the viruses for which there were *env* sequences.

Figure 3. Adult deer tick.

The Feulgen reaction is DNA-specific (15); therefore, it appeared illogical that inclusions of an RNA virus would be specifically stained in our microscopy-based assay. Experimental evidence suggests, however, that glycoproteins may also be stained (16,17) in what is known as the plasmalogen reaction. The inclusions detected in tick salivary glands may, therefore, represent envelope proteins from massively replicating virus.

DTV appears to be less infectious than other members of the TBE virus complex. Suspensions of suckling mouse brain tissue contained 10^4 adult mouse LD50 of virus; in contrast, similar preparations of Central European encephalitis may contain 10^8. However, early passage virus may increase in virulence with subsequent adaptation. Suckling or adult mice that had received control inocula but lived within the same cages as virus-infected mice did not become infected, which suggests that aerosol or contaminative transmission of virus between mice is rare.

The public health significance of DTV remains undescribed. TBE-like viruses tend to be virulent, causing an encephalitis with a case-fatality rate that approaches 40% or with debilitating neurologic sequelae (18,19). Although the consequences of human DTV infection are unknown, the related Powassan infection usually causes severe encephalitis (20). On the other hand, Central European encephalitis infection is frequently mild or silent (21). Our estimate of the prevalence of viral infection in adult *I. dammini* (0.43% of 465 ticks) is consistent with those for enzootic Central European encephalitis in present-day European sites where the risk for this infection is well described. TBE-group viruses, however, exist in microfoci (22), and local prevalence may be greater. Residents of New England sites where the other three members of the deer tick pathogen guild are zoonotic may thus be exposed to DTV, but the infection causes a self-limited febrile illness of unknown etiology.

Attempts to define the etiology of Lyme disease in the 1970s included an exhaustive search for evidence of arboviral infection in Connecticut *I. dammini*; none was found (23,24). Because deer ticks were less numerous then than they are now, the pathogens they transmitted might have been correspondingly infrequent. DTV may constitute an emerging fourth member of the guild of deer tick-transmitted zoonotic agents in eastern North America. The incidence of disease associated with the aggressively human-biting deer tick may continue to rise where deer populations increase.

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An Unusual Hantavirus Outbreak in Southern Argentina: Person-to-Person Transmission?

Hantavirus pulmonary syndrome is a rodent-borne zoonosis first recognized in the United States in 1993. Person-to-person transmission has not been reported; however, in the outbreak of 20 cases reported here, epidemiologic evidence strongly suggests this route of transmission.

In 1995, a novel hantavirus (Andes virus) was identified in samples from patients in southern Argentina (1). The patients had hantavirus pulmonary syndrome (HPS), a disease first described 2 years earlier in the United States, in association with Sin Nombre virus (a New World hantavirus) (2,3). Old World hantaviruses (Puumala, Seoul, Hantaan, and Dobrava) have been recognized causes of hemorrhagic fever with renal syndrome (HFRS) for at least 40 years and are responsible for more than 100,000 cases each year in Eurasia (4). Hantaviruses (family Bunyaviridae, genus *Hantavirus*) are rodent-borne viral zoonotic agents believed to be transmitted to humans primarily by inhalation of aerosols of infected rodent excretions (4). Person-to-person transmission of hantaviruses has not been reported despite extensive epidemiologic studies. Between September 22 and December 5, 1996, 18 cases of HPS occurred in residents of, or visitors to, the towns of El Bolsón, Bariloche, and Esquel in southern Argentina. Two additional persons who had contact with El Bolsón patients but had not visited the area, contracted HPS during this period (5). Five of the patients were physicians; three were directly responsible for the clinical care of an HPS patient. Epidemiologic links between all but four of the cases and evidence of low rodent population density in the area strongly suggest person-to-person transmission of HPS during this outbreak.

Sporadic cases of HPS and possible cases of HFRS from as early as 1987, have been retrospectively identified in Argentina (6). In 1995, genetic material from Andes virus, a unique hantavirus, was identified in the lungs of a patient from El Bolsón (population ~15,000) in southern Argentina (1). The outbreak of HPS reported in this dispatch began on September 22, 1996, when the 41-year-old index patient (patient I) (also from El Bolsón) became ill with HPS (Figure 1). El Bolsón, a rural town in Rio Negro Province, is approximately 350 meters above sea level in the foothills of the Andes. Patients requiring intensive care are transferred to Bariloche

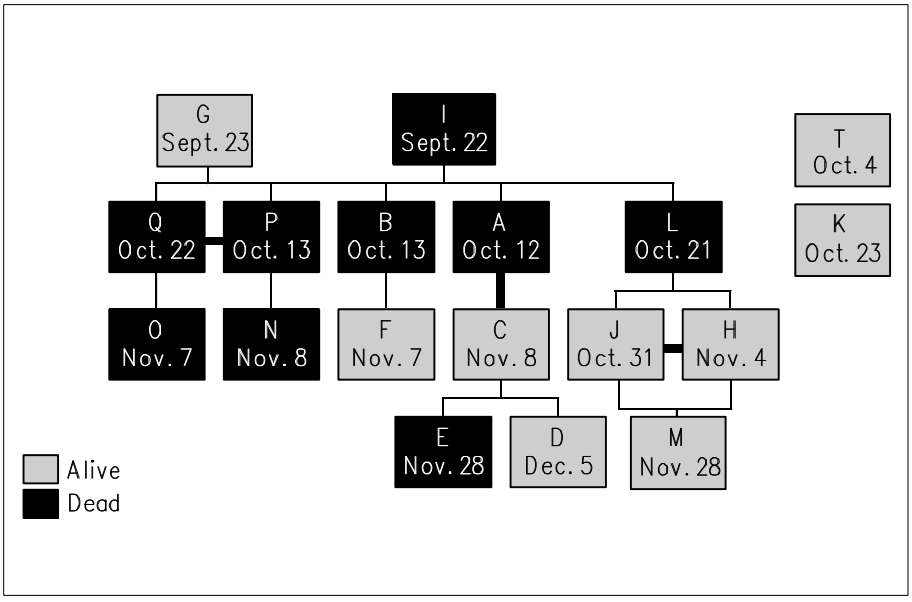

Figure 1. Transmission tree for HPS cases in southern Argentina, September–December 1996, indicating dates of onset of symptoms, survivor status, and proposed lines of transmission. Lines of transmission are hypothetical since many of the patients had contact with multiple HPS patients. Bold lines denote husband and wife. The two sporadic cases, U and R, are not shown.

(population 80,000), 150 kilometers to the north (Figure 2).

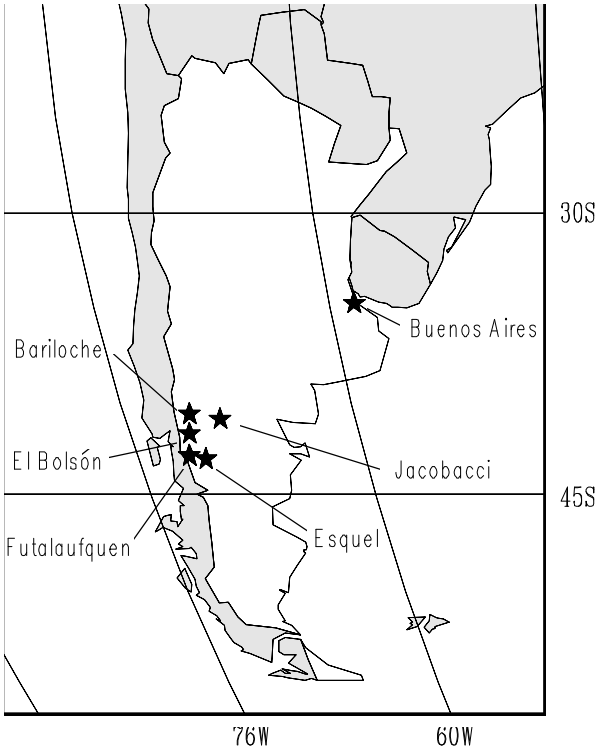

Figure 2. Towns involved in the 1996 HPS outbreak in southern Argentina.

Potential cases were identified by local physicians familiar with the clinical features of HPS (3) and were accepted as cases when enzyme-linked immunosorbent assay detected IgM antibodies reacting with Sin Nombre virus and/or when reverse transcriptase and polymerase chain reaction detected hantaviral RNA (1,2,6,7). Viral sequences indicated a sigmodontine-derived hantavirus related to, but clearly distinct from, Sin Nombre virus; additional sequencing and phylogenetic studies are under way.

Twenty-one and 20 days, respectively, after the index patient became symptomatic, his 70-year-old mother (patient B) and one of his doctors (patient A) contracted HPS. The doctor's spouse, also a doctor (patient C), became ill with HPS 27 days after her husband's first symptoms (19 days after his death). She traveled to Buenos Aires for medical care. In a Buenos Aires hospital, an admitting doctor (patient D) spent 1 hour taking a clinical history and examining her. The doctor (patient D) applied pressure to a venipuncture site on patient C's arm with multiple layers of gauze; no obvious blood contact occurred. The only other contact between this doctor and patient C occurred 2 days later, when the doctor briefly visited the hospital's intensive care unit to attend to another patient. Twenty-four days after attending patient C, the doctor became ill with HPS. The doctor, patient D, had not traveled outside Buenos Aires, and she reported no contact with rodents during the 2 months preceding her illness (5).

A 40-year-old doctor (patient E) from Buenos Aires contracted HPS 17 days after patient C was admitted to the hospital. This doctor was a friend of patients A and C and spent 3 days in El Bolsón after the death of patient A. She visited patient C often in the hospital but was not directly involved in the clinical management of any HPS patients. A fifth doctor with HPS (patient F) reported having close contact with several HPS patients. He intubated patient B and examined patients I and G. He also had daily contact with his colleague, (patient A), and spoke briefly with patient I's sister (patient H), brother-in-law (patient J), and friend (patient K).

At the funeral of patient B, patient I's housekeeper (patient L) was already symptomatic. She returned to Buenos Aires by car with patients H, J, and their daughter (patient M). Symptoms developed in the latter three occupants of the car 11, 15, and 29 days, respectively, after the drive back to Buenos Aires. No evidence of rodent infestation was found when the car was examined 3 weeks later. Although patients H and J had stayed at the home of patient I for several nights, patient M had not been to El Bolsón in the preceding months, traveling only as far as Jacobacci (~250 miles from El Bolsón) for patient B's funeral.

A second group of HPS cases occurred in Bariloche. Four people who had visited or worked in the hospital to which many of the El Bolsón patients were transferred (a modern 40-bed facility with a three-bed intensive care unit) contracted HPS: the hospital's night receptionist (patient N), a 40-year-old woman (patient O) who visited an unrelated patient, a 27-year-old man (patient P), and his wife (patient Q). Patients P and Q developed a close relationship with patient N during their many visits to the hospital between September 20 (when their 27-week-gestation baby was born) and October 27 (when the baby died). The three shared mate (a local tea drunk through a

communal metal straw), and patient P occasionally rested on patient N's camp bed. The baby got abdominal distention and shock 37 days after birth. The clinical signs suggested necrotizing enterocolitis, but no serum or tissue specimens from the infant were available for definitive diagnosis.

The remaining three HPS patients in El Bolsón during this period were men aged 44 (patient G), 29 (patient R), and 14 (patient T) years. Patients G and T were friends or acquaintances of one or more of the HPS patients, but did not recall contact with an HPS patient in the 6 weeks before the onset of their symptoms.

Both patients R and U likely had coincidental sporadic cases of HPS. Patient R lived in the Chilean mountains and had prodromal symptoms of HPS on arrival in El Bolsón. Patient U, a 33-year-old man living and working in Futalaufquén National Park, Chubut Province (150 km south of El Bolsón), also contracted HPS during this period but is unlikely to have had contact with HPS patients in El Bolsón.

Overall, 11 (55%) of the 20 patients were male, the mean age was 38 years (range 13-70 years), and the case-fatality rate was 50%. The death rate among cases for which epidemiologic links were identified (Figure 1) was lower during the latter stage of the outbreak (three of nine patients with onset of symptoms in November-December died compared with six of seven patients with onset of symptoms in October). The clinical picture was similar to that of HPS in the United States, although several atypical features, such as conjunctival injection and head and neck suffusion, were noted. Coughing did not occur earlier or more frequently in Argentinean HPS patients than in U.S. patients, which suggests that coughing was not the critical factor in person-to-person transmission.

Early in the investigation of this outbreak, attention was focused on the index patient's brick house, one of several dwellings located on a semirural property on the outskirts of El Bolsón. Several weeks before patient I became sick, he and patients B and L moved from a wooden cabin into the brick house. Two doctors (patients A and F) made visits to this house, and patients H and J stayed there at least one night during November 1996. The 13 other HPS patients in the outbreak did not visit the house. One month after the onset of patient I's symptoms, an examination of the brick house and other nearby structures found the buildings to be well constructed with no obvious sites for rodent entry and no evidence of rodent infestation (as confirmed by trapping studies). Trapping in and around the homesites of the El Bolsón and Bariloche patients yielded few rodents. Trap success (i.e., the number of rodents captured per 100 trap nights) in neighboring areas of disturbed or natural vegetation indicated a low rodent population density. Identification and serologic testing of captured rodents are under way. The low trap success rate contrasted with that of the 1993 HPS outbreak in the southwestern United States, when rodents were plentiful overall and significantly more numerous in and around homes of patients than in and around homes of controls (8).

The probable transmission of hantavirus from patient C to her doctor (Patient D) is the best epidemiologic evidence available to support the hypothesis that person-to-person transmission of hantavirus occurred during this outbreak. When considered separately, links between other Argentinean HPS patients are less convincing. However, when the contacts between patients are viewed collectively, the probability that person-to-person transmission played a major role in this outbreak strengthens. A comparison of the relative success of rodent trapping efforts over the last 20 years indicated that rodent population densities in the general area of the outbreak during the spring of 1996 were lower than average (O. Pearson, pers. comm. 1996), adding further support for the hypothesis of person-to-person transmission. It is highly unlikely that the low rodent population density observed was the result of a precipitous decline in rodent populations in late spring. Rodent populations in northwest Patagonia are at minimal levels in the early spring and represent only animals that have survived the winter. Renewed reproduction increases population density throughout the spring (N. Guthmann, pers. comm. 1997), as was corroborated by the high percentage of immature animals captured during trapping. Thus the rodent population density during our trapping in late spring is likely to have been higher than density when the outbreak began.

The unique pattern of person-to-person transmission in this outbreak has not been a feature of hantavirus epidemiology; in fact, a

study performed in New Mexico in 1993 found no disease or hantavirus antibodies in serum specimens taken from 396 health care workers, including 266 who had been exposed to patients with HPS or their body fluids 2 to 6 weeks earlier (9). The high case-fatality ratio has precluded a detailed description of the exact nature of contact between cases. Consequently, the likely mode of transmission—whether through direct contact, droplets, infectious aerosols, or contaminated fomites—is not known. Because hantaviruses are difficult to isolate and cultivate, few attempts have been made to study virus excretion from patients or to assess viral stability under different environmental conditions. Efforts to define the infectious stage of the disease and the type and duration of contact that may have led to transmission are continuing. Genetic sequences of viruses from patients and rodents are being determined to define more clearly the pattern of transmission. In the absence of any clear-cut evidence for nosocomial transmission of Sin Nombre or related viruses in the United States, it is premature to suggest changing the existing guidelines for care of HPS patients. The level of suspicion should be raised to investigate any suspected person-to-person spread of this rodent-borne zoonosis, however. Further epidemiologic and laboratory studies are needed to clarify requirements for protecting healthcare workers and others from hantaviral infections in Patagonia.

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Pertussis in the Netherlands: an Outbreak Despite High Levels of Immunization with Whole-Cell Vaccine

In 1996, a sudden increase in pertussis incidence was reported in the Netherlands (2.1 per 100,000 in 1995, 18 per 100,000 in 1996). Although not all potential surveillance artifacts could be excluded, it is highly probable that the data reflect a true outbreak. However, the cause of this increase has not yet been determined. Further research is directed to the severity of disease and a possible mismatch between the vaccine and the circulating Bordetella strains.

In 1996, 2,771 cases of pertussis were reported to the Inspectorate of Health in the Netherlands (population 15 million), compared with 319 cases in 1995. With epidemic cycles expected every 3 to 5 years and a recent outbreak in 1994, this rise was unexpected (1). After the introduction of pertussis immunization with a whole-cell vaccine in the National Immunization Program (1952), the incidence of pertussis in the Netherlands decreased significantly. Children are immunized at ages 3, 4, 5, and 11 months with a diphtheria, tetanus, pertussis, and inactivated polio vaccine (DTP-IPV), and vaccine coverage for pertussis, for at least three immunizations, is 96% at the age of 12 months. Until the 1980s, the incidence of pertussis seemed very low, because only incidental cases were reported. However, in the last two decades, pertussis has been endemic, and epidemic peaks have appeared.

The surveillance of pertussis is based mainly on notification, which was made obligatory by law in 1976. Because the availability and interpretation of serologic tests changed in the 1980s and the absence of a clear case definition for notification seemed to have influenced surveillance, a restrictive case definition that included criteria for laboratory diagnosis was introduced in 1988. Therefore, we have limited this report to notification data from 1989 onwards. The annual incidence of pertussis notification from 1989 to 1995 is shown in Figure 1. In this period, the case definition includes defined clinical symptoms and laboratory confirmation. The clinical symptoms are a serious cough lasting more than 2 weeks or cough attacks or cough followed by vomiting in combination with at least one of the following symptoms/findings: apnea, cyanosis, characteristic cough with whooping, subconjunctival bleeding, leukocytosis, or contact with a person with confirmed or suspected pertussis in the previous 3 weeks. Laboratory confirmation is defined as either positive culture of *Bordetella pertussis* or *Bordetella parapertussis* or positive two-point serology, i.e., the finding of a significant rise (> 4-fold) of IgG antibodies against pertussis toxin and/or IgA antibodies against *B. pertussis* in paired sera. The case definition is highly specific, but results in significant underreporting.

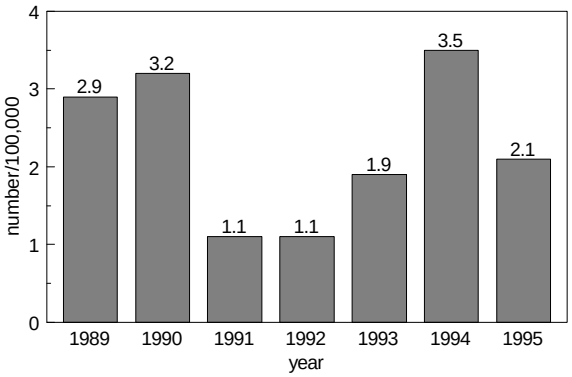

Figure 1. Annual incidence of pertussis estimated from notification by registration date, 1989–1995

We compared the number of cases reported per 4-week period in 1996 with the number reported in 1994, the year with the highest incidence in recent years (Figure 2). It appears that an unexpectedly large number of pertussis cases was reported (18 per 100,000) in 1996. The reports came from all over the country. No geographic clustering was observed, even in regions with pockets of low vaccination coverage or at the borders of

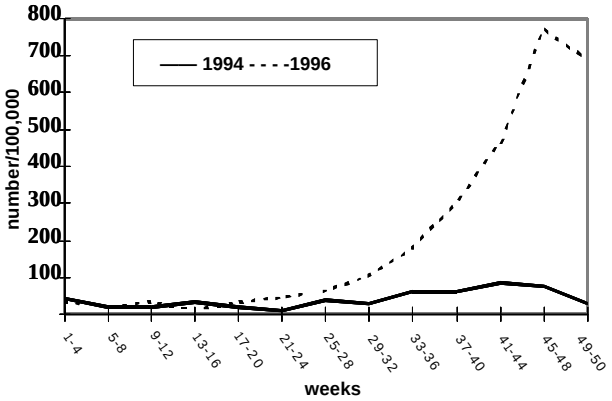

Figure 2. Number of cases reported by registration date, 1994 and 1996 (4-week periods).

the country (close to Germany, where vaccination coverage for pertussis is low).

The age distribution of patients in 1989 to 1996 shows a significant decrease in the proportion of infants less than 1 year old (from 21% in 1989 to 7% in 1996) and a significant increase in the proportion of 1- to 4-year-old children (from 21% in 1989 to 30% in 1996). A significant decrease (from 42% in 1989 to 30% in 1995) was also observed in 5- to 9-year-old children; however, in 1996, the proportion increased to 39%. No changes were observed in the 10- to 14- and 15- to 19-year-old groups, whereas the proportion of patients 20 years old or older increased from 5% to 11% (Table).

Table. Age distribution (percentages) pertussis notification in 1989 to 1996

Age	1989	1990	1991	1992	1993	1994	1995	1996
0	21	21	23	21	24	16	13	7
1-4	21	21	26	19	27	34	33	30
5-9	42	37	34	30	26	32	30	39
10-14	10	9	10	17	12	8	10	11
15-19	1	2	2	2	1	1	1	2
≥20	5	9	6	11	10	8	12	11
Unknown	0	0	1	1	0	1	1	0
Total (n)	434	471	164	169	294	536	319	2,771

Whereas the average vaccine coverage did not change, the proportion of vaccinated patients increased from 55% in 1989 to 85% in 1996. In all age groups, except that of 6- to 11-month old infants, the proportion of vaccinated patients is higher in the years 1994 to 1996 than in 1989 to 1993.

We estimated the annual incidence of pertussis for vaccinated and unvaccinated children ages 1 to 4 and 5 to 9 years, on the basis of reporting by registration date (1989 to 1996) (Figure 3). Calculation of incidence according to vaccination status was deliberately restricted to ages 1 to 4 years and 5 to 9 years (vaccine coverage estimated at 96%) for methodologic reasons. The changes in age-specific incidence over the years for vaccinated and unvaccinated children are identical for 1989 to 1993. However, from 1994 to 1996, and particularly in 1996, the incidence among vaccinated 1- to 4-year-olds was considerably higher than in 1989 and 1990, the years with the highest incidence in 1989 to 1993. In unvaccinated children, the incidence in 1994 and 1995 was lower than in 1989 and 1990. The difference in incidence between the period 1989 to 1990 and the year 1996 was much smaller in unvaccinated than in vaccinated persons. A similar discrepancy can be seen in the 5- to 9-year-old group.

In view of the increase in pertussis reporting in 1996, the first issue to be addressed was whether this reporting reflected a true increase in pertussis incidence or was a surveillance artifact. If considerable underreporting is assumed, an increase due to improved compliance to the notification system or increased alertness should be considered. However, in the first two quarters of 1996, there is no apparent reason for such an assumption. Nonetheless, after the first reports of the observed rise, both publicity and increased awareness of the disease by physicians and patients might have influenced reporting.

Serodiagnosis for the country as a whole is done by a single reference laboratory at the National Institute of Public Health and the Environment; thus, the data provided by the laboratory can be used for nationwide surveillance. Furthermore, the reference laboratory collects strains of *B. pertussis* from regional public health laboratories, and the number of strains received also reflects the epidemic curve. The epidemiologic curve derived from these sources over the past years is consistent with the notification data.

Since laboratory confirmation is part of the case definition, changes in serodiagnostic practice might have influenced notification. Frequently, no conclusive results are obtained from serology: a second serum sample may not be submitted, or the first serum sample may be taken late after onset of disease, thereby missing the dynamic phase of the immunoresponse. Recently, cut-off values for antibody titers characteristic for the acute immunoresponse after natural infection with *B. pertussis* have been determined by comparing the titer distribution in the healthy population,

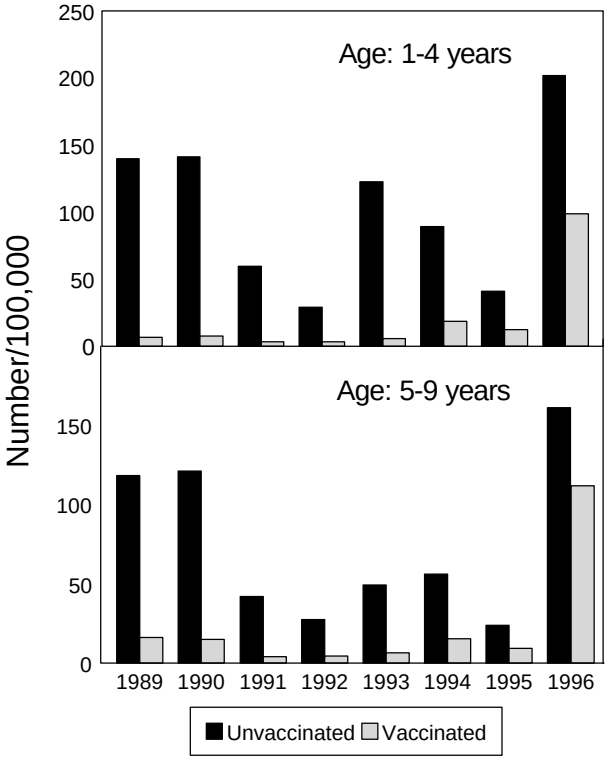

Figure 3. Estimated incidence of pertussis for unvaccinated and vaccinated children aged 1-4 years and 5-9 years based on notification records and assuming a coverage of 96% for the entire population.

in recently vaccinated children, and in sera longitudinally collected after natural infection. Since 1993, high titers in acute-phase sera are reported to be "suggestive for recent infection with *Bordetella pertussis*" (2). It can be imagined that such cases are reported, albeit not strictly in accordance with the case definition.

To exclude the possibility that the rise in notification was the result of reporting cases diagnosed with positive one-point serology only, we linked the notification and serodiagnosis databases. (The data were taken from January 1993 to September 1996.) Special permission to link the databases was given by the Inspectorate of Health. It appeared that some notification was based on positive one-point serology as early as 1993, and this proportion increased from 20% in 1993 to 33% in 1994. It remained at this level in the subsequent years. Thus, the 1996 increase in notification cannot be attributed to this change in serodiagnostic practice.

Our surveillance data suggest a decrease in vaccine efficacy, but estimation of vaccine efficacy from surveillance data should be interpreted with caution. However, there are no indications of significant bias from physicians' perceptions of vaccine efficacy that could have caused selective reporting of vaccinated patients; a higher probability of a positive serologic test result due to priming in vaccinated persons; or misclassification of cases with respect to vaccination status.

Although not all potential surveillance artifacts could be excluded, it is highly probable that the surveillance data reflect a true pertussis outbreak. The outbreak could have been caused by 1) decrease in vaccination coverage, 2) decrease in vaccine quality, 3) interference with other vaccines, 4) changes in circulating strains of *B. pertussis* that are not covered by vaccine-induced immunity, or 5) a combination of these factors. A decrease in vaccination coverage has not been observed in the (accurate) reporting system. The whole-cell vaccine used was produced in the National Institute of Public Health and the Environment and meets international standards. There was no sign of a gradual deterioration of vaccine quality as determined for product release by the mouse protection test.

In April 1993, vaccination against *Haemophilus influenzae* type b (Hib) was added to the immunization program. The polyribosylribitolphosphate tetanus toxoid conjugated vaccine (PRP-T) was administered simultaneously with the DTP-IPV, although on different limbs. Interference of Hib vaccination with the immunoresponse to pertussis vaccine has been described (3). In a prospective serologic study on the potential interference in the Netherlands, this effect was not observed (4). Furthermore, the increase of pertussis in 5- to 9-year-old vaccinated children, who never received Hib vaccine, was similar to the increase in the 1- to 4-year-old age group (Figure 3). Decreased vaccine efficacy due to interference of Hib vaccination is, therefore, very unlikely.

Molecular typing of *B. pertussis* isolates collected since 1945 indicated a shift in population structure, possibly due to the introduction of whole-cell vaccine (5). Further, antigenic variants of *B. pertussis* distinct from the strains incorporated in the vaccine appear to have emerged (6). It might be possible that circulating strains of *B. pertussis* have become less sensitive to vaccine-induced immunity. The abrupt increase in the proportion of vaccinated patients reported in all age groups suggests such a change.

Data from other countries in Europe indicate that the pertussis epidemic is restricted to the Netherlands. However, a resurgence of pertussis has been noted in the United States since the late

1980s and in Canada since 1991. No single factor has been found to explain this resurgence (7-10).

The decrease in pertussis notification in the Netherlands at the end of 1996 may indicate a seasonal variation in incidence. The 1996 data, including those on hospital admissions, are still being analyzed. These data will give insight into the severity of the pertussis epidemic among infants. Protection of these infants is the main reason for pertussis vaccination. In January 1997, active (monthly) surveillance by pediatricians of cases among hospitalized children was added to the routine surveillance based on notification and laboratory surveillance.

A prospective study is being considered to assess efficacy of the whole-cell vaccine, including differentiation of severity of illness and number of immunizations received, if the outbreak continues. Cooperative studies to determine the distribution of restriction fragment length polymorphism types and antigenic variants of *B. pertussis* in various countries in Europe, Asia, and North America are under way. Also, the immunogenicity of the Dutch whole-cell vaccine is being assessed: whether it has changed over time and how it compares to published immunogenicity data of whole-cell vaccines with known, prospectively determined, vaccine efficacy (11).

In conclusion, in the Netherlands a sudden increase of pertussis notification has been observed, which seems to reflect a true increase in incidence. Nevertheless, the cause of this increase has not been definitively determined. A possible mismatch between the vaccine and the circulating Bordetella strains is being investigated.

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Invasive *Haemophilus influenzae* type b Disease in Elderly Nursing Home Residents: Two Related Cases

We investigated two fatal cases of invasive *Haemophilus influenzae* type b (Hib) infection in a community nursing home in western Sydney, Australia. Two elderly women had lived in the same room, and the onset of their illness was 5 days apart. Hib isolates from blood cultures showed identical profiles by pulsed field gel electrophoresis. These findings suggest that Hib infection was transmitted within this nursing home. Serious Hib disease may be underrecognized in this setting. Continued surveillance and serotyping of invasive *H. influenzae* disease is essential for identifying groups at increasing risk that may benefit from immunization against Hib.

After the introduction of routine childhood immunization against *Haemophilus influenzae* type b (Hib), the incidence of invasive Hib disease fell dramatically in several countries, including Australia (1-3). This decline has been most evident among children aged less than 5 years. Meanwhile, serious Hib infections, such as pneumonia and epiglottitis, have been increasingly recognized among elderly, debilitated, and immunosuppressed adults (4-6). However, clusters of Hib infection have rarely been reported in adults. To examine their relatedness, we investigated two fatal cases of Hib septicemia in a community nursing home.

Case Reports

Case 1

In June 1996, a 71-year-old female nursing home resident was hospitalized after 3 days of unproductive cough, fevers, confusion, and increasing dyspnea. She had a history of cerebrovascular disease, muscular dystrophy, and congestive heart failure and was confined to a wheelchair. She had clinical signs of left lower lobe pneumonia and was afebrile. A blood film was normal, but a chest X-ray showed extensive bilateral pulmonary infiltrates. Arterial blood gases showed severe hypoxia in room air (PO_2 44 mm Hg, saturation 77%). Ceftriaxone 1 g and metronidazole 500 mg were given intravenously for suspected aspiration pneumonia, but the patient became severely hypotensive and died 8 hours after admission to the hospital. The following day, beta-lactamase-negative Hib was isolated from blood cultures collected before the patient died.

Case 2

Two days after the first patient was hospitalized, an 80-year-old female resident of the same nursing home became ill with fever and sore throat. She had a history of chronic airflow limitation, mild renal impairment, and dementia. Her sore throat rapidly worsened, and the next day she was hospitalized with severe dysphagia. On examination, she was febrile (38.5°C) and hypotensive (90/60 mm Hg) and had pretracheal cellulitis. No clinical evidence of pneumonia was observed, and a chest X-ray was normal. She was intubated, and extensive epiglottitis, laryngitis, and tracheitis were noted. Ceftriaxone 2 g, gentamicin 80 mg, flucloxacillin 1 g, and metronidazole 500 mg were administered intravenously. Inotropes were given for hypotension and acute renal failure, but the patient's condition deteriorated rapidly, and she died 9 hours after admission to the hospital. Beta-lactamase-negative Hib was subsequently isolated from blood cultures collected before the patient died.

Investigation

For several months, these two women had occupied beds in the same six-bed room (the largest room) in the 44-bed nursing home. The home employed 37 nursing and ancillary staff. During 3 weeks before the investigation, 20 residents and several staff had been ill with bronchitis, for which many had received oral antibiotics. No sputum samples had been collected for culture from residents or staff. Two other elderly residents had died during the preceding 10 days, but their case notes did not document any fever or symptoms suggesting

infection. On the first day of our investigation, two elderly residents were febrile. One woman had bilateral bronchopneumonia that was not responding to oral cefaclor and was hospitalized; blood and sputum cultures yielded no pathogens, and urine samples did not contain Hib capsular antigen. The other woman had been febrile for 1 week, with a cellulitic leg ulcer and pleuritic chest pain of uncertain etiology. Because of her other chronic illnesses she was treated palliatively and died 1 week later. A swab of her leg ulcer yielded group G streptococcus, blood cultures were negative, and sputum was not obtained.

Throat swabs were collected from all 81 residents and staff at the nursing home and cultured for Hib on bacitracin chocolate blood agar. We administered rifampicin chemoprophylaxis to all roommates of the index case patients and 28 staff who had been in regular close contact with the two case patients during the week before their illness. No new febrile illnesses occurred during 3 weeks of follow-up, and Hib was not isolated from any throat swab cultures. The Hib isolates from the first and second patients were examined by pulsed field gel electrophoresis and had identical profiles that were distinguishable from several epidemiologically unrelated isolates (Figure).

The relationship of these two fatal cases of Hib disease in time and place and the clonality of the isolates provide good evidence that transmission of Hib infection occurred in this nursing home. We were unable to demonstrate that Hib caused any of the associated reports of bronchitis and febrile illness among residents and staff, and our prevalence study did not show any asymptomatic Hib carriage. However, the survey was performed 10 days after the onset of the second case of septicemia following widespread use of antibiotics, and it may not have accurately reflected the prevalence of Hib carriage at the time these cases occurred. It is also difficult to evaluate whether chemoprophylaxis was a useful intervention in this setting.

To our knowledge, this is the first reported cluster of invasive Hib infection among adults in a community-based nursing home. Three other clusters of Hib infection in adults have been described: one occurred in a nursing home attached to a hospital, and two were nosocomial—including a cluster of bronchitic illness (7-9). Our report provides further evidence that Hib can cause clusters of serious disease among the elderly—at least in institutions. Given that diagnostic procedures are performed infrequently in many community nursing homes, Hib infection may be underrecognized in this setting.

We were especially interested in these two cases in adults as they were the only instances of

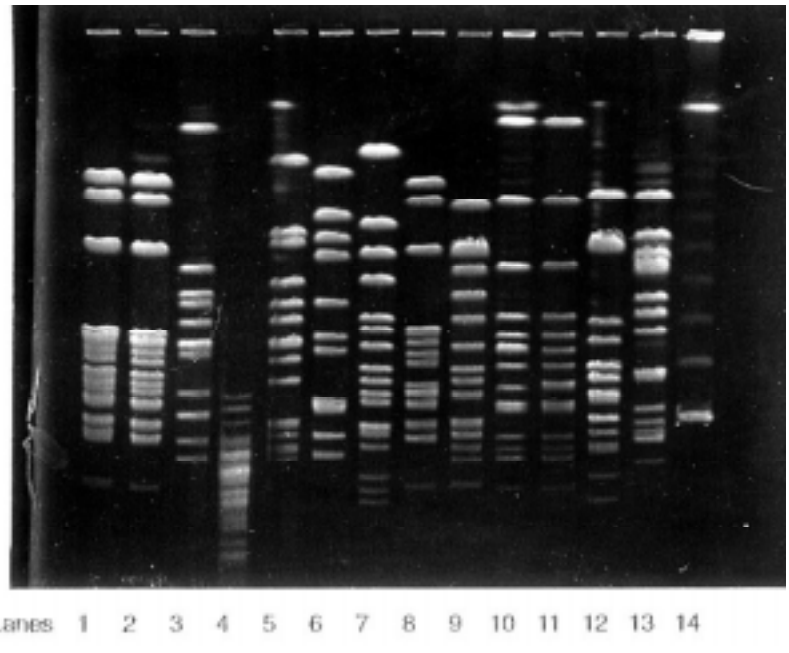

Figure. Pulsed field gel electrophoresis of *Haemophilus influenzae* type b (Hib) isolates from blood culture of two elderly nursing home residents (lanes 1 and 2) compared with epidemiologically unrelated *H. influenzae* isolates sent to our laboratory for typing: lane 3, laboratory Hib strain; lane 4, non-*H. influenzae*; lane 5 and 12, invasive nontypeable *H. influenzae*; lane 6-11, unrelated Hib isolates; lane 13, *H. influenzae* type a; lane 14, 1 kilobase molecular marker. The enzyme used for DNA digestion was *Apal*.

invasive Hib disease reported to this Public Health Unit from its population base of one million persons over 12 months—a rate of 0.3 per 100,000 population aged 15 years and older. This pattern of notification differs markedly from that observed in western Sydney at the time childhood Hib vaccination was first introduced in mid-1992. In 1992, 27 of 30 notifications of invasive Hib disease in this area were of children aged less than 5 years, or 36.9 per 100,000 population (New South Wales Health Department, unpub. data). Although invasive Hib infection remains uncommon in adults in communities where childhood Hib vaccination is routine, the relative frequency of invasive Hib infection is increasing in older age groups.

Population-based studies of acute epiglottitis undertaken in northern California and Rhode Island have also shown that the incidence of childhood epiglottitis is falling rapidly since the introduction of Hib immunization, while the incidence of epiglottitis in adults remains steady or is increasing (10,11). Acute epiglottitis in children is usually caused by Hib, and the falling incidence of epiglottitis in this age group is explainable by immunization. In adults, most culture-positive cases of acute epiglottitis are also caused by Hib (10,12). However, the incidence of Hib-related epiglottitis in adults does not appear to be changing appreciably—at least in Rhode Island—and Hib does not account for the increasing incidence of epiglottitis observed in this age group (10). Indeed, acute epiglottitis is frequently culture negative in adults, so explaining its increasing incidence and ascertaining the etiologic agents in older age groups require further study (10,12).

Invasive Hib disease should continue to be monitored in all age groups after the introduction of childhood immunization against Hib. Evidence suggests that Hib immunization reduces the acquisition of Hib carriage in children, and reduced carriage among children may indirectly prevent Hib disease in adults (13). However, surveillance data in western Sydney and metropolitan Atlanta have not supported this hypothesis; rates of adult Hib disease are remaining approximately unchanged, despite dramatic reductions in childhood disease (5; New South Wales Health Department, unpub. data). Only by serotyping isolates of *H. influenzae* that cause invasive disease will it be possible to detect changing patterns of invasive Hib disease in adults during this era of childhood immunization. Serotype-specific surveillance of invasive *H. influenzae* disease is also needed to assess progress towards the elimination of childhood Hib disease. In addition, periodic cross-sectional serotyping studies of *H. influenzae* isolates causing noninvasive disease would help measure the effects of Hib immunization on the incidence of less serious forms of Hib infection. These surveillance methods will identify groups at increasing or underrecognized risk that may benefit from immunization.

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Seroepidemiologic Studies of Hantavirus Infection Among Wild Rodents in California

A total of 4,626 mammals were serologically tested for antibodies to Sin Nombre virus. All nonrodent species were antibody negative. Among wild rodents, antibody prevalence was 8.5% in murids, 1.4% in heteromyids, and < 0.1% in sciurids. Of 1,921 *Peromyscus maniculatus* (deer mice), 226 (11.8%) were antibody positive, including one collected in 1975. The highest antibody prevalence (71.4% of 35) was found among *P. maniculatus* on Santa Cruz Island, off the southern California coast. Prevalence of antibodies among deer mice trapped near sites of human cases (26.8% of 164) was significantly higher than that of mice from other sites (odds ratio = 4.5; 95% confidence interval = 1.7, 11.6). Antibody prevalence increased with rising elevation (>1,200 meters) and correlated with a spatial cluster of hantavirus pulmonary syndrome cases in the Sierra Nevada.

In spring 1993, a cluster of unexplained severe acute respiratory illnesses associated with a high death rate was reported in the southwestern United States (1). The outbreak was linked to a newly recognized hantavirus strain, Sin Nombre virus (SNV), carried by the deer mouse, *Peromyscus maniculatus* (2-5). Sporadic cases of the illness, hantavirus pulmonary syndrome (HPS), were subsequently identified in other regions of North America, especially the western United States (6,7). Three additional pathogenic viruses associated with HPS were later discovered outside the usual range of *P. maniculatus*: 1) Black Creek Canal virus, harbored by the cotton rat, *Sigmodon hispidus*, in Florida; 2) Bayou virus, identified in the rice rat, *Oryzomys palustris*, in Louisiana; and 3) New York virus, isolated from the white-footed deer mouse, *Peromyscus leucopus*, in New York (8-12). These viruses have not been found in California; however, two novel hantaviruses, El Moro Canyon virus (EMCV) and Isla Vista virus (ISLA), were recently discovered in that state (13-15). Genetic studies identified the harvest mouse, *Reithrodontomys megalotis*, and the California meadow vole, *Microtus californicus*, as the reservoirs for EMCV and ISLA, respectively. Human infection with EMCV and ISLA has not been documented.

Through 31 January 1997, 156 cases of HPS were reported to the Centers for Disease Control and Prevention (CDC); the case-fatality rate is approximately 50% (T. Ksiazek, P. Rollin, unpub. data). HPS has been confirmed in 26 states, with California reporting the third largest number of cases (14 cases, 8 deaths), after New Mexico (29 cases) and Arizona (22 cases).

Hantaviruses are excreted in the urine, feces, and saliva of asymptomatic infected rodents (16). Transmission to humans occurs when aerosols contaminated with the virus are inhaled (16-18). Bites by infected rodents or exposure to broken skin or mucous membranes may represent alternative routes. Although specific risk factors are poorly defined, persons engaging in activities that bring them in contact with rodents and/or their excretions may be at a higher risk for infection (19).

The present study compiles retrospective and prospective serologic data to 1) confirm *P. maniculatus* as the primary reservoir of SNV and identify alternative reservoirs, if any, in California; 2) assess differences in SNV seroprevalence in vector populations from various geographic regions; and 3) compare seroprevalence rates of reservoirs collected near sites of human cases with those from other sites.

The Study

Mammal Surveys

The California Department of Health Services (CDHS) contacted 18 agencies (local vector control districts, local health and environmental health departments, universities, state and national park and forest services, the military, and wildlife refuges) involved in cross-sectional surveys of California mammals to participate in developing a centralized, statewide database of SNV serologic results and ecologic data.

Historical surveys used archived specimens collected in 1975, 1976, and 1988 and stored at the University of California, Berkeley, Museum of Vertebrate Zoology, and specimens collected from 1989 through early 1993 by local vector control districts. Prospective surveys and human case investigations were conducted from late 1993 through the end of 1995. Wild rodent trapping and processing methods were as previously described (20).

Reports by participating agencies included ecologic data on individual mammals (species, age, and sex). Information on survey sites included county, physical location of trapline, date of survey, elevation, and habitat. Habitat was categorized by a single dominant vegetation type: chaparral, conifer trees, grassland, hardwood trees, sage/scrub brush, or urban environment (21).

Human Cases

Within 2 weeks after a confirmed diagnosis of HPS, the patient or a surrogate was interviewed to determine potential sites of exposure to infected rodents. An environmental/ecologic investigation was conducted at possible sites of exposure (20,22).

Laboratory Studies

Rodent serum samples were tested by one or more of six laboratories (CDC; CDHS; University of New Mexico; University of California, Davis; and University of Nevada, Reno). Serum samples were examined for IgG antibodies to the SNV nucleocapsid protein by Western blot and/or enzyme-linked immunosorbent assay with CDC reagents (23,24). For archived specimens, liver tissue from frozen carcasses was used for polymerase chain reaction (PCR) (22,25).

Data Analysis

Descriptive data were analyzed by Epi Info, Version 6.0 (26). Frequency distributions were obtained, and chi-square tests of homogeneity for two-by-two contingency tables were used to examine the statistical significance of any association. A crude relationship between altitude and SNV-antibody prevalence was assessed by the Mantel-Cox test for trend. Adjusted odds ratios and 95% confidence intervals were calculated by logistic-binomial regression (27). A random effects model was chosen because of the presence of important clustering effects from the sampling design. The presence or absence of antibodies to SNV was the primary dependent variable, and characteristics that were identified in the descriptive analysis were the independent variables. Data from samples collected on the Channel Islands were analyzed separately from mainland data where indicated.

Sin Nombre Virus Antibody Prevalence

California Mammals

A total of 4,626 (4,549 rodent and 77 nonrodent species) mammals representing 47 species were collected between 1975 and 1995 in California and serologically tested for IgG antibodies to SNV (Table 1). Wild rodents (most 3,109, from the family Muridae) made up 98% of the sample, followed by Sciuridae (1,369), and Heteromyidae (71). Among the Muridae, 78% were deer mice or related species, 11% were wood rats, 6% were domestic rodents (house mice, rats), 3% were harvest mice, and fewer than 1% were meadow voles or cotton rats. Antibodies to SNV were found only among wild rodents. Prevalence of antibodies reactive with SNV was 8.5% among the Muridae, 1.4% among the Heteromyidae, and less than 0.1% among the Sciuridae. Nonrodent species tested included 74 carnivores (five domestic dogs, *Canis familiaris*; 26 coyotes, *Canis latrans*; 25 island foxes, *Urocyon littoralis*; six domestic cats, *Felis domesticus*; two opossums, *Didelphis virginiana*; three striped skunks, *Mephitis mephitis*; seven raccoons, *Procyon lotor*), one black-tailed jackrabbit, *Lepus californicus*, one Nuttall's cottontail, *Silvilagus nuttallii*, and one shrew, *Sorex* sp.

Deer Mouse Populations

Of 1,921 *P. maniculatus*, 226 (11.8%) had antibodies to SNV (Table 1). Other peromyscine-related species (e.g., cactus mice, canyon mice, California mice, and pinyon mice) also had antibodies to SNV, but prevalence was lower. In almost all instances, infected *P. maniculatus* were collected at the same site as SNV–antibody-positive animals of other species.

Retrospectively, antibodies to SNV were identified in 3 (6.0%) of 50 deer mice specimens collected in 1975 (1 of 22, 4.5%), 1976 (2 of 17, 11.8%), 1981 (0 of 2), 1992 (0 of 1), and early 1993 (0 of 8). The three seropositive *P. maniculatus* collected by the University of California, Berkeley in 1975 and 1976 were from Alameda, Kern, and Mono Counties. Frozen liver tissue available from the seropositive Kern County specimen yielded a PCR sequence of the G1 amplimer of SNV that differed from a *P. maniculatus*

Table 1. Prevalence of antibodies to Sin Nombre virus among wild rodents in California, 1975–1995

Family/ Species	Common Name	No. Tested	No. Pos.	% Pos.
Heteromyidae				
Dipodomys sp.	kangaroo rats	28	1	3.6
Perognathus sp.	pocket mice	43	0	0.0
SUBTOTAL		71	1	1.4
Muridae				
Microtus sp.	meadow voles	29	5	17.2
M. californicus	California meadow vole	22	5	22.7
M. montanus	Montane vole	3	0	0.0
M. townsendii	Townsend's vole	1	0	0.0
Mus musculus	house mouse	88	0	0.0
Neotoma sp.	wood rats	330	2	0.6
N. cinerea	bushy-tailed wood rat	20	0	0.0
N. fuscipes	dusky-footed wood rat	215	2	0.9
N. lepida	desert wood rat	95	0	0.0
Peromyscus sp.	deer mice	2430	236	9.7
P. boylii	brush mouse	98	0	0.0
P. californicus	California mouse	159	4	2.5
P. crinitus	canyon mouse	29	2	6.9
P. eremicus	cactus mouse	100	1	1.0
P. maniculatus	deer mouse	1921	226	11.8
P. truei	pinyon mouse	123	7	5.7
Rattus norvegicus	Norway rat	11	0	0.0
Rattus rattus	roof rat	99	0	0.0
Reithrodontomys megalotis	harvest mouse	108	16	14.8
Sigmondon hispidus	cotton rat	14	0	0.0
SUBTOTAL		3109	263	8.5
Sciuridae		1369	1	<0.1
Ammonospermophilus leucurus	antelope ground squirrel	4	0	0.0
Spermophilus. sp.	ground squirrels	1205	1	<0.1
S. beecheyi	California ground squirrel	856	1	0.1
Tamias sp.	chipmunks	152	0	0.0
Tamiascurus douglasii	Douglas' squirrel	5	0	0.0
SUBTOTAL		1369	1	<0.1
TOTAL		4549	245	5.4

collected in nearby Mono County in 1994 by only five residues out of 274. The largest comparative protein dissimilarity was with a *P. maniculatus* from the Channel Islands, which differed by seven amino acid substitutions.

Among the deer mice for which age (1,165 animals, 87% adults) and sex (1,239 animals, 57% male) were available, SNV antibody prevalence was, respectively, 13.5% in adults and 6.7% in juveniles and 13.0% in males and 10.1% in females. Differences in age (chi-square = 5.5) and sex (chi-square = 2.5) were not significant.

Geographic Distribution

Thirty-four of the state's 58 counties were surveyed (Table 2). Antibodies to SNV were identified in deer mice from 21 (62%) of these counties (Figure 1). Most samples were from coastal counties (56%), followed by foothill/mountainous (36%) and inland/valley (8%) counties. Among these, the prevalence was 14.5% of 684 in the foothills/mountains, 11.6% of 112 on the coast, and 0.7% of 151 in the inland/valley areas.

One hundred and thirty-nine individual sites were surveyed within 34 counties with a mean of four sites per county (Table 2). Antibody prevalence at individual sites was 0.0% to 71.4% (25 of 35 tested on Santa Cruz Island, Santa Barbara County). In the Sierras, the highest antibody prevalence (50.0% of 52) was found in deer mice captured in Truckee, Nevada County, during a human case-patient investigation (Table 3, Case 3). At least one SNV-antibody-positive mouse was detected at each mainland site where 38 or more mice were tested.

Channel Islands

The Channel Islands, a group of eight islands located south of the Santa Barbara-Los Angeles coast, are 20 km (Anacapa) to 98 km (San Nicolas) from the mainland and 5 km to 45 km from each other. Despite the proximity between them, SNV-antibody prevalence varied significantly between islands. Antibody prevalence in deer mice trapped on the islands (20.9% of 382) was significantly higher than that of deer mice from the mainland (chi-square = 40.9, $p < 0.001$). In addition, Channel Island sequences differed by approximately 17% to 19% from any mainland California sequences (22,25). The highest prevalence was found on Santa Cruz and Santa Rosa Islands, where, respectively, 25 (71.4%) of 35 and 47 (58%) of 81 deer mice were SNV-antibody positive. Antibody prevalence among deer mice on the other islands was 7 (17.9%) of 39 on San Miguel, 1 (14.3%) of 7 on Santa Catalina, and 1 (2.9%) of 34 on San Clemente. However, deer

mice sampled on Anacapa (n=37), San Nicolas (n = 91), and Santa Barbara (n = 58) Islands were all SNV-antibody negative.

Table 2. Prevalence of antibodies to Sin Nombre virus among *Peromyscus maniculatus* by county, California, 1975–1995

County	No. survey sites	No. tested	No. pos.	% pos.
COASTAL				
Alameda	2	6	1	16.7
Contra Costa	1	36	0	0.0
Del Norte	2	19	0	0.0
Los Angeles	10(8)	112(71)	13(11)	11.6(15.5)
Marin	2	153	3	2.0
Mendocino	1	13	0	0.0
Monterey	2	52	5	9.6
Orange	8	52	3	5.8
San Diego	32	131	7	5.3
San Francisco	1	30	0	0.0
San Luis Obispo	2	4	0	0.0
San Mateo	1	40	8	20.0
Santa Barbara	6(2)	294(81)	86(7)	29.3(8.6)
Sonoma	1	7	0	0.0
Ventura	5(3)	137(9)	0(0)	0.0(0.0)
SUBTOTAL	76(68)	1086(704)	126(45)	11.6(6.4)
INLAND/VALLEY				
Imperial	1	2	1	50.0
Riverside	10	57	0	0.0
Sacramento	1	36	0	0.0
San Bernardino	4	49	0	0.0
San Joaquin	1	7	0	0.0
SUBTOTAL	17	151	1	0.7
FOOTHILLS/MOUNTAINS				
Butte	12	115	14	12.2
El Dorado	1	25	0	0.0
Glenn	1	4	0	0.0
Inyo	2	3	1	33.3
Kern	5	66	7	10.6
Mariposa	1	46	7	15.2
Mono	8	107	17	15.9
Nevada	1	52	26	50.0
Placer	2	29	2	6.9
Plumas	4	35	1	2.9
Shasta	3	30	4	13.3
Siskiyou	4	117	12	10.3
Tehama	1	35	5	14.3
Tulare	1	20	2	10.0
SUBTOTAL	46	684	99	14.5
TOTAL	139	1921	226	11.8

*Numbers in parentheses exclude the Channel Islands in Los Angeles (Catalina, San Clemente), Santa Barbara (San Miguel, Santa Barbara, Santa Cruz, Santa Rosa), and Ventura (Anacapa, San Nicolas) counties.

Habitat

A higher SNV-antibody prevalence was observed in the Sierra Nevada, Great Basin, and southern coastal habitats (Figure 1). Likewise, antibody prevalence was higher among deer mice trapped in vegetation associated with these environments: 15.1% of 531 in conifer, 14.8% of 597 in grassland, 13.4% of 86 in hardwood, 11.2% of 165 in sage/scrub brush, and 5.8% of 474 in chaparral. In urban environments, only 2.9% of 68 deer mice were SNV-antibody positive.

Antibody prevalence among deer mice increased significantly ($p < 0.001$) with rising altitude (Figure 2). In the regression model, adjusted odds ratios steadily increased with elevation, peaking at 4.3 in the 1,800- to 2,100-meter range (95% confidence intervals = 1.3, 16.7) (Table 4). The increased prevalence of SNV antibodies in deer mice trapped at higher elevations correlated with an increased incidence of HPS cases at elevations above 1,200 meters (Table 3).

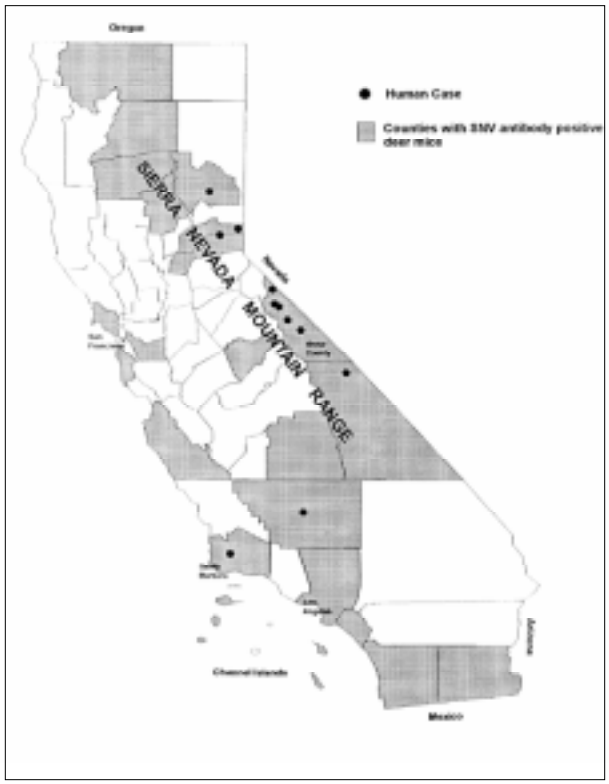

Figure 1. Geographic distribution of hantavirus pulmonary syndrome cases and occurrence of Sin Nombre virus antibodies among deer mice (*Peromyscus maniculatus*), California, 1975–1995 (n=1,921).

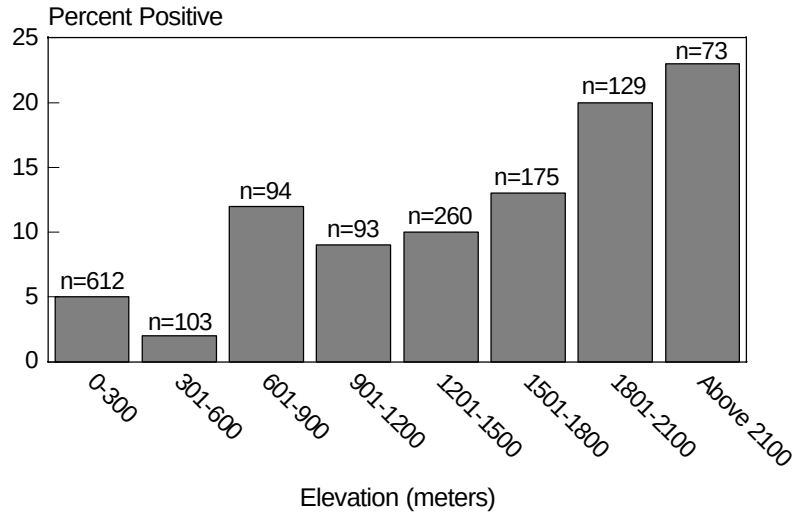

Figure 2. Prevalence of Sin Nombre virus antibodies among deer mice (*Peromyscus maniculatus*) by elevation, California, 1975–1995 (n=1,539, excluding Channel Islands).

Human Cases

HPS cases were spatially clustered in the state's Sierra Nevada region (Figure 1). Results from antibody prevalence studies among rodents collected during the investigation of six HPS cases in California are presented in Table 3. Antibody prevalence in deer mice trapped at these sites was significantly higher than at sites not associated with a human case (odds ratio = 4.5; 95% confidence interval = 1.7, 11.6) (Table 4).

Reservoirs of Hantavirus in California

Extensive serologic testing unequivocally identified *P. maniculatus* as the primary reservoir of SNV in California, as did previous studies in the western United States (2,20,28-30). The overall prevalence of SNV antibodies in deer mice (11.9%) in California is also comparable with results published by nearby western states such as Montana (8.0%) and Nevada (12.5%) (28,30). Other peromyscine rodents (e.g., canyon mice and pinyon mice) may harbor the virus in California, but at much lower levels. Because these related wild mice species frequently inhabit the same environment as *P. maniculatus*, their infections may repre-sent transmission from the primary reservoir population rather than from each other, as would occur in a permanent virus-species relationship. The extremely low prevalence of SNV antibodies (Table 1) in heteromyids (kangaroo rats, pocket mice), sciurids (ground squirrels, chipmunks), and Old World murids (domestic mice and rats), despite a large sample size (> 1,600), suggests that these animals are not important in the epidemiology of SNV in California.

Positive serologic test results from California meadow voles and harvest mice probably represent cross-reaction with SNV antigen by ISLA and EMCV, respectively (13-15). The high prevalence of antibodies identified in meadow voles (22.7% of 29) and harvest mice (14.8% of 108) is a cause for concern, even though human infection by these viruses has not been documented. Precautionary measures should be taken against exposure to hantaviruses through all potential

Table 3. Characteristics of selected hantavirus pulmonary syndrome cases and prevalence of antibodies to Sin Nombre virus in *Peromyscus maniculatus* collected at candidate sites of exposure, California, 1994-1995*

Patient	Onset Date	Outcome	Suspect County of Exposure	Predominant Vegetation	Elevation (meters)	No. Rodents Tested	% Pos.
1	09/94	Nonfatal	Mono	conifer, sage brush	1801-2100	34	14.7
2	03/95	Nonfatal	Mono	conifer, sage brush	1801-2100	22	13.6
3	04/95	Nonfatal	Nevada	sage brush	1801-2100	52	50.0
4	06/95	Fatal	Mono	conifer, sage brush	1801-2100	11	54.5
5	09/95	Fatal	Placer/Nevada	conifer	1501-1800	26	7.7
6	10/95	Nonfatal	Plumas	conifer	1201-1500	19	5.3
Total						164	26.8

*Rodent studies associated with California HPS cases described in greater detail (20,22,29).

wild rodent reservoirs until more is known about these newly discovered strains.

Temporal and Spatial Trends in Deer Mice Populations

Our data from historical surveys indicate that SNV was already circulating in deer mice 20 years ago in parts of California. The antibody-positive deer mouse originally trapped in Kern County in 1975 is the oldest documented evidence of SNV infection in wild rodents. Notably, SNV-antibody-positive deer mice identified retrospectively were captured almost 20 years before the first HPS cases were recognized in California and in some of the same geographic regions (20,22,29,31). The slight difference (approximately 2%) between sequences from the Kern County deer mouse trapped in 1976 and a Mono County deer mouse trapped in 1994 indicates a long-standing stability of the virus, as previously demonstrated by Nerukar et al. in Mono County (32).

Although SNV infection in deer mice is widespread in California, represented biotypes vary considerably in seroprevalence levels. For example, deer mice in foothill/mountainous (14.5% of 684) counties have a higher seroprevalence than those in inland/valley (0.7% of 151) and coastal (6.4% of 704, excluding the Channel Islands) counties. The trend of increasing prevalence with rising elevation found in this analysis has been observed in other states (Jim Mills, pers. comm.).

The Channel Islands offered a unique opportunity to study SNV infection in a relatively isolated population of deer mice. Deer mouse populations have been on the Channel Islands long enough that each island has its own subspecies and have considerable variation genetically within those subspecies (33,34). Likewise, Hjelle et al. found significant divergence between genetic sequences of virus from infected deer mice collected on the islands and those from the nearby coastal mainland (25). Although travel from the mainland to the islands and from island to island is common, evidence suggests that SNV coevolved separately within the deer mouse populations on each island (San Clemente, San Miguel, Santa Catalina, Santa Cruz, Santa Rosa). It appears that the virus is not endemic or is present at very low levels among deer mice on Anacapa, San Nicolas, and Santa Barbara Islands.

Human Cases

The antibody prevalence of SNV in deer mice collected at potential exposure sites during the investigation of sporadic HPS cases in California (26.8%) was similar to the antibody prevalence (30.0%) in deer mice observed during the 1993 HPS outbreak in the Four Corners region (2). Prevalence was significantly higher ($p = 0.002$) in deer mice trapped near human case exposure sites than in those from survey sites with no cases (Table 4). Together these findings imply that the percentage of infected deer mice may be a risk factor for human exposure to SNV. In addition, landscape features such as high elevation may be another important predictor of hantavirus in the state. The spatial clustering of HPS cases in the Sierra Nevada range supports this conclusion.

Characteristics of the mouse population, the environment, or human lifestyles may explain these geographic differences. The climate and vegetation in the mountains could be conducive to large populations of deer mice, a factor which might influence SNV prevalence. In addition, the occupational and recreational activities of the inhabitants of rural, mountainous environments bring them into frequent contact with rodents. In addition, local residences (e.g., old log cabins) are prone to rodent infestation, a possible risk factor for HPS infection (35).

Otteson et al. documented a higher SNV antibody prevalence among rodents trapped near

Table 4. Odds ratios for Sin Nombre virus antibody prevalence among *Peromyscus maniculatus* by association with human cases and elevation, California, 1975–1995[a]

	Cases (+SNV)	Controls (-SNV)	Adj. OR[bc]	95% CI[c]	p value
Human case[d]					
Absent	101	1274	1.0	NA[c]	NA
Present	44	120	4.5	1.7-11.6	0.002
Elevation (meters)					
0-300	32	580	1.0	NA	NA
301-600	2	101	0.4	0.4-3.5	0.423
601-900	11	83	0.6	0.2-1.8	0.390
901-1200	8	85	2.4	0.7-8.6	0.168
1201-1500	26	234	3.2	1.0-10.0	0.044
1501-1800	23	152	2.6	0.7-9.2	0.151
1801-2100	26	103	4.3	1.3-16.7	0.032

[a]Excludes Channel Islands; [b]Adjusted for trapline location and survey date, vegetation, sex and age of deer mouse; [c]OR, odds ratio; CI, confidence interval; NA, not applicable; [d]Deer mice collected at candidate sites of exposure during human case investigations.

buildings, regardless of the presence of a human case; a case-control study of the Four Corners outbreak had similar findings (30,36). Information on proximity to human dwellings was not available for our analysis; however, most mice were collected near buildings during investigation of human cases. Since other survey sites were probably less likely to be located near human dwellings, this factor may represent a source of bias in our study. Other biases may have been introduced because of nonrandom sampling and small sample size at some survey sites. Systematic longitudinal studies of SNV infection in deer mice at the key locations identified in this analysis are needed to further develop a predictive model for hantavirus infection in California which could elucidate the natural history of SNV and enhance prevention efforts.

Public Health Implications

Preliminary results indicate a need for health education of residents, visitors, and workers at high risk, especially in the Sierra Nevada range. Human dwellings in the mountains may be more vulnerable to deer mice infestation, especially if the buildings are older and/or intermittently occupied. In addition, persons working in or cleaning these structures may be at an even higher risk (22,29,35,36). Local health care providers and tertiary care centers should be aware of the potential for HPS cases in the state.

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Gestational Psittacosis in a Montana Sheep Rancher

In humans, psittacosis is primarily a flulike illness following exposure to psittacine birds. In rare cases, pregnant women exposed to *Chlamydia psittaci* can contract gestational psittacosis: atypical pneumonia, sepsis, and placental insufficiency resulting in premature birth or miscarriage. In the United States, only two cases of gestational psittacosis have been reported, both from exposure to psittacine birds. Eleven other cases have been reported worldwide, mostly in the United Kingdom, all from exposure to infected birth fluids and membranes of farm mammals, notably sheep and goats. In these mammals, *C. psittaci* inhabit the reproductive tract, are transmitted sexually or by the fecal-oral route, and cause miscarriages. The case of gestational psittacosis in a Montana sheep rancher is the first farm animal–related case reported in the United States. Pregnant women should avoid close contact with *C. psittaci*–infected animals, particularly sheep and goats during the birthing season. Obstetricians should consider this diagnosis along with early antibiotic treatment and cesarean section delivery in the context of the patient's case history.

Psittacosis is a flulike systemic infection, often with fever, headache, and atypical pneumonia, caused by *Chlamydia psittaci*. The number of reported cases varies from year to year because of periodic outbreaks, although the true baseline incidence in the United States is thought to be 75 to 100 cases per year with 1 or 2 deaths (1). Montana typically reports one case per year (Montana Dept. of Health surveillance data). Many cases are probably not diagnosed. In most reported cases, transmission occurs by inhaling infectious material from diseased psittacine birds (2).

However, not all cases of psittacosis result from inhaling avian strains of *C. psittaci*, nor are they all manifested as a simple flulike illness or atypical pneumonia. In rare cases, pregnant women contract severe chlamydial disease after exposure to the infected birth fluids and membranes of goats and sheep (3). This "gestational psittacosis" can be defined as a flulike syndrome leading to sepsis, placental infection, and fetal compromise (4). Only 13 cases have been reported worldwide: Eleven after exposure to gravid sheep and goats (United Kingdom and France) and two after exposure to psittacine birds (United States). This is the first farm animal–related case reported in the United States.

Case Report

In April 1996, a previously healthy 25-year-old Montana sheep rancher, pregnant for the first time, had cough and congestion for 14 days at weeks 19 to 21 of gestation; the symptoms were followed by 4 days of high fever (104°F), myalgia, headache, fatigue, and backache, and 2 days of abdominal pain. Upon hospitalization, a chest X-ray was normal, but laboratory tests showed anemia (Hg 10.7 g/dl), thrombocytopenia (platelets 42,000/µl), and hepatic dysfunction (SGOT 351 IU/L), as well as proteinuria (2+) and hematuria (1+). Other tests showed a positive lupus anticoagulant and positive anticardiolipin antibodies. Blood, urine, and sputum cultures (collected before antibiotics were administered) yielded negative results. Admitting diagnosis was "early severe and low platelet count) syndrome" with a possible concurrent viral infection and poor hydration accounting for the fever and lack of hypertension.

On the second day of hospitalization, the patient underwent emergency termination of the pregnancy (22-23 weeks gestation). On the following day, the patient still had high fevers; moderate respiratory distress with tachypnea developed, and oxygen was required. A chest X-ray showed diffuse bilateral alveolar infiltrates; antibiotic treatment (IV Ancef) was started for presumed pneumonia. The fever subsided, the patient's condition improved remarkably, and she was discharged within 3 days. The discharge diagnosis was early severe HELLP syndrome (probably related to lupus anticoagulant and anticardiolipin antibodies) with coincident pneumonia. On follow-up, the patient was feeling well and had resumed her activities around the ranch.

Suspicious of a diagnosis related to environmental exposure and motivated by a need to explain all of the patient's symptoms, the obstetrician sent placental and fetal tissue to California and Oklahoma for specialized pathologic examination, after which patient sera were sent to the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, and animal sera were sent to the Veterinary Diagnostic Laboratory in Bozeman, Montana. A concomitant epidemiologic investigation was initiated.

Hematoxylin and eosin stain of the placenta showed placentitis: an intense acute inflammation of the intervillous space, more on the maternal side, which suggested infection from hematogenous spread rather than from ascension from the birth canal. Numerous basophilic intracytoplasmic inclusions in the cytoplasm of the trophoblast appeared to contain minute cocci-shaped organisms. The inclusions were identified by genus-specific fluorescein-tagged monoclonal antibody staining as masses of *Chlamydia* (either *pneumoniae* or *psittaci*) in various stages of development. Electronmicrographs further defined the round, rather than pear-shaped, morphologic features of the elementary bodies, which suggested *psittaci* rather than *pneumoniae* as the specific species. The pathologic findings are reviewed in greater detail, including photographs, in a concurrent publication (4).

Because traditional complement-fixation serologic tests do not adequately differentiate the chlamydial species in humans, sera were sent to CDC for a microimmunofluorescence assay, which is more sensitive and specific (5). The patient's sera had very high (1:512) IgG antibody titer to *C. psittaci*; identical titer was seen on sera collected several months later. No IgM to *C.psittaci* was detected. Tests for the other chlamydia, as well as for other placental-acquired infections (e.g., Q fever and brucellosis) were negative.

The epidemiologic investigation showed that, during the week before hospitalization, the patient's husband had a similar flulike illness with mild respiratory symptoms. The spring lambing season began 4 to 6 weeks before hospitalization, during which the patient and her husband worked closely with gravid sheep.

Out of a flock of 24 sheep, 19 gave birth to 30 lambs. There were six lamb deaths but no reported abortions. The patient assisted in most deliveries; however, she had direct contact on just three occasions: pulling one lamb during a twin birth, pulling one kid during the twin birth of her pet goat, and assisting in the premature delivery of a yearling ewe. The latter resulted in considerable placental exposure. She spent approximately 1 hour manually extracting the retained placenta, wearing only plastic gloves; she did not cover the mouth or nose. Serologic testing of five sheep and two goats was performed by complement fixation for *C. psittaci*. Only sera from two sheep were positive, including the yearling ewe from which the patient manually extracted the retained placenta.

The sheep were purchased from a neighboring flock with reported "pink eye" and abortions, both of presumed chlamydial etiology. The flock's abortion problem resolved after tetracycline-fortified feed was administered just before yearly breeding. Neither antibiotic feed nor the available chlamydia vaccine was used for the sheep on the patient's ranch. During her pregnancy, the patient had minimal contact with other ranch animals, including cattle, horses, and poultry; none of these animals were ill or had abortions. She had no contact with psittacine birds.

HELLP is a variant of the preeclampsia-eclampsia syndrome, which also includes epigastric pain and liver tenderness, as well as hypertension, edema, and proteinuria. An underappreciated early symptom is generalized malaise and/or flulike illness (6,7). Antiphospholipid antibodies, such as the lupus anticoagulant and anticardiolipin antibodies, have been associated with thrombosis, thrombocytopenia, and pregnancy complications, particularly decidual vasculopathy, placental infarction, fetal growth retardation, early-onset preeclampsia, recurrent miscarriages, and fetal death (8). Initially, in this patient, except for the high fever and lack of hypertension, the symptoms reasonably conformed to those of HELLP syndrome. However, in this case, epidemiologic, clinical, laboratory (human and animal), and histopathologic data support the diagnosis of gestational psittacosis. The most likely mechanism of transmission was inhalation of *C.psittaci* while manually extracting the retained placenta from the *C. psittaci*–infected sheep that lambed prematurely just 2 weeks before the patient's hospitalization. The usual incubation time for psittacosis is 1 to 4 weeks (2).

In the United States, pneumonia acquired through contact with birth membranes or fluids of farm animals is more commonly caused by *Coxiella burnetti* (Q-fever) or *Brucella sp.*

(brucellosis) (9). In both diseases, the flulike syndrome does not lead to fetal compromise in pregnant women. However, in gestational psittacosis, the flulike illness results in sepsis, placental infection, and fetal compromise. The disease is relatively new, first reported in the United Kingdom in 1967, and rare, just 13 cases reported worldwide (4,10,11). Gestational psittacosis has occurred from exposure to psittacine birds (the United States: two previous cases; 12,13) and from exposure to infected birth fluids and membranes of sheep or goats (the United Kingdom: nine cases, all sheep [10,11,14-19]; France: two cases, both goats [20,21]). This is, therefore, the third case reported in the United States, the first related to farm animals (4).

Further review of the clinical details of the 13 other reported cases shows remarkable similarity between them and the case reported here. For example, in all 14 reported cases (including this case) the illness is manifested as a flulike febrile syndrome. Similarly, in the 14 cases, thrombocytopenia and/or coagulopathy were reported (12 [85%]), as well as pulmonary disease (12 [85%]) and liver disease (11 [78%]). The outcome of pregnancy is also similar: 11 (78.5%) of 14 pregnancies ended in fetal death, while the three surviving neonates were born by cesarean section before 34 weeks gestation (4). Maternal outcome was much better: 13 of the 14 women recovered fully after pregnancy termination. The other woman died of overwhelming sepsis. Erythromycin was administered to six of the 14 pregnant women, including the mothers of the three surviving infants, early in the illness (4,10,12,13). Tetracycline, the drug of choice for psittacosis, is usually contraindicated during pregnancy, although it is still recommended for cases of gestational psittacosis that appear refractory to erythromycin (12,13).

C. psittaci is known worldwide as the primary cause of abortion in sheep, often referred to as "enzootic abortion of ewes", "chlamydial abortion," or simply "enzootic abortion." Enzootic abortion in sheep was first reported in Scotland in 1936, where *C. psittaci* became known as the "ewe abortion agent" (10,22). Abortion usually occurs within 2 years of contact with other aborting sheep in the flock. Indeed, pregnant sheep experimentally infected with an ovine abortion isolate of *C.psittaci* either abort or give birth to weak lambs and maintain a persistent systemic antibody response to the organism for up to 2.5 years postinfection; for sheep that aborted, detectable amounts of chlamydial antigen are excreted from the reproductive tract during subsequent estrus cycles (23). Postpartum (or postabortive) infected sheep are considered protected from subsequent *C. psittaci*–induced pregnancy complications, and they remain healthy, fertile, and therefore able to transmit the organism within the flock, perhaps through sexual contact (23,24).

Moreover, *C. psittaci* organisms are found in eye, respiratory, oral, and fecal material, which suggests other modes of transmission. Indeed, environmental contamination from diseased placentae or vaginal discharge is considered the primary source of infection to other sheep, perhaps through ingestion of infected tissue or contaminated feed during the previous or current lambing season and subsequent intestinal colonization (23,24). The carrier state is not evident but is often presumed if a high incidence of abortions or premature lambing occurs in the flock. Sometimes a high rate of "pink eye" in the flock indicates high *C. psittaci* prevalence, although this is usually caused by a serovar different from the one causing abortion. Nevertheless, ranchers who suspect *C.psittaci* in the flock typically respond by physically isolating infected sheep, giving antibiotic feed, and/or using the chlamydial vaccine. However, these methods rarely eliminate the disease from the flock (23).

In Montana, 50 to 100 aborted sheep fetuses are examined each year, more than 50% of which test positive for *C. psittaci* by complement fixation on sera or enzyme-linked immunosorbent assay on placental tissue. For this reason, masks are always worn by laboratory workers during sheep (and psittacine bird) autopsies (L. Stackhouse, Montana Veterinary Diagnostic Laboratory, pers. comm.). It is difficult to estimate the overall rate of colonization/infection within sheep flocks, although it is presumed to be high, particularly in the United Kingdom, and in areas of the United States where sheep abortions have already occurred and feed-antibiotics or chlamydial vaccine is not routinely used. High rates of sheep infection, thus far, have not led to high rates of miscarriage in female sheep ranchers. Nevertheless, given the increasing number of small family farms or hobby farms in the western United States, a growing number of farmers could be raising sheep (and other animals) without knowing the potential hazards to human health and how to prevent them.

Thus, it is strongly recommended that pregnant women avoid contact with membranes or birth fluids of sheep and goats and close contact with psittacine birds during pregnancy. Moreover, obstetricians should consider the diagnosis of gestational psittacosis in pregnant patients initially presumed to have HELLP syndrome and/or a flulike illness, particularly during the spring lambing season, so that appropriate early antibiotics and cesarean section delivery can help reduce illness and death from the disease.

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Lack of Serologic Evidence for an Association between Cache Valley Virus Infection and Anencephaly and other Neural Tube Defects in Texas

We tested the hypothesis that Cache Valley Virus (CVV), an endemic North American bunyavirus, may be involved in the pathogenesis of human neural tube defects. This investigation followed a 1990 and 1991 south Texas outbreak of neural tube defects with a high prevalence of anencephaly and the demonstration in 1987 that in utero infection by CVV was the cause of outbreaks of central nervous system and musculoskeletal defects in North American ruminants. Sera from 74 women who gave birth to infants with neural tube defects in south Texas from 1993 through early 1995 were tested for CVV neutralizing antibody. All tested sera did not neutralize CVV. These data suggest that CVV is not involved in the induction of human neural tube defects during nonepidemic periods but do not preclude CVV involvement during epidemics. Other endemic bunyaviruses may still be involved in the pathogenesis of neural tube defects or other congenital central nervous system or musculoskeletal malformations.

Anencephaly, spina bifida, and encephalocele (the major types of neural tube defects) are generally due to the failure of the neural tube to close during early embryonic development (1). Neural tube defects are among the most common and most severe major birth defects. Anencephaly is caused by failure of the anterior neuropore to close during embryonic development and results in total or partial absence of the cranial vault, the covering skin, and the brain. Infants with anencephaly are stillborn or die shortly after birth. Spina bifida is caused by a disturbance in the normal closure of neural walls and results in spinal cord defects. Most infants with spina bifida survive surgical repair of the defect with residual neurologic handicaps of varying severity. Encephaloceles are skull defects through which skin-covered meninges, with or without brain tissue, herniate. Small to moderately sized encephaloceles are surgically correctable (1-4).

The prevalence of neural tube defects in the United States has been steadily declining (5) and is currently estimated to be six per 10,000 births. The prevalence of anencephaly in the United States has likewise declined and is now approximately three per 10,000 live births (4). A vital record study of anencephaly in Texas showed that from 1981 to 1986 the prevalence rate was 3.8 to 4.3 cases per 10,000 births (6). This study also showed that in south Texas during this period the average annual prevalence of anencephaly was approximately 4.9 per 10,000 births. Women with Hispanic surnames, three or more previous live births, history of stillbirth, or residence in east or south Texas were at increased risk for neural tube defect–affected pregnancies. On the basis of vital record data, the annual prevalence of anencephaly in south Texas from 1981 through 1986 was approximately 4.9 per 10,000 live births.

In 1991 three babies with anencephaly were born over a 36-hour period at a single hospital in Cameron County, the southernmost county in Texas (Brownsville is the county's largest city). The ensuing study, which used active multisource case finding rather than vital records, showed that the neural tube defect prevalence rate increased from 14.7 per 10,000 births in 1986 to 1989 to 27.1 per 10,000 births in 1990 to 1991. The higher rate was due largely to an increase in anencephaly cases. From 1986-89 to 1990-91, the average annual anencephaly prevalence rate rose from 9.6 to 19.7 per 10,000 births (7,8).

Despite the high prevalence of neural tube defects and the significant rate of illness associated with them, much remains to be learned about their complex multifactorial etiology. Evidence suggests that these defects are etiologically heterogeneous and may follow fetal

insults such as maternal diabetes, hyperthermia, folic acid deficiency, and anticonvulsant (valproate) therapy (9-13).

In 1987, before the increase in prevalence of human anencephaly in south Texas, an outbreak of severe congenital malformations of the central nervous system and musculoskeletal system of lambs occurred in San Angelo, Texas (14). At the time of the outbreak, there was no active surveillance of human birth defects in San Angelo, and no reports were received of human birth defects in the area. The ovine problem was later found to be caused by in utero infection by Cache Valley Virus (CVV). Although this insect-borne bunyavirus had been known to commonly infect North American ruminants (15), it was not thought to be of clinical significance.

Experimentally, it was determined that CVV infection of the dam in early gestation and transplacental infection of the ovine fetus could produce severe brain malformations and arthrogryposis multiplex congenita, an anomaly characterized by limbs fixed in contracture (14). Central nervous system malformations associated with experimental and spontaneous CVV infection include hydrocephalus, hydranencephaly, porencephaly, micrencephaly, and micromelia. After the syndrome was characterized, outbreaks of CVV-induced malformations in ruminants were diagnosed throughout North America, and work by Calisher and Sever (16) also linked CVV to congenital cases of human macrocephaly in the United States.

Other bunyaviruses can cause identical congenital malformations of the central nervous system in experimentally infected livestock (14). Human congenital morbidity has also been correlated with maternal antibody to bunyaviruses (16). A recent study correlated both human microcephaly and macrocephaly with antibody to Tenshaw virus in mothers of infants with these illnesses.

We decided to test the hypothesis that CVV infection was related to human neural tube defects. Public concern regarding the 1990 to 1991 cluster in Brownsville, Texas, (7) had resulted in an ongoing project in the 14 Texas counties that border Mexico. A neural tube defect surveillance and folic acid intervention project were implemented in 1993, and a case-control study was begun in mid-1995. Sera from case patients had been banked before the case-control study began.

Sera from 74 women who lived in the Texas border counties and had neural tube defect–affected pregnancies (36 with spina bifida, 34 with anencephaly, and 4 with encephalocele) from 1993 through early 1995 were examined for a possible link between CVV and neural tube defects. With a standard microtiter serum dilution neutralization test (17), the sera were screened at final dilutions of 1:2, 1:4, 1:8, and 1:16. The virus used in all tests was the prototype CVV (strain 6V-633, provided by the Centers for Disease Control and Prevention [CDC], Ft. Collins, Colorado), which had been passaged one time in Vero cells after receipt from CDC. Controls included sera from women of undetermined CVV status who gave birth to healthy infants in south Texas [8]; sera collected from sheep before CVV infection [3]; and normal macaque [4], horse [1], and bovine [1] sera. Positive controls included CVV–convalescent-phase ovine sera [3] and CVV antibody–positive sera from a horse and a cow. No serum neutralization activity for CVV was detected in sera from women who gave birth to healthy infants or infants with neural tube defects. Had CVV infection been present in these women during gestation, CVV antibody would have been detectable postpartum.

Before this study, the relationship between CVV and human neural tube defects was unknown. Testing an adequate number of controls is critical when seroepidemiology is used to establish a causal relationship between an agent and a low frequency event or malformation, particularly when case patients have evidence of antibodies against the agent of interest. In this study, there was no evidence that CVV was related to the neural tube defect cases observed in Texas from 1993 through early 1995. Had CVV antibodies been detected in serum from women with neural tube defect–affected pregnancies, it would have been necessary to test control sera from age- and location-matched women with normal births.

The average annual neural tube defect prevalence rate in Cameron County, Texas, for 1992 to 1995 has returned to the 1986 to 1989 rate of approximately 14-15 cases per 10,000 births. These data suggest that CVV is not involved in the induction of human neural tube defects during nonepidemic periods but do not preclude CVV involvement during epidemics. CVV may still be involved in induction of other human malformations. Other endemic bunyaviruses may be involved in the pathogenesis of neural tube defects and of other congenital nervous system or musculoskeletal malformations (18,19). It would

seem valid to continue to investigate the relationship of CVV and other arboviruses to human developmental illness and death rate. Because of the wide variety of defects caused by these viruses, laboratory models of fetal infection by the Bunyaviridae would facilitate the understanding of viral teratogenesis mechanisms in humans.

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Rabies Postexposure Prophylaxis Survey—Kentucky, 1994

A survey of rabies postexposure prophylaxis administered by local health departments for a 1-year period showed that very few patients received treatment as a result of exposure to a confirmed rabid animal. Most prophylaxis was administered for contact with domestic animals in situations where existing recommendations for quarantine or laboratory testing of the animal were not followed. Because rabies in domestic animals in Kentucky is uncommon, these findings suggest that had the existing recommendations been followed, the prophylaxis would have been unnecessary in most cases.

Rabies postexposure prophylaxis (PEP) is expensive, not totally free of risk, and overused (1). A national public health objective for the year 2000 is to reduce the number of prophylaxis treatments by 50% (2). In Kentucky, where PEP is administered in public and private settings, there are no baseline data on PEP use.

A survey of local health departments was used to determine the nature of each patient's exposure to rabies. The number of PEP treatments administered by all providers in Kentucky was estimated from local health department information on rabies biologics purchases and use.

Survey and Sales Summary

In May 1995, the 1994 invoices of the Kentucky Department for Health Services, Vaccine Depot, were reviewed to determine which local health departments received 1.0 ml doses of human diploid cell vaccine for PEP. (Local health departments used 1.0 ml human diploid cell vaccine for PEP only, and 0.1 ml human diploid cell vaccine intradermally for all rabies preexposure prophylaxis). Data from two large health departments that acquired their vaccine directly from the manufacturer rather than from the Vaccine Depot were included in the survey. In June 1995, local health departments that had administered at least one PEP during 1994 were asked to review the records of patients receiving PEP. Information (patient's age and sex, the number of doses of human diploid cell vaccine, whether human rabies immune globulin was administered, exposure information, and method of payment for the treatment) collected on each patient was recorded on a standardized form by the same telephone surveyor during a follow-up telephone call. All data were entered into an Epi Info Version 5.0 record file and analyzed in either the Analysis or Statcalc Programs for summary statistics and/or odds ratios, confidence intervals, Fisher's exact test, or Chi-square at the .05 significance level (3).

A sales record summary for human diploid cell vaccine sold to all providers in Kentucky was obtained from the only manufacturer of human rabies vaccine recording any sales in Kentucky that year (Connaught Laboratories, Inc., Swiftwater, PA). The number of PEPs administered in the state by all providers was estimated by comparing local health department purchases and use with the total number of human diploid cell vaccine 1.0 ml doses sold to other providers with Kentucky addresses.

PEP Administration Profile

Vaccine Depot records indicated that 28 health departments treated a total of 97 patients. The number of PEP regimens administered per health department ranged from 1 to 23 with a median of 1 PEP for the year. Fifty-two (53.6%) of the patients were male (Table 1); the median age was 28 years (range 2 to 71); 34 (35.1%) patients were younger than 18 years of age; 59 (60.8%) were older than 18 years of age; and for 4 (4.1%), age was unknown. No significant differences were observed in the type of animal exposure by sex or age. Seven patients (7.2%) had previously received PEP and were treated with two to three doses of human diploid cell vaccine and no human rabies immune globulin.

Urban health departments (in the three metropolitan statistical areas of the state) were more likely to administer PEP than rural health departments (odds ratio = 1.54, confidence interval = 1.01, 2.33) (4). Patients did not significantly

Table 1. Characteristics of local health department patients receiving rabies postexposure prophylaxis

Sex		
	Male	52
	Female	43
	Unspecified	2
Age[a]	Youth (2 - 10)	19
	Adolescent (11 - 17)	15
	Adult (18-71)	59
	Unspecified	4
Health department location[b]		
	Urban	48
	Rural	49
Previously immunized		6
Animal exposure		
	Wild	15
	Domestic (50 dogs, 29 cats, 1 horse)	80
	Unspecified	2
Type of exposure		
	Bite or contact with saliva	72
	No contact with saliva	17
	Unspecified	8
Treatment payer		
	Private insurance	39
	Medicaid	7
	Medicare	3
	Patient	14
	Other (employer, worker's compensation)	3
	Unspecified	6
	No reimbursement	25

(N=97)

[a] $\bar{x}$ = 28 yrs.

[b] Health departments in urban areas, as defined by the 1990 census of population for Kentucky. Metropolitan statistical areas were more likely to administer PEP than rural departments. (p=.033)

differ in age, sex, or type of exposure between urban and rural health departments.

For 25 (25.8%) of the patients, local health department funds covered the expense of PEP treatments; no payment was received from private insurance, Medicaid, Medicare, or the patient. There were no significant differences in payment characteristics between urban and rural health department patients.

Bite exposures were responsible for 71 (73.2%) of the 97 PEP treatments, 18 (18.6%) exposures were scratches, licks, or "other," and 8 (8.2%) exposure types were not recorded. Domestic animals accounted for 80 (82.5%) of the exposures treated.

Type of Animal Exposure

Sixty-four (77.1%) of 83 animals involved in these incidents were not available for observation or testing. For wild animals, testing was performed in 3 (20%) of 15 incidents. Testing or observation occurred in only 16 (20.0%) of 80 domestic animal exposures.

Stray domestic animals accounted for 26 (26.8%) of all exposures. Another 19 (19.6%) of the incidents involved owned dogs that were unavailable for testing or observation. Unavailability for testing was due to severe brain damage caused by clubbing or gunshot by irate owners, death and disposal of the animal without testing, or the animal's escape. For 36 (37%) incidents, the reason for not testing or observing the animal was not specified.

Thirteen (13.4%) of the patients were exposed to an animal that was tested and found to be positive for rabies, and two of these patients had bite exposures. The remaining exposures to these rabies-positive animals were either low-risk exposures or not true exposures (Table 2).

Table 2. Patients receiving postexposure prophylaxis for exposure to a confirmed rabid animal in Kentucky, 1994

Species	Type of exposure	Previous history of prophylaxis
Bat	Bite	No
Cat[a]	Mucus & Saliva	Yes[b]
Cat[a]	Mucus & Saliva	No
Cat[a]	Cleaned exam table	No
Cat[a]	Cleaned exam instruments	No
Dog[c]	Bite	Yes
Dog[c]	Touch	Yes
Dog[c]	Touch	Yes
Dog[c]	Touch	Yes
Dog[c]	Touch	No
Dog[c]	Touch	No
Horse	Sutured wound	Yes[b]
Skunk	Touch	No

[a] Same cat

[b] Veterinarian with history of preexposure prophylaxis

[c] Same dog

Total Estimate of State Rabies Postexposure Prophylaxis

Kentucky sales in 1994 for human diploid cell vaccine 1.0 ml to nonmilitary providers and distributors totaled 1,603 doses. The health departments ordered 700 of these doses, of which

445 were used for PEP in that same year. The other doses remained as inventory. Assuming that other users administered human diploid cell vaccine 1.0 ml in a similar proportion (445/700 = 0.64), the private sector administered 578 doses (903 x .64) of human diploid cell vaccine 1.0 ml. Comparing actual local health department use of human diploid cell vaccine 1.0 ml and estimated use by others, local health departments administered 43.5% (445/([445+578]) of the human diploid cell vaccine 1.0 ml used in the state in 1994. Therefore, the estimated total number of PEP patients in the state is 223 (97/.435) for 1994.

Exact total costs for PEP administration cannot be calculated since most treatments were made by private providers. The actual cost of biologics to local health department patients in 1994 was $68,850. Estimated costs of biologics used by private providers (based on estimates of hospital pharmacy costs in Connecticut in 1994) would be $180,180 for a typical patient (126 patients x $1,430) (5). Estimated total costs of biologics is $249,030. Unknown costs include medical and hospital care, local health department investigation of the incident, state health department consultations, and loss of work income by the patient.

Study Limitations

Because records at the local health departments were not always complete or as detailed as desired, certain variables could not be analyzed for all 97 cases; information about why the suspect animal was not tested or observed for rabies was absent from more than 10% of the cases. Since no detailed information was obtained from the private sector, we assumed that the number of doses used per patient, inventory, waste, spoilage, and other factors influencing PEP use in the private sector were similar to those in the public sector. Kentucky residents receiving PEP in another state and out-of-state residents receiving PEP in Kentucky would not be specifically accounted for in our estimate.

The difference in urban versus rural PEP administration could be due to differences in the number of animals or bite incidents; however, the number of animals or animal bites statewide is not known. An investigation of prescribing practices of full-time physicians at large, urban health departments and part-time or contract physicians at small, rural health departments might determine if these practices contributed to treatment disparity.

Guidelines and Noncompliance

Guidelines for determining exposures that warrant PEP exist (6,7). Ideally, any animal involved in a human exposure should be confined and observed or tested for rabies, whichever is appropriate. It is understandable that most of the wild animals might have escaped and not be available for testing. However, the large proportion of domestic animals unavailable for testing indicates inappropriate handling of the incident or a breach of existing laws (5-7).

Six people received PEP due to exposure to a single dog with laboratory-confirmed rabies. This particular incident illustrates how "anything that can go wrong will go wrong." First, the dog had been vaccinated by the owner. It is illegal for individual owners to vaccinate their own dogs in Kentucky (8). Second, the vaccine may have failed for any number of reasons, including vaccine failure, improper handling/administration of the vaccine, or failure to vaccinate. Third, only one of these patients was bitten; the other five reported only touching the dog and probably were not exposed. Fourth, none of these patients had insurance or was able to pay for treatment; thus the local health departments spent several thousand dollars in unbudgeted expenses. Furthermore, four of these patients had received PEP before.

Noncompliance with existing public health recommendations and laws contributes to the number of rabies exposure incidents in Kentucky. PEP administration in Kentucky could be reduced if existing recommendations and laws were adhered to by the public and health care providers. Accurate and complete record keeping is essential for assessing the use of PEP. Additionally, making PEP a notifiable (reportable) condition would allow public health agencies to assess PEP administration in the private sector.

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Biologic Terrorism — Responding to the Threat

The growing awareness of the possibility that a terrorist organization might use a biologic agent in an attack on a civilian target in the United States raises important questions about our capability as a nation to respond effectively to the threat and to deal with the consequences of an attack. The article by Kaufmann et al. in this issue of *Emerging Infectious Diseases* describes three possible biologic attack scenarios and uses an economic analysis to describe the benefits of a rapid medical response and early intervention. The authors conclude that major reductions in morbidity and mortality and consequent cost savings can be achieved by early intervention. The effectiveness of postattack intervention depends on a rapid response which requires prior planning, preparation, and training. Achieving the level of preparedness implied by the assumptions stated in the article will require a major national effort. This discussion of possible bioterrorist attack scenarios adds to a growing concern about our willingness as a nation to commit the effort and resources necessary to protect our citizens.

Biologic warfare and use of biologic weapons by terrorists have only recently been discussed openly and realistically. The fall of the Soviet Union and the defeat of Iraq uncovered extensive biologic weapons programs of surprising sophistication and diversity. The threat to the nation from biologic weapons is no longer a debate issue. Now the questions are how immediate and serious is the threat and how do we respond effectively?

Protecting the armed forces against biologic weapons, although complex and difficult, is less challenging than protecting the civilian population. The armed forces are relatively small populations that can be vaccinated against the major threat agents. Aerosols containing biologic materials can be detected at a distance, and protective masks and suits are effective. Military medical personnel are trained to recognize and treat casualties, and antibiotics, antiviral drugs, and antitoxins can be stockpiled for military contingencies. The preponderance of scientific expertise for many of the threat agents is within the military medical research laboratories, although this capability is now being seriously compromised by budget cuts and personnel reductions.

The civilian population cannot be protected in the same manner as the armed forces. We must rely heavily on our intelligence and criminal investigation agencies and on international efforts to identify specific threats and deter terrorists. We must also recognize the possibility that a determined terrorist organization may not be deterred, may evade detection, and may succeed in releasing an aerosol of a virulent bacterium, virus, or toxin in a susceptible target area such as an airport or stadium. Our current capability to effectively respond to such a scenario and minimize the impact is far less than needed.

The U.S. Armed Forces and the Department of Defense have the greatest capability in biologic defense, but the responsibility for dealing with the threat of biologic weapon use by a terrorist falls on multiple federal, state, and municipal agencies and the civilian health care community. Most of the organizations are inadequately prepared to deal effectively with the problem.

The organizational aspects of dealing with an attack on our civilian population are daunting. Responsibility for recognizing an unusual outbreak of illness that may be the result of the deliberate release of a biologic warfare agent will fall on the health care community. Early recognition will be an important factor in determining the overall outcome and will depend on the level of suspicion and knowledge of the health care providers that see the initial cases. Rapid, precise, and reliable diagnosis will be the responsibility of the federal and state public health laboratory system with help from their military colleagues. Organizing and managing the care of patients and mounting the appropriate public health response will involve local health care and municipal agencies and authorities and state public health authorities. The effectiveness of coordination, support, and leadership at the federal level may make huge differences in reducing death rates and containing the possible secondary spread of a communicable disease. The Federal Emergency Management Agency has the major responsibility for planning and coordinating the consequences phase of a federal response, but the level of preparedness at all levels will ultimately determine the outcome.

If we take the biologic warfare threat seriously, a major effort will be needed to develop contingency plans and initiate coordinated and mutually supportive programs in all involved agencies. Training and education of the health care community will require a major effort involving several major professional organizations. Developing and improving diagnostic and identi-

fication capability is essential for medical care, public health, intelligence, and law enforcement agencies and should be a national priority.

The science base needed to deal with the broad spectrum of agents on the threat list, bacteria, viruses, toxins, and parasites, is widely distributed among several federal laboratories in the Department of Health and Human Services, the Department of Defense, and the Department of Energy, as well as in universities and state public health laboratories. In addition, since many of the biologic agents are not normally large public health problems or popular subjects of scientific research, critical areas have inadequate research capability and limited expert personnel. Deficiencies in our scientific knowledge and a paucity of experts will ultimately limit our capability to rapidly and precisely identify agents and respond effectively in a crisis. For example, the global molecular epidemiology of the agents at the top of the threat list is critically important for identifying the organisms accurately and differentiating local from exotic strains. Current databases are inadequate, and no organized effort is being made to fill in the gaps.

The current public discussion of the threat of biologic terrorism is an opportunity to evaluate our collective capabilities and to assess weaknesses and vulnerabilities. Raising the level of national preparedness will require leadership and action by responsible federal agencies. A thoughtful analysis of the consequences of unpreparedness provides a mandate for action.

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The Hantaviruses of Europe: from the Bedside to the Bench

In Europe, hantavirus disease can hardly be called an emerging zoonosis; it is rather a rediscovered disease. Since 1934 an epidemic condition with primarily renal involvement has been described in Sweden. Nowadays, hundreds to thousands of cases per year are registered in Fennoscandia, fluctuating with the numbers of the specific *Arvicoline*-rodent reservoir, the red bank vole, which carries the main European serotype, Puumala (PUU). In the early 1980s, the rat-transmitted serotype, Seoul (SEO), caused laboratory outbreaks throughout Europe, and recent reports also suggest sporadic, wild rat-spread hantavirus disease. In the Balkans, at least four serotypes are present simultaneously: PUU, SEO, the "Korean" prototype Hantaan (HTN) or HTN-like types, and Dobrava, the latter causing a mortality rate of up to 20%. Moreover, recent genotyping studies have disclosed several PUU-like genotypes spread in Europe and/or Russia by other genera of the *Arvicoline*-rodent subfamily: Tula, Tobetsu, Khabarovsk, and Topografov. Their importance for human pathogenicity is still unclear, but serologic cross-reactions with PUU antigen might have caused their misdiagnosis as PUU-infections in the past.

Hantaviruses are often heralded as new or at least as emerging pathogens, particularly in the New World. However, even on the American continent, application of the newest genotyping techniques (the bench) comparing RNA sequences found in both human cases and rodent reservoirs shows a long-standing coevolution of each newly discovered hantavirus serotype in its specific rodent host; this coevolution results in remarkable genetic stability across time and in a certain genetic differentiation in geographic spread. Hantaviruses inducing hantavirus pulmonary syndrome emerge not through genetic reassortment or a recent mutation, but through increased exposure to infected rodents and their excreta. While the same lines of evidence also apply to the European situation, the problem there is totally different, from a historical point of view.

History

In most European countries, hantavirus disease has long been known by various (mostly geographic) names, which suggests a long-standing clinical presence (the bedside). An epidemic of "trench nephritis" during World War I may in fact have been hantavirus induced. Thousands of cases of this illness, considered an entirely new renal disease, were noted on both sides of the front (Kriegsnephritis or néphrite de guerre). Already in 1934, the typical mild renal form of hantavirus disease had been described in Sweden (1); it was then described in all other Scandinavian countries, where the disease was appropriately called nephropathia epidemica from 1945 on.

During World War II, more than 10,000 cases of a rodent-borne leptospirosislike disease were noted during the 1942 German campaign in Finnish Lapland (2). Because the snow melted, great numbers of lemmings and field mice invaded the German bunkers. Examinations in Munich and Berlin of these rodents, air-lifted from the war theater, offered no clue. Confronted with some distinctive clinical symptoms (e.g., acute myopia and localized edema) and with repeatedly negative findings for leptospirosis in his patients, a researcher concluded it was a new field-like fever disease (2).

"Old" Hantaviruses in the Old World

The Puumala (PUU) serotype, carried by the red bank vole (*Clethrionomys glareolus)*, remains the most important Western and Central European serotype, with at least 1,000 serologically confirmed nephropathia epidemica cases per year in Finland and hundreds per year in Sweden (3). The number of documented cases in other European countries was more than 1,000 in the former Yugoslavia (3), 531 in France by the end of 1994 (4), approximately 250 in Belgium by the end of 1996 (J. Clement, unpub. obs.), some 200 in Germany by the end of 1995 (5), 138 in Greece by the end of 1993 (6), and 39 in the Netherlands by the end of 1994 (7).

IgG seroprevalence rates reported from some of these countries, measured mostly by immunofluorescence assay (IFA) and/or enzyme-linked immunosorbent assay (ELISA) both for PUU and HTN, were 6% for Finland (8), 8% for Sweden (9), 1.7% for Germany (10), 0.9% for the Netherlands (11), 1.6% for Belgium (12), <1% for France (4), 0.3% for Spain (13) and 4.0% for Greece (6). Most of the PUU infections were subclinical. A study comparing nephropathia epidemica incidence (recorded over 14 years) with IgG IFA PUU-antibody prevalence in an area of Sweden with high rates of endemic disease found that the antibody prevalence rate for men and women in the oldest age groups (>60 years) was 14 to 20 times higher, respectively, than the accumulated life-risk of being hospitalized with nephropathia epidemica (14). Thus, hospital admissions for the disease are only the tip of the iceberg.

In nephropathia epidemica, acute renal failure serum creatinine values peak above 4.5 mg (>400 mol/L) in only one-third of the cases (15). Early thrombocytopenia, however, is present in 75% of the cases. Eye symptoms, and particularly acute myopia, are rare (25%) but distinctive first symptoms of PUU infections. Mild transient hepatitis is frequent (40%), but icterus is rare (7%) (15). Noncardiogenic pulmonary edema, the hallmark of Sin Nombre (or Sin Nombre-like)-induced hantavirus disease in the New World, has been described in a milder, nonlethal form (acute lung injury) in some rare European PUU (and Seoul [SEO] virus) cases (16,17).

In the former Soviet Union, hantavirus disease has been recognized since 1934 and officially registered since 1978. Seroprevalence studies carried out by IFA or direct blocking radioimmunoassay involving 115,765 persons resulted in an overall seropositivity rate of 3.3%, ranging from 3.5% in the European part to 0.9% in the Far Eastern part (18). A total of 68,612 cases were registered between 1988 and 1992 (65,906 from the European part and 2,706 from the Far Eastern part), with morbidity rates of 1.2 (1982) to 8.0 (1985) per 100,000 inhabitants. The peak year was 1985 with 11,413 registered cases (19). In the European part of Russia, most cases were due to milder infection with PUU-related viruses, with mortality rates of 1% to 2%; whereas in Far East Russia, more severe HTN-like cases also occurred (19).

The Wild Rat: Omnipresent, but Often Overlooked

In Europe, the parallel spread of PUU and HTN (or HTN-like) viruses has been noted in such countries as Belgium and the Netherlands (11,20), Germany (10), and European Russia (21). A partial explanation could be that the HTN-like infection is due to serologic cross-reaction with SEO. The wild rat is the only hantavirus reservoir with a worldwide distribution (22), including Europe, and SEO infections are probably underestimated. The first documented hantavirus disease in Portugal was an HTN-like infection with acute renal failure and icterus (23). In Portugal *Clethrionomys glareolus* is not prevalent, but hantavirus-seropositive wild rats have been documented (24). Sixteen cases of acute disease, mostly with acute renal failure and reacting almost exclusively in IFA against a SEO strain (R22VP30), have been described in North Ireland (25), another country where *C. glareolus* is not prevalent, but the most important hantavirus vector seems to be the wild rat (26). In France, three SEO-induced cases of acute renal failure have been reported south of the PUU-endemic region, from rural areas where the rat is an agricultural pest (4). Moreover, 14 SEO-like cases were detected between December 1991 and February 1992 in the Tula region (300 km south of Moscow) and confirmed by plaque reduction neutralization tests and positive virus isolation in three of the cases (27); these cases are awaiting further confirmation.

An often overlooked fact is that hantavirus has been transmitted from laboratory rats to animal keepers first in Belgium (1979) and later in France, in the United Kingdom, and in the Netherlands (28). In the earlier days, these rat-transmitted infections were described as HTN-like by IFA or

ELISA because of cross-reactions with the prototype screening antigen HTN 76-118, but they were later confirmed as SEO-like by ELISA and blocking ELISA (20). Because of the now established close relationship between each hantavirus serotype and its rodent vector, these earlier laboratory infections can all be regarded as SEO-induced.

The Balkans: A Complicated Situation

In the Balkans, and particularly the former Yugoslavia, outbreaks of hantavirus disease have been recorded since the early 1950s, often with a death rate of 5% to 10% or even higher (29). The elevated rates of illness and death in early reports suggested the spread of one (or several) hantaviral strains, in addition to the mild PUU serotype prevalent in the rest of Europe. These HTN-like viruses were later called Plitvice and Fojnica. In 1987, an HTN-like virus (Porogia) was isolated from the urine of a Greek soldier who became ill after a military exercise near the border in northern Greece and had both acute renal failure and severe pulmonary edema. Extensive cross-reactivity with HTN 76-118 was demonstrated by IFA with a panel of Mabs and in plaque reduction neutralization tests (30). No polymerase chain reaction (PCR) genotyping was available at that time. However, in Slovenia in 1994, a hantavirus was isolated that was indistinguishable from the prototype Korean strain HTN 76-118 by PCR genotyping and other serologic techniques (31). Moreover, a hantavirus (close but not identical to HTN 76-118) that caused a mortality rate of up to 20% was first described as a human isolate (Belgrade) in Serbia (32) and confirmed as a new hantavirus serotype called Dobrava (DOB) after isolation from its rodent vector, an *Apodemus flavicollis* (yellow-necked field mouse) captured in Dobrava, Slovenia (33).

The first genetic evidence for the association between DOB and severe hantavirus disease was demonstrated by nested reverse transcriptase-PCR on RNA extracted from whole blood of a Greek and an Albanian patient (34). During the recent conflict in Bosnia, more than 300 patients, most of them soldiers exposed in the field, were hospitalized in the Tuzla region (northeast Bosnia) with acute hantavirus disease due either to PUU or to DOB, as first documented by IgG and IgM ELISA (35) and later confirmed by focus reduction neutralization tests (36). These findings suggest that at least three distinct serotypes (PUU, DOB, and maybe also HTN) are endemic throughout the Balkans and that easily accessible serologic tests are needed to permit a differential diagnosis, given the totally different prognosis for each infection. Moreover, preliminary evidence implicated a fourth serotype, i.e., SEO, spread by wild rats (*Rattus norvegicus* or *Rattus rattus)*. Severe hantavirus disease, apparently due to SEO, was documented in 1992 in a Canadian soldier (37) and later in 1996 in a British soldier (17), both stationed in Bosnia. These two patients are clinically interesting, in that the former had a clear exposure to wild rats inside an infested building, whereas the latter had acute renal failure and hemodynamically documented acute lung injury, a complication hitherto undocumented in SEO cases (38). The exact serotype involved in both these cases needs to be defined, however, by confirmatory tests such as plaque reduction neutralization tests or (if technically possible) by PCR genotyping.

That so many of these Balkan cases are in persons on active military duty should come as no surprise. Exposure to rodents has been confirmed as the most important risk factor for developing hantavirus diseases and seems an unavoidable aspect in the life of the soldier at war. Even exercises imitating war conditions can put the soldier at risk: the most important cluster of hantavirus disease in Americans abroad was reported in U.S. soldiers exercising in January 1990 in southern Germany and camping under tent in a mice-infested area. Within 2 weeks, 24 acute PUU infections were documented, and 14 soldiers had to be hospitalized with varying degrees of acute renal failure (no deaths), whereas no outbreak occurred in the civilian population of the surrounding area (5).

"New" Hantaviruses: Tula (TUL), Tobetsu (TOB), Khabarovsk (KBR), and Topografov (TOP)

Apart from the long-standing clinical experience with hantavirus strains in Europe and Asia, an explosive growth in the number of newly discovered lineages or genotypes has further complicated matters. All these new genotypes appear more or less related to PUU (Figure, Table). TUL virus was first detected by RT-PCR in European common voles (*Microtus arvalis* and *Microtus rossiaemeridionalis*) captured in the Tula region (39). TUL virus was later also detected in voles from Moravia, the Czech Republic, and Slovakia (Figure). The three viruses most closely related

to TUL have been detected on the American continent: Prospect Hill, isolated in 1982 from *Microtus pennsylvaticus* (meadow vole); Isla Vista virus, recently detected in *Microtus californicus* (Californian meadow vole) (40); and Bloodland Lake virus, detected in *Microtus ochrogaster* (prairie vole) (Hjelle et al., unpub. data). TUL was isolated from the lungs of infected *M. arvalis* and showed in cross-focus reduction neutralization tests and cross-hemagglutination inhibitor tests at least eightfold higher homologous to heterologous titers when compared with PUU, PH, KBR, and HTN (41). None of these *Microtus*-derived hantaviruses is a known pathogen in humans, in contrast to the PUU viruses, which are spread by another genus of the same rodent subfamily *Arvicolinae* (Table, Figure). However, serum of a blood donor living in Moravia, the Czech Republic, possessed a focus reduction neutralization test titer to TUL at least 16-fold higher than to PUU or other hantaviruses, thereby giving the first solid evidence that these viruses carried by *Microtus* rodents can infect humans (41). As in other hantavirus serotypes, antibody response to the TUL N-antigen appeared highly reactive and cross-reactive. Thus, part of the so-called PUU infections in European (and particularly in Central European and Russian) patients may have been due to related TUL viruses. Under that hypothesis, it would be very remarkable that the even more closely related North American viruses (Prospect Hill, Isla Vista, and Bloodland Lake) would appear to be apathogenic to humans. Already in the early 1930s, Tula fever was one of the many regional synonyms used in Russia for describing epidemics of a feverish condition, which later appeared to be a hantavirus infection.

TOB was the name preliminarily given to a PUU-like virus detected in *Clethrionomys rufocanus* (grey-sided vole) captured in Hokkaido, an island in the north of Japan (44). Its putative rodent reservoir, *Cl. rufocanus*, has a very broad geographic range, extending almost over the whole of Eurasia: in the north from northern Scandinavia to Kamtchatka, and in the south from the Urals to Manchuria and down to Korea. Moreover, PUU-like human infections have been noted in Korea (18) (H.W. Lee, pers. comm.).

KBR (not in the Figure), a hantavirus close to PUU, was recently isolated from a *Microtus fortis* (reed vole) captured in Far East Russia (46). Although no pathogenicity for humans has been

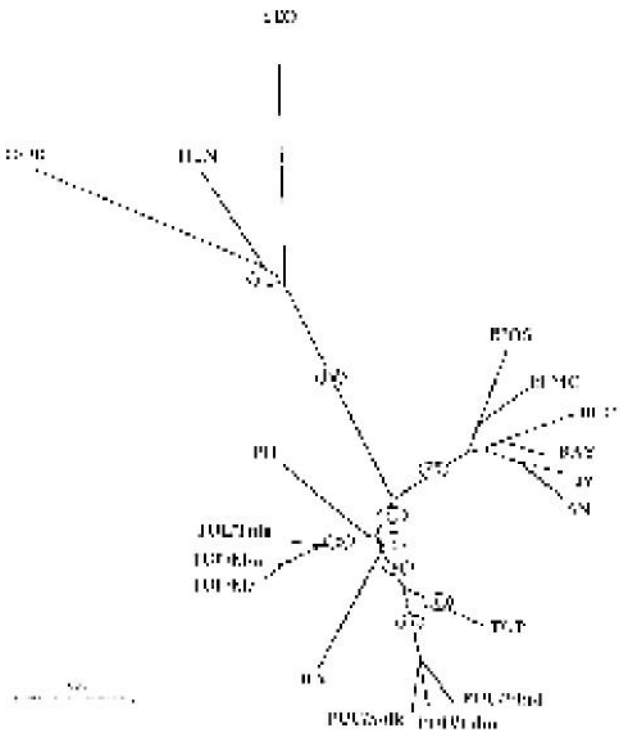

Figure: Dendrogram of Old World hantaviruses (upper, left, and lower part of the tree) vs. New World hantaviruses (right part of the tree). Reproduced with permission (42). Branch lengths are proportional to genetic distances. The bootstrap support percentages of particular branching points calculated from 500 replicates are given in ovals. HTN = Hantaan virus, strain 76-118; SEO = Seoul virus, strain SR-11; DOB = Dobrava virus, PH = Prospect Hill virus; TUL = Tula virus, strains Tula/76Ma/87, Moravia/5286Ma/94 and Malacky/Ma32/94; ILV = Isla Vista virus, strain MC-SB-1; TOP = Topografov virus; PUU = Puumala virus, strains Sotkamo, Vindeln/83-L20 and Udmurtia/458g/88; RIOS = Rio Segundo virus, strain RMx-Costa-1; ELMC = El Moro Canyon virus, strain RM-97; BAY = Bayou virus, strain Louisiana; BCC = Black Creek Canal virus; SN = Sin Nombre virus, strain H10; NY = New York virus, strain RI-1; KBR = Khabarovsk virus, another PUU-like virus, is not depicted here. PH & ILV (and BLL, not depicted here) are the only New World genotypes with PUU-like characteristics, hence their position close to the TUL-clade.

documented so far, this new agent could partially explain PUU-like infections described for many years in this region and in China, together with the findings of PUU-positive *M. arvalis* rodents (19). KBR is more closely related to PUU than to Prospect Hill, which may reflect that both KBR and PUU are viruses from the Old World, whereas Prospect Hill has been documented so far only in North America. The first reports in Russia that describe a hantaviruslike disease are found in the 1913 archives of a hospital in Vladivostok, Siberia.

Table: Classification of old and new PUU-like genotypes, according to respective rodent host and geographic spread.

Rodent reservoir (*Arvicolinae* Subfamily)	Hantavirus (geographic spread)	Reference
Clethrionomys		
glareolus	Puumala (Europe)	(43)
rufocanus	Tobetsu (Japan)	(44)
Lemmus		
sibiricus	Topografov (Russia)	(42)
Microtus		
pennsylvaticus	Prospect Hill (N. America)	(45)
californicus	Isla Vista (N. America)	(40)
ochrogaster	Bloodland Lake (N. America)	Hjelle et al., unpublished data
arvalis	Tula (Eurasia)	(39)
rossiaemeridionalis	Tula (Eurasia)	(39)
fortis	Khabarovsk (Eurasia)	(46)

TOP is another PUU-like genotype, detected in the lemming (*Lemmus sibiricus*) (42). Lemmings, the most important small mammals in the Arctic tundra regions, are also present in Alaska and Canada. Together with the wild rat, the lemming is the only rodent reservoir harboring a newly recognized hantavirus genotype, and living in both the Old and the New World. Of the newer European viruses, TOP is the most closely related to PUU (Figure). No human pathogenicity has been recognized. However, lemming fever has traditionally been reported by Nordic inhabitants, particularly during lemming years (42); this link was made already in 1942 (another lemming year) by Stuhlfauth (2) when describing an epidemic in German troops plagued by lemmings.

With the current explosive growth of knowledge concerning hantaviruses, a tendency is emerging to globalize at least some strains and symptoms: 1) The distribution of various new and old strains is giving an ever more confused picture of infection in Eurasia, with HTN-like strains (HTN and DOB) in the West, and PUU-like strains (TUL, TOB, KBR, and TOP) in the East. Moreover, Bloodland Lake and Isla Vista have joined the prototype North American isolate Prospect Hill as PUU-like strains in the Americas. 2) The wild rat SEO-strain remains probably the most underestimated (pathogenic) hantavirus strain worldwide, despite recent reports of SEO-like infections throughout Europe and the Americas. 3) The clinical symptoms tend also to grow to each other on the global scene: whereas in the Americas, non-Sin Nombre virus cases of hantavirus pulmonary syndrome, i.e., induced by Black Creek Canal virus and/or Bayou virus, have renal as well as lung involvement, and whereas even mild cases have recently been described, we also find now, albeit rarely, evidence of lung involvement under the form of acute lung injury in documented PUU and SEO cases in Europe. 4) Careful reading of the earlier literature, often containing astute clinical or epidemiologic descriptions of viral hemorrhagic fevers, can still teach us many lessons, both for the bedside and for the bench.

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Brucellosis: an Overview

Brucellosis remains a major zoonosis worldwide. Although many countries have eradicated *Brucella abortus* from cattle, in some areas *Brucella melitensis* has emerged as a cause of infection in this species as well as in sheep and goats. Despite vaccination campaigns with the Rev 1 strain, *B. melitensis* remains the principal cause of human brucellosis. *Brucella suis* is also emerging as an agent of infection in cattle, thus extending its opportunities to infect humans. The recent isolation of distinctive strains of *Brucella* from marine mammals has extended its ecologic range. Molecular genetic studies have demonstrated the phylogenetic affiliation to *Agrobacterium, Phyllobacterium, Ochrobactrum,* and *Rhizobium.* Polymerase chain reaction and gene probe development may provide more effective typing methods. Pathogenicity is related to production of lipopolysaccharides containing a poly N-formyl perosamine O chain, Cu-Zn superoxide dismutase, erythrulose phosphate dehydrogenase, stress-induced proteins related to intracellular survival, and adenine and guanine monophosphate inhibitors of phagocyte functions. Protective immunity is conferred by antibody to lipopolysaccharide and T-cell-mediated macrophage activation triggered by protein antigens. Diagnosis still centers on isolation of the organism and serologic test results, especially enzyme immunoassay, which is replacing other methods. Polymerase chain reaction is also under evaluation. Therapy is based on tetracyclines with or without rifampicin, aminoglycosides, or quinolones. No satisfactory vaccines against human brucellosis are available, although attenuated *purE* mutants appear promising.

Brucellosis has been an emerging disease since the discovery of *Brucella melitensis* by Bruce in 1887. Subsequently, an increasingly complex pattern of strains has emerged with the identification of *Brucella abortus, Brucella suis, Brucella neotomae, Brucella ovis, Brucella canis,* and, more recently, types infecting marine mammals. Because each type has distinctive epidemiologic features, with each new type, the complexity of the interaction with humans has increased. Because new strains may emerge and existing types adapt to changing social and agricultural practices, the picture remains incomplete.

This synopsis reviews major advances in the knowledge of certain aspects—genetics, antigenic structure, mechanisms of pathogenicity, diagnosis, treatment, and prevention of the disease—of the *Brucella* genus and its host interactions.

Epidemiology

Worldwide, brucellosis remains a major source of disease in humans and domesticated animals. Although reported incidence and prevalence of the disease vary widely from country to country, bovine brucellosis caused mainly by *B. abortus* is still the most widespread form (Tables 1-5). In humans, ovine/caprine brucellosis caused by *B. melitensis* is by far the most important clinically apparent disease. The disease has a limited geographic distribution, but remains a major problem in the Mediterranean region, wes-tern Asia, and parts of Africa and Latin America. Recent reemergence in Malta and Oman indicates the difficulty of eradicating this infection (1). Sheep and goats and their products remain the main source of infection, but *B. melitensis* in cattle has emerged as an important problem in some southern European countries, Israel, Kuwait, and Saudi Arabia. *B. melitensis* infection is particularly problematic because *B. abortus* vaccines do not protect effectively against *B. melitensis* infection; the *B. melitensis* Rev.1. vaccine has not been fully evaluated for use in cattle. Thus, bovine *B. melitensis* infection is emerging as an increasingly serious public health problem in some countries. A related problem has been noted in some South American countries, particularly Brazil and Colombia, where *B. suis* biovar 1 has become established in cattle (2). In some areas, cattle are now more important than pigs as a source of human infection.

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Table 1. Brucellosis in animals, Europe, 1994

Country	Bovine (*B. abortus*)	Ovine/ caprine (*B. melitensis*)	Porcine (*B. suis*)	Ovine (*B. ovis*)
Albania	-	+	+	+
Belgium	+	-	-	-
Bulgaria	-	-	+	+
Croatia	-	-	+	+
Czech Republic	-	-	?	-
France	+	++	?	+
Germany	+	-	?	+
Greece	+	++	ND	ND
Ireland	+	-	-	-
Italy	+	+	-	ND
Latvia	-	-	+	-
Lithuania	-	-	-	?
Macedonia	+	+	-	-
Malta	+	+	-	-
Poland	+	+	?	-
Portugal	+	+	-	+
Romania	-	-	+	-
Russia	++	++	+	+
Slovakia	-	-	ND	-
Slovenia	-	-	-	+
Spain	+	+	-	+
Ukraine	ND	ND	ND	ND
Yugoslavia	+	+	+	-

\- not present
\+ low sporadic incidence
++ high incidence
? presence uncertain
ND no data

None of the four types of brucellosis is present in Austria, Denmark, Estonia, Finland, Hungary, Iceland, Luxembourg, Moldavia, Netherlands, Sweden, Switzerland, and the United Kingdom

Source for Tables 1-8: FAO-WHO-OIE Animal Health Yearbooks, 1994, 1995.

Table 2. Brucellosis in animals, Africa, 1994

Country	Bovine (*B. abortus*)	Ovine/ caprine (*B. melitensis*)	Porcine (*B. suis*)	Ovine (*B. ovis*)
Algeria	+	?	ND	+
Angola	?	?	?	?
Botswana	+	ND	-	ND
Cape Verde	?	?	?	+
Central African Republic	++	ND	+	ND
Chad	++	?	?	ND
Congo	+	-	-	-
Côte d'Ivoire	+	-	-	+
Egypt	+	+	ND	-
Eritrea	+	?	ND	+
Ghana	+	-	-	-
Guinea	+	ND	-	ND
Kenya	+	+	ND	ND
Libya	+	+	-	-
Mauritius	-	-	-	-
Morocco	+	?	-	-
Mozambique	++	+	++	+
Namibia	+	-	-	?
Niger	+	+	ND	+
Nigeria	++	+	+	ND
Seychelles	+	-	-	-
South Africa	++	+	-	+
Sudan	++	+	-	-
Tanzania	+	ND	ND	ND
Tunisia	+	++	-	-
Zaire	+	ND	+	ND
Zimbabwe	+	+	-	+

\- not present
\+ low sporadic incidence
++ high incidence
? presence uncertain
ND no data

No data on any of the four types of brucellosis are available for Gambia, Mali, and Mauritania

The true incidence of human brucellosis is unknown. Reported incidence in endemic-disease areas varies widely, from <0.01 to >200 per 100,000 population (3). While some areas, such as Peru, Kuwait, and parts of Saudi Arabia, have a very high incidence of acute infections, the low incidence reported in other known brucellosis-endemic areas may reflect low levels of surveillance and reporting, although other factors such as methods of food preparation, heat treatment of dairy products, and direct contact with animals also influence risk to the population.

Consumption of contaminated foods and occupational contact remain the major sources of infection. Examples of human-to-human transmission by tissue transplantation or sexual contact are occasionally reported but are insignificant (4). Prevention of human brucellosis depends on the control of the disease in animals. The greatest success has been achieved in eradicating the bovine disease, mainly in industrialized countries (Table 6); however, most countries have control programs. *B. melitensis* infection has proved more intractable, and success has been limited (Table 7).

Although few recent outbreaks of disease caused by *B. suis* biovar 4 have been reported (5), foci of the infection persist in the Arctic regions of North America and Russia and constitute a potential hazard for the local population. *B. ovis* has not been demonstrated to cause overt disease in humans, although it is widespread in sheep (Tables 1-5). *B. canis* can cause disease in humans, although this is rare even in countries where the infection is common in dogs (6). Precise

Table 3. Brucellosis in animals, Asia, 1994

Country	Bovine (*B. abortus*)	Ovine/ caprine (*B. melitensis*)	Porcine (*B. suis*)	Ovine (*B. ovis*)
Afghanistan	+	+	ND	ND
Bangladesh	+	+	ND	ND
Bhutan	+	-	-	ND
China	+	+	+	+
Hong Kong	ND	ND	?	ND
India	+	+	?+	-
Indonesia	+	ND	+	+
Iran	+	+	-	-
Israel	-	+	-	-
Iraq	+	+	ND	ND
Jordan	-	++	-	-
Korea (S)	++	-	?+	-
Kuwait	++	++	-	-
Malaysia	+	-	?-	-
Mongolia	++	+	-	+
Myanmar	+	ND	+	ND
Oman	++	ND	ND	ND
Qatar	ND	ND	ND	ND
Sri Lanka	++	+	-	+
Syria	+	ND	ND	ND
Thailand	+	-	+	-
Turkey	++	++	-	ND
UAE	-	+	-	+
Yemen	+	+	-	-

- not present
+ low sporadic incidence
++ high incidence
? presence uncertain
ND no data

None of the four types of brucellosis is present in Bahrain, Cyprus, Japan, Malaysia (Sabah), Philippines, or Singapore
No data for countries of the former Soviet Union or Qatar

Table 4. Brucellosis in animals, the Americas, 1994

Country	Bovine (*B. abortus*)	Ovine/ caprine (*B. melitensis*)	Porcine (*B. suis*)	Ovine (*B. ovis*)
Antigua/ Barbuda	?	-	-	-
Argentina	++	-	+	++
Belize	-	-	-	ND
Bolivia	++	+	+	ND
Brazil	++	-	+	-
Canada	-	-	-	+
Chile	++	-	-	+
Colombia	+	-	-	-
Cuba	?	-	++	-
Dominican Republic	++	-	+	-
Ecuador	++	ND	ND	ND
El Salvador	++	ND	+	ND
Guatemala	+	-	+	-
Haiti	+	-	-	-
Honduras	?	-	++	-
Jamaica	?+	-	-	-
Mexico	+	+	ND	-
Nicaragua	++	ND	ND	ND
Peru	++	ND	ND	++
Paraguay	+	ND	-	+
Uruguay	+	-	-	+
United States	+	-	(+)	+
Venezuela	++	-	++	?

- not present
+ low sporadic incidence
++ high incidence
? presence uncertain
ND no data

None of the four types of brucellosis is present in Barbados, Falkland Islands, Surinam, or St. Kitts/Nevis

information on prevalence is lacking, but *B. canis* has been recorded in the United States, Mexico, Argentina, Spain, China, Japan, Tunisia, and other countries. The recent isolation of distinctive *Brucella* strains, tentatively named *Brucella maris*, from marine animals in the United Kingdom and the United States extends the ecologic range of the genus and, potentially, its scope as a zoonosis (7,8). A hitherto unreported incident of laboratory-acquired infection suggests that this type is pathogenic for humans. Infection could result from occupational contact with infected seals or cetaceans.

Table 5. Brucellosis in animals, Oceania, 1994

Country	Bovine (*B. abortus*)	Ovine/ caprine (*B. melitensis*)	Porcine (*B. suis*)	Ovine (*B. ovis*)
Australia	-	-	(+)	+
Cook Island	-	ND	-	ND
New Caledonia	-	-	-	-
New Zealand	-	-	-	++
Samoa	+	ND	ND	ND

++ high prevalence
+ present
(+) limited presence
- not present
ND no data

None of the four types of brucellosis is present in Vanuatu

Molecular Genetics

Characterization of the molecular genetics of *Brucella* has taken place almost entirely within the past 10 years. The average molecular complexity of the genome is 2.37 x 10^9 daltons and the molar G + C 58-59% (9). The genus itself is highly homogeneous with all members showing >95% homology in DNA-DNA pairing studies, thus classifying *Brucella* as a monospecific genus (10). However, the nomenclature proposed by Verger

Table 6. Countries reporting eradication of bovine brucellosis, 1994

EUROPE		
Bulgaria (1958)	Croatia (1965)	Czech Republic (1964)
Denmark (1962)	Estonia (1961)	Finland (1960)
Hungary (1985)	Iceland (never recorded)	Latvia (1963)
Lithuania (1952)	Luxembourg (1993)	Netherlands (1993)
Romania (1969)	Slovak Republic (1964)	Slovenia (1970)
Sweden (1957)	Switzerland (1963)	U.K. (1993)
AFRICA		
Mauritius (1986)		
AMERICAS		
Belize (1980)	Canada (1989)	
ASIA		
Cyprus (1932)	Israel (1984)	Japan (1992)
Jordan (1992)	N Korea (1959)	Papua New Guinea (1974)
Philippines (1989)	U.A.E. (1992)	
OCEANIA		
Australia (1989)	French Polynesia (1984)	
New Zealand (1989)	Vanuatu (1992)	

and colleagues, in which all types would be regarded as biovars of *B. melitensis*, has not been generally adopted on practical grounds. For this reason, although its shortcomings are well known, the old nomenclature has been retained with the former species' names *B. abortus*, *B. melitensis*, *B. suis*, *Brucella neotomae*, *B. ovis*, and *B. canis* being used for the corresponding nomen species (11,12). Within these, seven biovars are recognized for *B. abortus* (1,7-10,12,13), three for *B. melitensis* (1, 7,8), and five for *B. suis* (1,7-10,12). The other species have not been differentiated into biovars, although variants exist (14). The current biotyping system does not encompass all known variants even of the principal species. Thus, variants of *B. melitensis* have been described; this suggests that the scheme should be extended (11,13,15). The strains isolated from marine animals clearly form a separate group and have been unofficially designated *B. maris* (E. S. Broughton, unpub. data). At least two subdivisions of this strain can be distinguished, corresponding approximately to strains isolated from cetaceans and seals, respectively (7,8).

Restriction fragment patterns produced by infrequently cutting endonucleases provide support for the current differentiation of the nomen species (16). Restriction endonuclease analysis has generally been unsuccessful for typing when applied to the whole genome (17) but polymerase chain amplification of selected sequences followed by restriction analysis has provided evidence of polymorphism in a number of genes including *omp 2*, *dnaK*, *htr*, and *ery* (the erythrulose-1-phosphate dehydrogenase gene) (18-20). The *omp2* gene is taxonomically important because it determines dye sensitivity, one of the traditional typing methods for biovar differentiation (21). Its polymorphism and capacity for posttranslational modification of its product may explain the tendency for variation in dye sensitivity patterns and have been used as the basis for a genetic classification of *Brucella* (22,23). The *dnaK* gene

Table 7. Countries reporting eradication of other forms of brucellosis, 1994

Region	Ovine/caprine (*B. melitensis*)	Porcine (*B. suis*)	Ovine (*B. ovis*)
Europe	Bulgaria (1941) Croatia (1991) Czech Rep. (1951) Germany (1986) Switzerland (1963)	Denmark (1951) Estonia (1988) Lithuania (1991) Sweden (1957)	Czech Rep. (1951) Germany (1986) Latvia (1989)
Africa	Ghana (1993) Namibia (1990)	None	Ghana (1993)
Americas	United States (1972) Chile (1987)	Belize (1985) Honduras (1992) Colombia (1982)	Falkland Is. (1991) Mexico (1991)
Asia	Cyprus (1993)	Singapore (1989)	Yemen (1989)
Oceania	Not present	None	None

of *B. melitensis* is cleaved into two fragments by Eco RV endonuclease, whereas the genes of the other nomen species all produce a single fragment (24). The *ery* gene is reported to have undergone a 7.2 kbp deletion in *B. abortus* strain 19 (20). This could explain this strain's erythritol sensitivity, a major factor in its attenuation.

The genome of *Brucella* contains two chromosomes of 2.1 and 1.5 mbp, respectively. Both replicons encode essential metabolic and replicative functions and hence are chromosomes and not plasmids (25,26). Natural plasmids have not been detected in *Brucella,* although transformation has been effected by wide host range plasmids after conjugative transfer or electroporation (27).

rRNA sequencing has defined the phylogenetic relationship of *Brucella*. Its closest known relation, *Ochrobactrum anthropi*, is an environmental bacterium associated with opportunistic infections (28); this organism is also detected by a polymerase chain reaction (PCR) procedure that is otherwise specific for *Brucella* (29). Possibly more closely related is the incompletely characterized *Vibrio cyclosites,* which displays >90% similarity of 5S rRNA sequence (30). Less closely related but within the same subgroup of the -2 Proteobacteria are *Agrobacterium, Phyllobacterium,* and *Rhizobium,* which also possess multiple replicons and a capacity for intracellular growth. The *Bartonella* group also shows some affinity to *Brucella* on the basis of rRNA, but not DNA, similarity (31). Other similarities have been noted in cell membrane lipid composition and intracellular growth.

Antigenic Composition

A substantial number of antigenic components of *Brucella* have been characterized. However, the antigen that dominates the antibody response is the lipopolysaccharide (LPS). In smooth phase strains (S), the S-LPS comprises a lipid A (containing two types of aminoglycose); distinctive fatty acids (excluding ß-hydroxymyristic acid); a core region containing glucose, mannose, and quinovosamine; and an O chain comprising a homopolymer of approximately 100 residues of 4-formamido-4,6-dideoxymannose (linked predominantly α-1,2 in A epitope-dominant strains with every fifth residue linked α-1,3 in M dominant strains) (32).

The difference in linkage influences the shape of the LPS epitopes. The A-dominant type is rod-shaped and is determined by five consecutive α-1,2 linked residues, whereas the M-dominant type is kinked and determined by four residues, including one linked α-1,3 (33). Strains that react with antisera to both A and M epitopes produce LPS of both types in approximately equal proportions (30), consistent with the origi-nal hypothesis of Wilson and Miles (34). The presence of 4-amino, 4,6 dideoxymannose in the LPS is also responsible for the antigenic cross-reactivity with *Escherichia hermanni* and *Escherichia coli* O:157, *Salmonella* O:30, *Stenotrophomonas maltophilia, Vibrio cholerae* O:1, and *Yersinia enterocolitica* O:9 LPS (32). The structure of the LPS of nonsmooth strains (R-LPS) is basically similar to that of the S-LPS except that the O-chain is either absent or reduced to a few residues. The specificity of the R-LPS is, therefore, largely determined by the core polysaccharide.

Numerous outer and inner membrane, cytoplasmic, and periplasmic protein antigens have also been characterized. Some are reognized by the immune system during infection and are potentially useful in diagnostic tests (35). Hitherto, tests based on such antigens have suffered from low sensitivity as infected persons tend to develop a much less consistent response to individual protein antigens than to LPS. Thus, tests such as immunoblotting against whole-cell extracts may have some advantages over more quantitative tests that employ purified individual antigens (36).

Recently, ribosomal proteins have reemerged as immunologically important components. Interest in these first arose more than 20 years ago when crude ribosomal preparations were demonstrated to stimulate both antibody and cell-mediated responses and to confer protection against challenge with *Brucella* (37). However, the individual components responsible for such activity were not identified until recently. It has been established that the L7/L12 ribosomal proteins are important in stimulating cell-mediated responses. They elicit delayed hypersensivity responses as components of brucellins (38), and as fusion proteins, they have been shown to stimulate protective responses to *Brucella* (39). They appear to have potential as candidate vaccine components.

Mechanisms of Pathogenicity

Virulent *Brucella* organisms can infect both nonphagocytic and phagocytic cells. The mechanism of invasion of nonphagocytic cells is not clearly established. Cell components specifically promoting cell adhesion and invasion have not

been characterized, and attempts to detect invasin genes homologous to those of enterobacteria have failed. Within nonphagocytic cells, brucellae tend to localize in the rough endoplasmic reticulum. In polymorphonuclear or mononuclear phagocytic cells, they use a number of mechanisms for avoiding or suppressing bactericidal responses. The S-LPS probably plays a substantial role in intracellular survival, as smooth organisms survive much more effectively than nonsmooth ones. Compared with enterobacterial LPS, S-LPS has many unusual properties: a relatively low toxicity for endotoxin-sensitive mice, rabbits, and chick embryos; low toxicity for macrophages; low pyrogenicity; and low hypoferremia-inducing activity. It is also a relatively poor inducer of interferon (and tumor necrosis factor) but, paradoxically, is an effective inducer of interleukin 12 (40,41).

S-LPS is the main antigen responsible for containing protection against infection in passive transfer experiments with monoclonal and polyclonal antibodies. The protection is usually short-term and incomplete, however. The elimination of virulent *Brucella* depends on activated macrophages and hence requires development of Th1 type cell-mediated responses to protein antigens (42).

An important determinant of virulence is the production of adenine and guanine monophosphate, which inhibit phagolysosome fusion; degranulation and activation of the myeloperoxidase-halide system; and production of tumor necrosis factor (41,43). The production of these inhibitors is prevented in *pur E* mutants, which are substantially attenuated in consequence. Cu-Zn superoxide dismutase is believed to play a significant role in the early phase of intracellular infection (44). However, conflicting results have been reported, and this role needs to be confirmed.

Survival within macrophages is associated with the synthesis of proteins of molecular weight 17, 24, 28, 60, and 62 kDa. The 62 kDa protein corresponds to the Gro EL homologue Hsp 62, and the 60 kDa protein is an acid-induced variant of this. The 24 kDa protein is also acid-induced, and its production correlates with bacterial survival under acidic conditions (<pH4). The 17 and 28 kDa proteins are apparently specifically induced by macrophages and correlated with intracellular survival (45).

Another stress-induced protein, HtrA, is involved in the induction of an early granulomatous response to *B. abortus* in mice and is associated with a reduction in the levels of infection during the early phase. Howevr, it does not prevent a subsequent increase in bacterial numbers, and *htrA*-deficient mutants ultimately produce levels of splenic infection similar to those given by wild-type *B. abortus* (46). Similarly, *recA*-deleted mutants produce a lower initial spleen count than *recA*-positive strains but still establish persistent infection (47). The role of iron-sequestering proteins or other siderophores in the pathogenesis of brucellosis is still unknown. In general, the low availability of iron in vivo restricts microbial growth. However, high iron concentrations promote the killing of *Brucella*, probably by favoring production of hydroxylamine and hydroxyl radical.

The mechanisms of pathogenesis of *Brucella* infection in its natural host species and in humans are still not completely understood, and further studies are needed.

Diagnosis

The clinical picture in human brucellosis can be misleading, and cases in which gastrointestinal, respiratory, dermal, or neurologic manifestations predominate are not uncommon (48-52). Because unusual cases with atypical lesions continue to be reported, diagnosis needs to be supported by laboratory tests (52). Blood culture is still the standard method and is often effective during the acute phase; the lysis concentration method gives the best results (53). Automated incubation-detection methods are effective, but allowance should be made for the relatively slow growth of the organism (54). Presumptive identification is made on the basis of morphologic, cultural, and serologic properties. Confirmation requires phage-typing, oxidative metabolism, or genotyping procedures. Reliance should not be placed on gallery type rapid identification systems as these have misidentified *Brucella* as *Moraxella phenylpyruvica*, with serious consequences for laboratory staff (55).

PCR with random or selected primers gives promising results, but standardization and further evaluation are needed, especially for chronic disease (56). Similarly, antigen detection methods are potentially useful but have not been validated. Combinations of these with PCR, such as immuno-PCR, have considerable potential but require evaluation. Enzyme immunoassay is now widely used for serologic diagnosis of the disease in humans and other species. IgA and IgG antibodies seem the most useful indicators of

active infection (57,58). Western blotting against selected cytoplasmic proteins may be useful in support of screening tests to differentiate active from past or subclinical infection (35).

Treatment

Despite extensive studies over the past 15 years, the optimum antibiotic therapy for brucellosis is still disputed. The treatment recommended by the World Health Organization for acute brucellosis in adults is rifampicin 600 to 900 mg and doxycycline 200 mg daily for a minimum of 6 weeks (59). Some still claim that the long-established combination of intramuscular streptomycin with an oral tetracycline gives fewer relapses (60). There is some evidence of physiologic antagonism between rifampicin and tetracyclines, but recent studies suggest that the two regimens have very similar results given adequate time. Quinolones in combination with rifampicin seem as effective as either of these regimens (61). Controlled clinical trials with other antibiotics, including new macrolides and ß-lactams, have either give inferior results or involved too few patients for proper evaluation.

Infections with complications, such as meningoencephalitis or endocarditis, require combination therapy with rifampicin, a tetracycline, and an aminoglycoside (62). Rifampicin has been recommended as the treatment of choice for uncomplicated disease in children, with cotrimoxazole as an alternative. Both are associated with a high relapse rate if used singly, and best results are achieved by using them in combination (63). Cotrimoxazole is an alternative but also has a high relapse rate. A combination of the two agents gives the best results.

Prevention

Prevention of brucellosis in humans still depends on the eradication or control of the disease in animal hosts, the exercise of hygienic precautions to limit exposure to infection through occupational activities, and the effective heating of dairy products and other potentially contaminated foods. Vaccination now has only a small role in the prevention of human disease, although in the past, various preparations have been used, including the live attenuated *B. abortus* strains 19-BA and 104M (used mainly in the former Soviet Union and China), the phenol-insoluble peptidoglycan vaccine (formerly available in France), and the polysaccharide-protein vaccine (used in Russia). All had limited efficacy (64) and in the cases of live vaccines, were associated with potentially serious reactogenicity. Subunit vaccines against brucellosis are still of interest. The live vaccines have provoked unacceptable reactions in individuals sensitized by previous exposure to *Brucella* or if inadvertently administered by subcutaneous rather than percutaneous injection. These will probably require a combination of detoxified lipopolysaccharide-protein conjugate and protein antigens such as the L7/L12 ribosomal proteins presented in an adjuvant or delivery system favoring a Th1 type immune response. *pur E* mutants of *B. melitensis* appear safe in animals (65) and may have potential application as human vaccines if their safety and efficacy is confirmed in clinical trials. New vaccines have been evaluated for use in animals, including the *B. suis* strain 2 live vaccine given either orally or parenterally (66,67). This vaccine has proved inferior to the Rev.1. strain for the prevention of *B. melitensis* infection in sheep and goats and ineffective against *B. ovis* infection in sheep. *B. abortus* strain 19 still appears to be as effective as any for the prevention of *B. abortus* infection in cattle. However, the RB51 strain of *B. abortus*, an R mutant used as a live vaccine, has been licensed in the United States. This does not interfere with diagnostic serologic tests, but in laboratory trials, its efficacy appeared comparable with that of strain 19 (68). Similar *rfb* mutants of *B. melitensis* and *B. suis* are under development for the prevention of ovine/caprine and porcine brucellosis.

Substantial progress has been achieved in understanding the molecular basis of the genetics of *Brucella* and the pathogenesis of the infection. However, further progress is needed, especially in relation to diagnostic procedures and therapy. An effective and safe vaccine against human brucellosis is also some way in the future.

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1st International Conference on Emerging Zoonoses
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Global Aspects of Emerging and Potential Zoonoses: a WHO Perspective

Many new human pathogens that have emerged or reemerged worldwide originated from animals or from products of animal origin. Many animal species as well as categories of agents have been involved in the emergence of diseases. Wild (e.g., bats, rodents) as well as draught animals (e.g., horses) and food animals (e.g., poultry, cattle) were implicated in the epidemiologic cycles of these diseases. Many of the agents responsible for new infections and diseases in humans were viruses (e.g., hantaviruses, lyssaviruses, and morbilliviruses), but bacteria, especially enteritic bacteria (e.g., Salmonellae and *Escherichia coli*) and parasites (e.g., *Cryptosporidium*) of animal origin, were also involved in major food and waterborne outbreaks. The public health relevance of some of these agents (e.g., new lyssaviruses and morbilliviruses) is not yet fully assessed. In addition the zoonotic nature of some other human diseases, such as Ebola and the new variant form of Creutzfeldt-Jakob disease, is suspected but not yet demonstrated. Finally, the possible future use of xenografts may lead, if precautions are not taken, to the emergence of new diseases called xenozoonoses.

Emerging and Reemerging Zoonotic Diseases

In both the developing and industrialized worlds, a number of zoonoses have emerged either as new pathologic entities or as already known agents, appearing in areas or species in which they had not been previously reported. In addition known zoonotic agents have reemerged sometimes after many years of absence in areas where they had been reported before. In this connection, limited and sometimes important outbreaks of otherwise endemic zoonoses such as rabies, brucellosis, leptospirosis, anthrax, a number of zoonotic foodborne diseases (caused by *Salmonella enteritidis, Salmonella typhimurium,* and *Escherichia coli*) and arbovirus infections involving production animals (Venezuelan equine encephalitis, Congo-Crimean hemorrhagic fever) have continued to appear in many industrialized and developing countries. Some examples of both emerging and reemerging zoonotic infections are given below.

Emerging Zoonoses

S. typhimurium in the United Kingdom

Multidrug-resistant *S. typhimurium* DT 104 initially emerged in cattle in 1988 in England and Wales. Subsequently, the strain has been isolated from poultry, sheep, pigs, and horses. Antimicrobial therapy is used extensively to combat *S. typhimurium* infection in animals. The evolution of a strain resistant to the commonly used antibiotics has made infections with *S. typhimurium* DT 104 in food animals difficult to control and likely to remain a zoonotic problem. Furthermore, in *S. typhimurium* DT 104 of R-type ACSSuT, multiple-drug resistance has become an integral part of the genetic material of the organism. Unlike other salmonella serotypes, multidrug-resistant *S. typhimurium* DT 104 is, therefore, likely to retain its resistance genes even when antimicrobial drugs are no longer used. Unlike *S. enteritidis*, which is mainly associated with poultry and eggs, multidrug-resistant *S. typhimurium* DT 104 can be found in a broad range of foods. Outbreaks in the United Kingdom have been linked to poultry, various meats and meat products, and unpasteurized milk. In addition to infections from contaminated food, cases have also occurred from human contact with infected cattle. A small proportion of cases may have been caused by pets such as cats and dogs, which can also be infected with this strain of *Salmonella*. Like humans, pets probably acquire the infection through consumption of contaminated raw meat, poultry, or poultry-derived products. The emergence of *Salmonella* strains resistant to antibiotics in common use is important to clinicians, microbiologists, and those responsible for the control of communicable disease.

Equine Morbillivirus in Australia

Equine morbillivirus (EMV) was isolated in September 1994 in Queensland, Australia. Fourteen horses died as a result of infection with EMV in southeast Queensland during September 1994. Seven additional horses were infected by the virus and were humanely killed. The first death (of a mare) attributed to the infection occurred on 9 September 1994. The virus produced severe damage to the lungs with the accumulation of massive amounts of fluid. Two persons who had close contact with the sick mare became infected with the virus and also had respiratory illness, in one case with fatal consequences. During October 1995, a third human infection with EMV was diagnosed. The infected man came from a property in Mackay where two horses had been diagnosed as dying from avocado poisoning and snakebite during August 1994.

Rabies in Australia

On 24 May 1996, a black flying fox with signs of neurologic illness was found in Ballina, New South Wales, Australia, and was submitted for autopsy. Histopathologic examination of the brain showed severe nonsuppurative encephalitis. Tissues were examined for EMV infection at the Animal Research Institute, Brisbane. Additional fixed tissues were sent for EMV and rabies testing. Results were negative for EMV. However, immunoperoxidase testing on fixed brain tissue was positive for *Lyssavirus* antigen and was subsequently confirmed by immunofluorescence testing. Subsequent investigations have indicated that the *Lyssavirus* present is not classic rabies, serotype 1. A range of cell cultures and mice inoculations were carried out to characterize the specific *Lyssavirus* involved.

Rabies in the United Kingdom

Since 30 May, four people in the vicinity of Newhaven, on the south coast of England, were thought to have been in contact with an insectivorous bat with rabies. After the bat was caught and humanely killed, on 3 June, the carcass was examined, and the diagnosis of rabies was confirmed. In the past 30 years, three human deaths have been associated with bat rabies in Europe, one in Finland and two in the former Soviet Union. Bat rabies is mainly confined to serotine bats in Europe, particularly in Denmark, northern Germany, and the Netherlands. Serotine bats are relatively uncommon in the United Kingdom. In the United States, four cases of human rabies, caused by different variants of rabies viruses associated with insectivorous bats, were reported in 1995.

Reemerging Zoonoses

Venezuelan Equine Encephalitis in Colombia and Venezuela

An outbreak of Venezuelan equine encephalitis (VEE), which began in early September 1995, was reported on the border between Colombia and Venezuela. As of 21 September 1995, 825 human cases of VEE had been reported, with five confirmed by laboratory analysis and four resulting in death in Venezuela, mainly in the state of Zulia. In Colombia, more than 450 cases have been reported in the Department of La Guajira. VEE is the most severe of several viruses causing disease in horses and can be transmitted to humans by mosquito bites. Most infections are relatively mild, and symptoms include abrupt onset of severe headache, chills, fever, muscular pain, nausea, and vomiting. Clinical cases occur in 11% to 20% of the exposed population, and death occurs in fewer than 1% of those cases. Large-scale epidemics of VEE occurred in Colombia, Peru, Trinidad, and Venezuela in the 1950s and Central America and Mexico in the late 1960s, reaching the United States (Texas) in 1971. Outbreaks can be prevented by the regular immunization of horses in the framework of public health programs.

Leptospirosis in Nicaragua

On 1 November 1995, health authorities in Nicaragua established a task force to determine the cause of an unidentified illness that affected hundreds of residents of the Achuapa area, causing some 15 deaths. A task force investigated the nature and origins of the unknown disease, whose symptoms ranged from high fever to internal bleeding. On 6 November 1995, tests conducted by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA, indicated that the cause of the disease that killed at least 16 people in Nicaragua was an unusual form of leptospirosis. Initial reports that the disease was dengue fever, which has been common in the region, were ruled out after several autopsies showed severe respiratory hemorrhaging, and laboratory tests for dengue fever proved negative. Tests on three of the four

specimens were positive for leptospirosis. Leptospirosis, while not rare in the Americas or Asia, usually affects the liver or kidneys, rather than the respiratory system.

Enterohemorrhagic *E. coli* Infection in Japan

An outbreak of enterohemorrhagic *E. coli* (EHEC) infection among schoolchildren was announced in Sakai City (population ca. 800,000), in the region of Osaka, Japan, in July 1996. Most of the reported cases were in children 6 to 12 years of age from 62 public elementary schools of the municipality. EHEC serotype O157 was detected in patients' stool samples. School lunch, which was prepared in individual schools from the foods delivered by a central supply station, was the suspected cause of this outbreak; the responsible food is still unknown. Epidemiologic investigation has shown that fresh radish sprouts (*kaiware-daikon*) were among the foods eaten by the schoolchildren. However, samples of radish seeds and sprouts, water, and soil from the environment of the farm concerned and stool samples from the farm workers showed no trace of this organism. As of 26 August 1996, 9,578 cases of *E. coli* serotypes O157:H7 and O157:H- infection, in both outbreaks and sporadic infec-tions resulting in 11 deaths, had been reported in Japan. Although most of the cases are believed to be foodborne, the responsible foods have not been identified with certainty except in a few isolated cases. Investigations of outbreaks in other countries have established that animals, especially cattle, are major reservoirs of Shiga-like toxin producing *E. coli* and that foods of bovine origin, such as ground beef and unpasteurized milk, are the principal sources of human infection. The possibility that the source of the responsible agent is of animal origin should be further investigated in Japan.

Potential Zoonoses

The natural cycle of Ebola is not known, and its reservoir (i.e., the vertebrate playing an essential role in the maintenance of the agent and its life cycle, if one exists) remains to be identified. On the other hand, exposure to the agent of bovine spongiform encephalopathy (BSE), a known disease of cattle, is suspected to be the origin of a new variant form of Creutzfeldt-Jakob disease (CJD) in humans in the United Kingdom. These potential zoonoses have very recently had a considerable impact on public health, animal production, and laboratory animal sectors worldwide in view of the need to minimize the risks for humans.

Bovine Spongiform Encephalopathy

BSE is a fatal disease of cattle that first came to the attention of the scientific community in November 1986 with the appearance in cattle of a newly recognized form of neurologic disease in the United Kingdom. Between November 1986 and 31 May 1996, approximately 160,000 cases of this newly recognized cattle disease were confirmed on approximately 33,400 farms. The disease is now on the decline in the United Kingdom. Epidemiologic studies suggested that the source of disease was cattle feed prepared from carcasses of dead ruminants. Modifications to the process used for preparing cattle feed introduced in 1981 to 1982 may have been a risk factor by not eliminating the agent from the feed. By May 1996, BSE had been reported from 10 countries and areas outside the United Kingdom. In one group of countries—France, Portugal, the Republic of Ireland, and Switzerland—the disease occurred in native cattle and could be linked to importation of potentially infected cattle feed from the United Kingdom. In another group—the Falkland Islands, Oman Sultanate, Germany, Canada, Italy, and Denmark—cases were only identified in cattle imported from the United Kingdom.

BSE is associated with a transmissible agent, the nature of which is not yet fully understood, and is one of several forms of transmissible brain disease of animals. A number of similar severe and fatal neurologic human diseases are due to non-conventional agents. These diseases include kuru, a disease that was transmitted by human ritual handling of bodies and brains of the dead and was identified in Papua, New Guinea in the 1950s, and the various forms (i.e., sporadic, familial, and iatrogenic) of CJD, a rare disease with a worldwide distribution.

After the identification of BSE, because the above human and animal diseases shared a number of characteristics, including their ability to be transmitted experimentally to a range of animals, the World Health Organization (WHO) held, or coorganized as a regular activity, four consultations during 1991 to 1995 to study these diseases (called transmissible spongiform encephalopathies). An express purpose of these consultations was to review the possible human public health implications of animal spongiform encephalopathies, with a special emphasis on BSE.

The sudden announcement on 20 March 1996 by the United Kingdom of a cluster of 10 human cases identified with what appears to be a variant of CJD necessitated a fifth and sixth consultation at WHO because the results of the investigation indicated that, although there was no direct evidence of a link, the most likely hypothesis was that these cases might be related to exposure to BSE in the United Kingdom. The fifth consultation reassessed current recommendations in light of these new developments and issued recommendations to further minimize risks for consumers particularly in relation to foods and food products of animal origin. This was achieved through the organization of expert consultations first held in April 1996. The sixth consultation by WHO was organized in May 1996 to propose to its member states a protocol for global surveillance of the new variant form of CJD and identify the main areas for research on these diseases. Other consultations may be held when new findings with a bearing on public health become available.

Ebola and Ebola-related Viruses

In 1976, Ebola attracted worldwide attention with the outbreaks of Yambuku, Zaire, with 318 cases and Nzara, Sudan, with 284 cases, characterized by very high case-mortality rates (53% in Sudan and 88% in Zaire). In the first cases at the origin of the outbreaks (index cases) the patients may have had contacts with infected animals or their products (bats and/or rodents in Sudan, meat from monkeys or wild antelopes in Zaire), but contact could not be proven, and investigations of the possible animal reservoir remained inconclusive.

During 1977 to 1989 a small number of suspect, sporadic cases, or relatively small outbreaks were reported (e.g., in Nzara, 1979). In 1989, a new Ebola-related virus was isolated from macaques originally captured in the wild in the Philippines dying from acute hemorrhagic fever and imported into the United States. The same virus, called Reston, was isolated again in different animal colonies in macaques imported from the same country in 1990 (United States), 1992 (Italy), and 1996 (United States).

In 1994, 15 years after the last Ebola outbreak (Nzara, 1979) reported in Africa, a Swiss ethologist became infected by a new Ebola variant after doing a postmortem on a chimpanzee from the Tai forest in Côte d'Ivoire. The infection was demonstrated in this chimpanzee, and a number of deaths reported in these animals in 1994 in the Tai forest were associated with this agent. Other outbreaks may have occurred in the chimpanzee population of the Tai forest before 1994. In January 1995, a new Ebola epidemic was reported in Kikwit, Zaire, with 315 cases and 244 deaths. The initial source of the infection of this outbreak may have been a forest worker involved in charcoal making. A year later, in January 1996, an outbreak occurred in Makokou, Gabon, with 37 cases and 21 deaths. The investigation of this outbreak showed that most patients had contacts with dead chimpanzees that they butchered. In October 1996, a second outbreak was reported in the Makokou region. The number of cases was 24 with 17 deaths on 12 November 1996. The index case was a hunter who fell ill in July 1996 and died in August in Booue hospital 200 km from Makokou.

Nothing definite is known on the reservoir of Ebola virus. A number of hypotheses have been proposed involving rodents or insects and even viruses of plant origin. It is generally agreed that the species of monkeys and apes in which the virus stains have been isolated so far are only victims of the disease, because in view of the high mortality rates usually observed, these populations could not sustain themselves or the agent for very long. These animals, however, participate in virus amplification by facilitating further virus transmission, including to humans, through handling of monkey carcasses or consumption of their meat. The identification of a new variant of Ebola in chimpanzees in its natural environment is a unique opportunity; so far the agent was only isolated from monkeys captured in the wild but (at time of the disease) located far from their natural environment. WHO, in collaboration with scientists from many countries including Switzerland, France, the United Kingdom, Sweden, Canada, and the United States, has, therefore, initiated a multidisciplinary study in the Tai forest in Côte d'Ivoire to identify the natural reservoir of the virus on the basis of the behavior of the chimpanzees. This identification would be essential to understanding the mechanisms for transmission in nature and facilitating the prevention of future Ebola outbreaks.

Xenografts and Xenozoonoses

Successful xenotransplantation (the transfer of animal organs or tissues into human recipients) may quickly become a biomedical reality. The development of immunosuppressive drug protocols to prevent both organ rejection and graft vs.

host disease, as well as advances in surgical techniques is opening the door to a field that can save thousands of human lives each year.

A risk of disease transmission is present in any transplantation system. In allotransplantation, disease transmission is a major cause of illness and death in recipients. This is largely due to the required level of recipient immunosuppression but is also attributable to infected, but usually asymptomatic, human donors. Cases of viral, bacterial, fungal, and parasitic transmission are documented. In some cases, the presence of the infectious agent was known before transplantation. The decision was made to proceed because of the lack of alternative donors. The field of xenotransplantation also brings with it the potential for introducing unwanted animal-origin infectious agents, both known and as yet unknown, into the human population. These agents could cause disease in their new host. Dissemination beyond the original recipient into the general population could lead to epidemics. Xenotransplantation, therefore, has the potential for being both of great benefit and of detriment to humans. WHO is developing guidelines to ensure the development of xenotransplant donors free of zoonotic and other agents representing a potential risk for the recipient.

Reasons for the Increasing Trend–Control Measures

As shown in this paper, in both the developing and industrialized worlds, a number of zoonoses have emerged either as new pathologic entities or as already known agents, appearing in areas or species where they had not been previously reported or where they had seemingly disappeared. The reasons for this increasing trend are complex, but some have been identified as follows: 1) alteration of the environment affecting the size and distribution of certain animal species, vectors, and transmitters of infectious agents of humans; 2) increasing human populations favoring an increased level of contact between humans and infected/affected animals; 3) industrialization of foods of animal origin; changes in food processing and consumer nutritional habits; and, 4) increasing movements of people as well as trade of animals and animal products and decreasing activities for the surveillance and control of major zoonoses.

Although the prevalence of major zoonoses, such as brucellosis, tuberculosis, and dog rabies, has been greatly reduced in the industrialized world, these diseases have not been eliminated altogether; zoonoses prevention and control will remain an area of major concern in most developing countries. Recent observations show that expenses related to the prevention of zoonotic diseases in humans are likely to increase dramatically in these countries in the near future if no programs for their control and elimination in animal reservoirs are implemented. Diseases such as brucellosis, rabies, and bovine tuberculosis will certainly be brought under control during the first decade of the second millennium, but this will require constant efforts for the next 15 to 20 years. Also, as the trade of animal products and the movements of people become more intense, the risks of introduction/reintroduction of certain diseases in a country increase. It is very likely, in view of the foreseeable global changes over the next few decades (e.g., population growth, urbanization, and climatic changes), that this trend will continue and even increase. Human disease patterns will be affected by high densities and movements of human populations within and between countries and changes in lifestyles; animal disease patterns will be affected by changing land-use patterns, new farming practices, displacement of animals, and environmental contamination.

Infectious diseases, including zoonoses, will remain the major health problems in most developing countries, together with opportunistic infections, including zoonotic diseases as a very important component. In industrialized countries, where cardiovascular diseases and cancers will remain the main causes of illness and death in spite of the AIDS pandemic, special attention will need to be paid to human-animal relationships especially with groups at higher risk such as the elderly, who already represent a significant part of the population of industrialized countries, as well as children and childbearing women, HIV-infected persons, and AIDS patients.

As zoonoses and animal diseases with the potential to affect human health likely will continue to emerge and reemerge, zoonoses surveillance will need to be reinforced and maintained at the national and international levels. In view of the foreseeable unplanned urbanization phenomenon and the associated lack or shortage of basic facilities, a strategy to respond to human and animal health problems in an urban environment will need to be developed for cities of

both industrialized and developing countries. Comprehensive plans will need to be developed to reduce the rural-urban migration flow to reverse the current and anticipated trend. Major components of these plans should include improving rural employment and food availability by promoting animal production projects and improving health through zoonoses control and reduction of environmental pollution related to animal rearing.

Further collaboration is essential between all professions involved in food technology development, food industry control, and promotion of new production techniques at the primary production level in order to ensure food protection and response to the needs of the world population.

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Epidemiology of Emerging Zoonoses in Israel

The epizootiologic, climatic, and ecologic conditions in the Middle East, combined with socioeconomic and agricultural structures in Israel, have created a unique and hazardous veterinary public health situation in this country. In this paper, emerging zoonotic diseases are newly introduced disease agents or endemic zoonoses whose previous epidemiologic patterns have changed.

As of 1996, 15 zoonotic diseases of animals, anthrax, brucellosis, cysticercosis (bovine), erysipelas, leishmaniasis (canine), leptospirosis, listeriosis, malleus (glanders), psittacosis (chlamydiasis), Q fever, rabies, Rift Valley fever (RVF), salmonellosis, trichinosis, and tuberculosis (bovine and avian) (Table 1), have been declared notifiable in Israel. When diagnosed in animals, these diseases are officially reported to the state veterinary officers, who in turn report them to the medical officers. Of these notifiable zoonoses, RVF has never been recorded in Israel, and glanders has not been reported since 1951; ten are notifiable in humans at the national level.

This article examines five of the reportable animal diseases whose epidemiologic features have changed in Israel during the last decade: *Brucella melitensis* in cattle, *Salmonella enteritidis* in poultry, rabies, canine leishmaniasis, and trichinosis in wild boars. Two nonreportable diseases in animals—botulism in ruminants and echinococcosis—will also be discussed briefly. (Table 1). Recently, the possibility that bovine spongiform encephalopathy (BSE) may be a zoonotic disease has been discussed worldwide. Thus, we also describe steps adopted by the Veterinary Services and Animal Health (VSAH) to prevent the introduction of BSE into Israel. The data on zoonotic diseases in animals are from VSAH's annual reports to the Office International des Epizootics in Paris. If not otherwise mentioned, the data on the diseases in humans are from the weekly and monthly reports of the Ministry of Health in Jerusalem.

Table 1. Selected zoonotic diseases in Israel, 1996

	Animal		Human	
Disease	Year Notifiable	Occurrence	Year Notifiable	Occurrence
Anthrax	1945	1993	1949	1984
Brucellosis	1952	+++	1949	+
Cysticercosis (bovine)	1981	+	NN	+...
Erysipelas	1945	(+)	NN	+...
Leishmaniasis (canine)	1945	( )	1949	1994
Leptospirosis	1945	+	1949	(+)
Listeriosis	1961	+	1993	+...
Malleus (glanders)	1945	1951	NN	never
Psittacosis	1952	+	NN	+...
Q fever	1952	+	1949	(+)
Rabies	1955	+	1949	1960
Rift Valley fever	1978	never	NN	never
Salmonellosis	1959	+++	1949	++
Trichinosis	1982	( )	No	+...
Tuberculosis (bov., av.)	1945	1993	1949	–
Botulism (types C,D)	NN	+	1993	–
Echinococcosis	NN	++	1980	(+)

NN	Not notifiable
0000	Never reported
-	not reported, probably no occurrence
Year	Year of last occurrence
(+)	exceptional occurrence
+	low sporadic occurrence
++	enzootic/endemic
+++	High occurrence

Brucella melitensis in Dairy Cattle

Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and rarely by *B. suis*. The disease is manifested by abortion and excretion of the organisms in uterine discharges and in milk. Brucellosis is highly pathogenic for humans, *B. melitensis* is regarded as more pathogenic than *B. abortus*. *B. melitensis* is the main causative agent of caprine and ovine brucellosis; it appears to occur mainly in the Mediterranean regions, but infection occurs worldwide.

As of 1996, the dairy cattle of Israel, approximately 120,000 lactating cows and 230,000 younger animals on 1,500 farms, are free of infection with *B. abortus*. Since 1984, VSAH's ongoing, compulsory surveillance of all farms has reported no cases of *B. abortus*. The surveillance includes periodic bulk milk testing. In the event of a positive reaction, the test is repeated on bulk milk from groups of cows, and the cows of a suspected

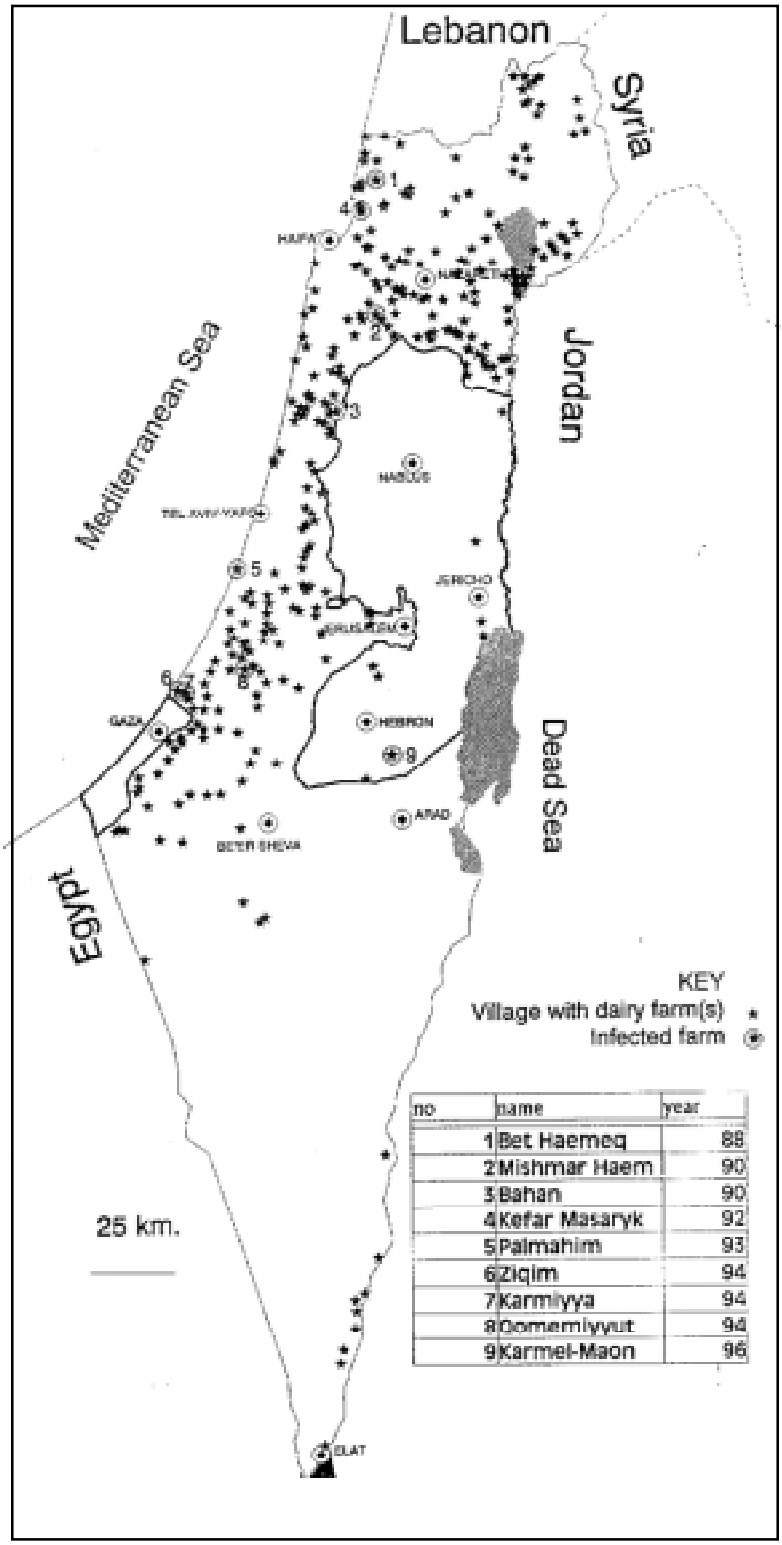
group undergo individual blood testing. Cows with positive test results are slaughtered with full state compensations, and their organs are bacteriologically examined in the State Reference Laboratory for Brucellosis in the Kimron Veterinary Institute. In spite of the absence of *B. abortus,* vaccinations of all 2- to 6-month-old dairy heifers with *B. abortus* strain 19 vaccine has not been discontinued.

The status of *B. melitensis* in small ruminants, however, is different. Brucellosis, which has been present in sheep and goat flocks (mainly in the Bedouin sector) for decades, has increased considerably since the mid-1980s A countrywide serologic survey was carried out during 1994–1995, in which 906 (10.1%) out of the 4,738 examined flocks were found infected. The total number of reacting animals was 4,785 (3.16%) out of 151,409 examined sheep and goats. Accordingly, a sharp rise in the occurrence of *B. melitensis* in humans has been recorded from one case per 100,000 in 1983 to 11 cases per 100,000 (total 498 cases) in 1988 to 11 cases per 100,000 (total 498 cases) in 1988. The most common sources of human infection are locally prepared unpasteurized goat and sheep cheeses and milk (1).

An important phenomenon regarding *B. melitensis* in Israel is its penetration into cattle herds. Until the late 1980s, these were mainly small herds of locally bred Baladi beef cattle. In sporadic exceptional cases, introduction of *B. melitensis* into dairy cattle farms was also observed. Such a case was recorded for the first time in 1977 when raw unpasteurized whey, a by-product of sheep cheese, from a central dairy plant was fed to the lactating cows in one of the largest and finest dairy herds in Israel, in the centrally situated Valley of Yezreel (2). The case came to the attention of VSAH only after three dairy workers were hospitalized with Malta fever. It took 2 years and the slaughter of 28 cows for the herd to become disease-free again. According the VSAH "test and slaughter" policy, all cattle in a farm where the seropositivity rate does not exceed 50% are subject to monthly repeated serologic examinations. Animals with positive test results are slaughtered, and compensations are paid to the owners. The farm may be declared free of brucellosis, and qua-rantine measures are discontinued after three consecutive negative serologic tests of the entire herd. If more than 50% of the cows react positively to the serologic test, the entire herd is slaughtered. Since this incident, only pasteurized whey has been permitted as animal feed; however, a second major outbreak occurred in late 1988, when cattle on a dairy farm with 765 animals in western Galilee tested positive on the periodic milk-ring test and was infected with *B. melitensis* (3). Circumstantial evidence implicated contaminated whey from sheep or goat milk as the most likely vehicle of infection. The epidemiologic investigation was inconclusive, but researchers hypothesized that the pasteurized whey could have been recontaminated when transported in tankers not properly disinfected after having transported raw goat's milk. The dairy herd was put under quarantine, and test and slaughter policies were applied; implementation of these policies led to the compulsory slaughter of 175 animals (±23% of the herd). The farm was declared free of brucellosis a year after the initial infection and two consecutive herd tests with negative results. No human cases were involved. From this outbreak, it could be concluded that vaccination of cattle with strain 19 vaccine did not confer adequate protection against infection with *B. melitensis,* although it may have prevented abortions. These observations were later supported by additional data from other outbreaks in dairy cattle.

Since 1988, eight additional outbreaks of *B. melitensis* in large dairy cattle farms throughout Israel have been recorded (Figure 1). The largest outbreak occurred in 1994 when the entire cattle population (761 cattle) of a large kibbutz dairy farm had to be slaughtered because the infection rate exceeded 60%. In this outbreak, more than 30 members of the kibbutz community, as well as the attending veterinarian, were infected, most by direct contact with the infected cows or placenta and by the consumption of unpasteurized milk. At present, an additional outbreak with human cases is being investigated.

The last eight outbreaks have been attributed to indirect contact with infected nomadic sheep and goat flocks, which apparently contaminated the pasture from which fodder was supplied to the lactating cows. Another route of infection might have been dogs, most probably infected by consuming infected placentas. Serologic examinations of dogs were carried out on two of the farms, and antibodies were found in samples of 9 of the 39 dogs tested. The infected dogs were then euthanized. It should be noted that Israel is free of *B. canis*. The introduction of *B. melitensis* into



no	name	year
1	Bet Haemeq	88
2	Mishmar Haem	90
3	Bahan	90
4	Kefar Masaryk	92
5	Palmahim	93
6	Ziqim	94
7	Karmiyya	94
8	Domemiyyut	94
9	Karmel-Maon	96

Figure 1. *Brucella mellitensis* in dairy cattle, Israel 1988–1996.

dairy cattle farms, a serious public health hazard, may be almost inevitable in a highly contaminated environment.

Salmonella enteritidis

Salmonellosis is an infectious disease of humans and animals caused primarily by intestinal bacteria of which 2,375 serovars are recognized, a number constantly increasing. Salmonellosis appears to be most prevalent in areas of intensive animal husbandry, especially of poultry or pigs. *S. enteritidis*, especially of the phage-type TP4, emerged as a serious public health hazard in Europe during the late 80s, mainly as a contaminant of table eggs because of its transovarian infection in laying hens (4). National programs have been implemented in some countries to control *S. enteritidis* in poultry and protect the consumer.

Since the initial report from Europe, in 1988, of the emerging animal and public health problem caused by *S. enteritidis* PT4 in poultry, Israeli policy and activities have undergone a three-phase evolution:

1) 1988–1991: To prevent introduction of *S. enteritidis,* Israel implemented strict quarantine conditions, including testing (serologic and bacteriologic), on the import of breeding poultry, chicks, and hatching eggs. From 1988 to 1991**,** 11 imported breeding flocks (five broiler chickens, five laying chickens, one turkey), 103,100 birds, and 149,970 hatching (layer) eggs were found infected with *S. enteritidis* PT4 and destroyed. The policy of strict border control of imported day-old chicks and hatching eggs probably contributed to the delay in the appearance of human infection until 1992.

2) 1992–1993: For the first time local breeding flocks were found infected with *S. enteritidis* PT4 and destroyed along with their offspring laying chick flocks. A surveillance

program was implemented in the 200 local breeding flocks. The program included bacteriologic examinations of dead birds forwarded to the regional poultry disease laboratory, as well as monthly visits of state poultry disease officers to each breeding farm for sanitation control that included drag swab tests, sampling of caecal contents for bacteriologic examinations, and in several flocks, rapid slight agglutinations. Hatcheries were included as well. After the surveillance, infected flocks (31 [10 broilers, 20 layer, one goose] which comprised 272,188 birds and 2.8 million hatching eggs) were destroyed. The state had to compen-sate the owners approximately 1.5 million U.S. dollars; $300,000 in 1992, $1.17 million in 1993). "Competitive exclusion," a preventive-curative technique developed in Finland and tried experimentally on six breeding flocks produced inconclusive results (5). During 1992 and 1993, a significant increase in laboratory-contracted human cases of *S. enteritidis* PT4 infection was recorded by the Ministry of Health: nine cases in 1991, 203 in 1992, and 473 in 1993.

During 1993, to evaluate the *S. enteritidis* infection rate on commercial farms, VSAH surveyed 88 broiler and layer breeding farms and found 18 (26.8%) of the 67 surveyed broiler breeder farms and 6 (28.5%) of the 21 layer breeder farms infected by direct cultures. In another investigation, 35 flocks of laying hens were examined in slaughterhouses, of which 12 (30.8%) were infected with *S. enteritidis*. Out of the said 10 isolates, five were identified as PT4. In addition, 39 hatcheries throughout the country were surveyed, of which 12 (30.8%) were infected with *S. enteritidis*. These findings, combined with the alarming increase human *S. enteritidis* infection rates, led to a reevaluation of the general policy, which examined experience in other countries and local data. One of the main decisions was to concentrate the efforts in the layer line and discontinue the stamping out policy in the broilers line. The first step was to prohibit the simultaneous incubation of layer- and broiler-hatching eggs in the same hatchery.

The updated policy to concentrate and intensify the control efforts upon the layers breeding stock while generally improving sanitary conditions in all lines and to implement the control upon handling of table-eggs seems to have contributed to the improvement of the public health situation regarding *S. enteritidis* PT4.

3) 1994–

In 1993, after the publication of state regulations forbidding the simultaneous incubation of layer and broiler hatching eggs, VSAH discontinued intensive surveillance and, eventually, stamping out of broiler breeding farms, to concentrate efforts on the layer line. Consequently, the number of stamped-out broiler poultry flocks decreased from 24 in 1993 to nine in 1994 to only one in 1995. VSAH implemented four steps: a) Strict control of the layers breeding stock and hatcheries, including bacteriologic examinations of meconium, offspring, and laying birds and monthly sampling of drag swabs from the hatcheries and the breeding farms; b) improvement of sanitary conditions in poultry farms; c) introduction of an (inactivated) *S. enteritidis* vaccine, combined with the live attenuated *Salmonella typhimurium* vaccine in parent-stock breeding flocks; and d) enforcement of new state regulations regarding the handling of table-eggs, their collection, transportation, packing, marking, chilling, and marketing.

These measures were possible because of a single, centralized veterinary system that combines modern diagnostic facilities, field activities, and legal enforcement tools. The results of the measures described are reflected in the number of reported human *S. enteritidis* isolates (Table 2).

Table 2: Reported *Salmonella enteritidis* isolates in humans

	S. enteritidis	*S. enteritidis (PT4)*
1989	357	-
1990	878	-
1991	987	9
1992	926	203
1993	1376	473
1994	1450	750
1995	997	557

Furnished by Dr. Vered Agmon, Ministry of Health, Jerusalem. Preliminary figures for 1996 indicate that decline continues.

Rabies

Rabies, a major zoonosis caused by the neurotropic Lyssavirus, transmissible to all mammals, occurs in all parts of the world. It is especially prevalent in the temperate zones and where there is a large canine population. Generally, in countries where the virus reservoir is predominantly within the stray dogs which are

the main vectors, the term "urbanic rabies" is applied. On the other hand, when the reservoir is mainly the wildlife population, the term "sylvatic rabies" is used. Urban rabies predominates in Asia, Africa, and South America.

The evolution of rabies in vector animals, since 1948 is presented in Table 3. Annual vaccination of owned dogs became compulsory in 1957; vaccination coverage is estimated at ±60%. In 1979, rabies became sylvatic (involving mainly fauna [foxes] rather than dogs) and remained so until 1990. A transitory change was observed during 1991–92 when a sharp rise in the number of rabid dogs converted the picture into "urban rabies" (dogs/fauna ratio:2/1). This change, combined with the penetration of the disease into the densely populated coastal region where rabies had been practically absent for more than 30 years, could have been an outcome of the Gulf war at the beginning of 1991 when many people (nearly 50% of the inhabitants of Tel Aviv and other coastal cities) left their homes for safer areas during the Iraqi missile attacks. Many of them abandoned their pets, establishing a large stray-dog population. Subsequent steps to eliminate stray dogs may have reversed the situation during 1992. Since 1993 (up to November 1996), the pattern of predominantly sylvatic rabies has been reestablished.

Rabies is practically endemic in most of Israel; clusters of rabies cases in foxes are observed mainly in areas around the cities of Beer-Sheva, Arad, Jerusalem, and Nazareth, but sporadic cases are scattered in other areas (Figure 2).

The wildlife potentially involved with rabies comprises mainly foxes (*Vulpes vulpes*) but in recent years, a significant increase in the number of jackals (*Canis aureus*) has been observed in the central and northern parts of the country. During 1995, 63 foxes and 14 jackals were tested at the central rabies diagnostic laboratory at the Kimron Veterinary Institute; 30 (47.6%) foxes and 2 (14.3%) jackals were infected. The figures for 1996 are similar. Other wildlife species, involved with rabies are the mongoose (*Herpestes ichneumon ichneumon*), badgers (*Meles meles canescens*), and the stone marten (*Martes foina syriaca*). During 1986 to 1996, rabies was diagnosed in 251 foxes, 31 jackals, four mongooses, four badgers, and three martens, which were sent to the Kimron Veterinary Institute. The actual incidence of the disease was much higher. In view of the growth of the jackal population, rabies—if spread within that species—may increase considerably the already serious threat to humans and farm animals. Recently, seven cattle herds in northern Israel were found infected, more than twice the mean annual number for decades. This might reflect the increased rabies incidence among the extremely dense populations of foxes and jackals in that region.

Oral vaccination of wildlife in Israel has been considered, but vaccine and baits evaluation is still in its experimental stages. At any rate, vaccination will only be practical if it is effective for both foxes and jackals and involves entire ecologically related regions (the West Bank, the Jordan Valley, and probably the western parts of Jordan and southern Lebanon).

Canine Leishmaniasis

Canine leishmaniasis is a chronic visceracutaneous disease caused by *Leishmania infantum* (in the Old World), of which the dog acts as the source reservoir. The vectors of leishmaniasis are phlebotomine sandflies belonging to the genera Phlebotomus (Old World) and Lutzomyia (New World).

Canine leishmaniasis was common before 1948 but seemed to disappear after that. Since 1955, no cases have been reported in dogs. However, four human cases of visceral leishmaniasis (Kala-azar) (two in 1980 and two in 1982) were reported by the Ministry of Health in two restricted areas in eastern and western Galilee. The disease became regarded as endemic in these areas, although no canine cases were detected there.

Table 3. Mean annual number of rabies cases in animals and humans, 1948–1995

		Domestic animals		Wildlife				
Years	No. of years	Dogs	Cats	Foxes	Jackals	Other fauna	Fauna (%)	Human cases
1948-1957	10	72.0	3.9	0.1	9.9	0.4	12.0	23
1958-1966	9	19.4	0.9	0.3	0.8	0.1	5.6	2
1967-1978	12	8.6	0.3	1.3	0.3	0.16	16.5	2
1979-1990	12	6.2	0.25	12.6	1.25	1.3	70.1	0
1991-1992	2	27.0	0	11.0	2	0	32.5	0
1993-1995	3	14.25	1.25	35.25	4	4	72.2	0

From January 1995 through November 1996, 30 clinical cases of canine leishmaniasis were reported from five communities in the Judean hills northwest of Jerusalem and from one location in western Galilee (Figure 3). Diagnosis was carried out by the Koret Veterinary School and the Kuvin Center of the Hebrew University, which reported that the cases were caused by *L. infantum* (G. Benet, pers. comm.).

Trichinosis

Trichinosis is a parasitic disease. Human infections are established through the consumption of insufficiently cooked pork infected with the causative nematode *Trichinella spiralis*.

No cases of trichinellosis (trichinosis) have been recorded in domestic pigs in Israel since 1948, though a substantial number of pigs are slaughtered (more than 100,000 in 1995) and each batch is sampled for representative microscopy examinations. In light of the accumulated data about the absence of infection in the housed pigs, the sample rate is limited. Trichinosis in animals was declared notifiable in 1982 when its potential hazard was reflected by the admission to Israeli hospitals of 20 clinically infected persons from southern Lebanon, close to the northern Israeli border.

According to a state regulation published in 1977, the entire carcass of hunted wild boars should be presented to accredited veterinary inspectors at specified inspection stations. Until 1991, 100–200 such carcasses, approximately 10% of the actual number of killed boars, were annually presented for microscopy examinations without detecting any infections. However, in 1992, hunters from northern Galilee were hos-pitalized with symptoms of acute trichinosis after eating pork derived from game. Consequently,

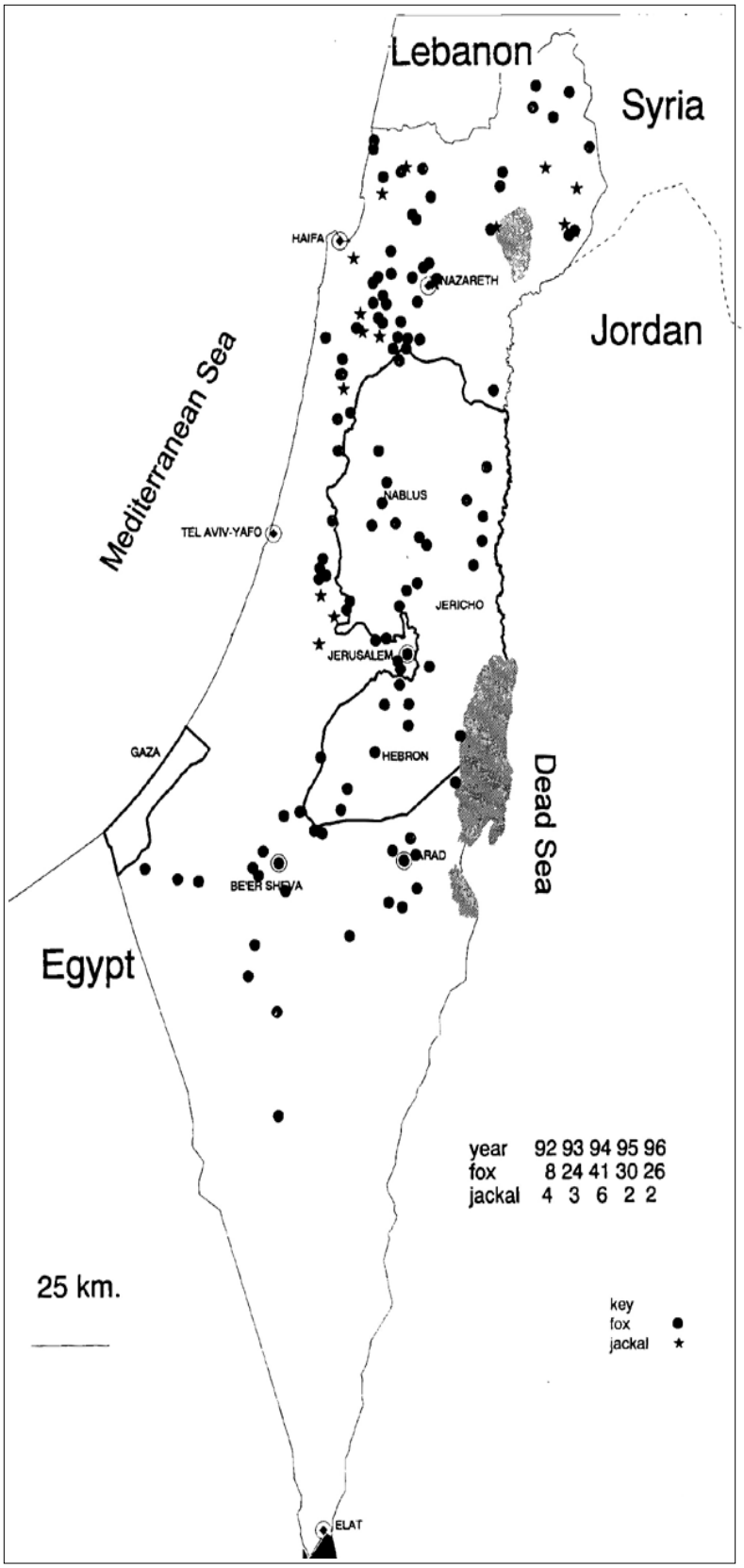


Figure 2. Rabies in fauna in Israel, 1992–1996.

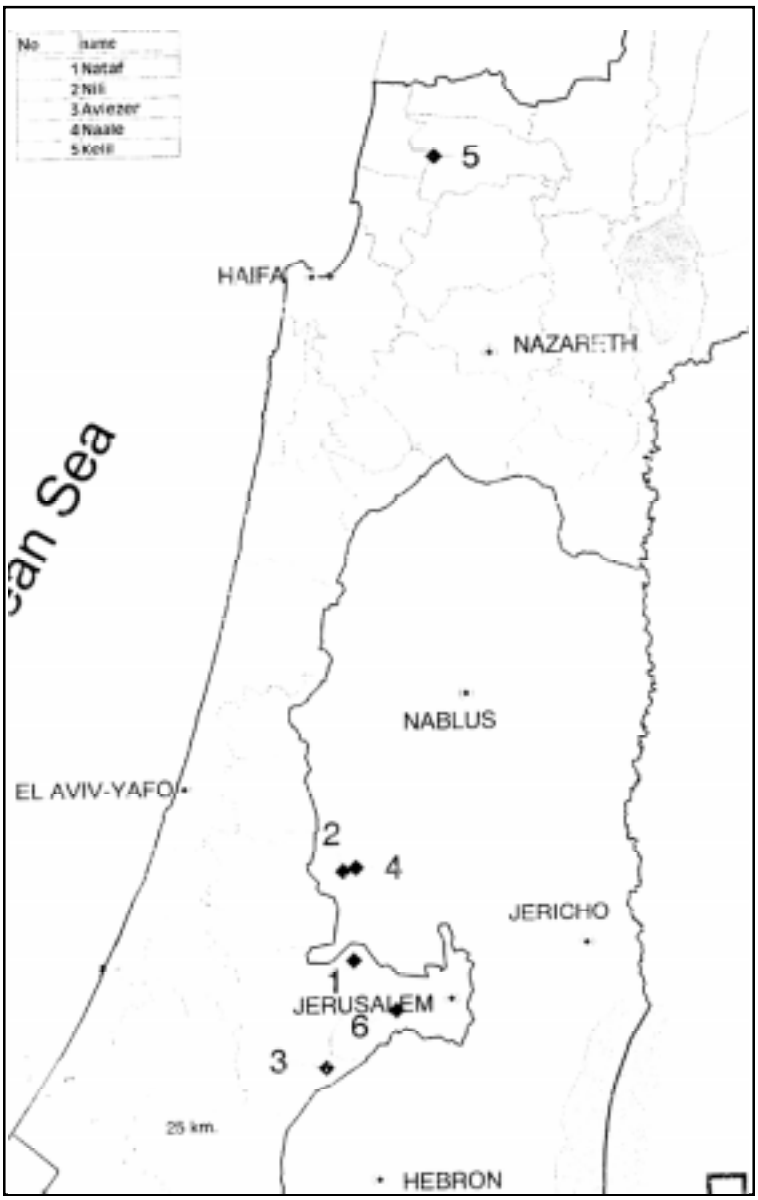


Figure 3. Canine Leishmaniasis in Israel, 1995–1996.

public awareness of the disease grew, and the number of samples from wild boars submitted for laboratory examination increased two- to three-fold from previous years. In May 1992, the first case was found in a wild boar from the Lebanese frontier, followed by 19 additional cases be-tween 1993 and 1996, all in wild boars shot in three areas: (upper Galilee; central and western Galilee, and the Carmel Hills). By October 1996 alone, no fewer than nine cases of trichinosis were diagnosed.[1] Accor-ding to the Animal Diseases Regu-lations, carcasses of animals found infected are condemned; no alter-native measures, such as cooking or freezing, are officially permitted.

Botulism

Botulism is a highly fatal toxemia caused by ingestion of the toxin of *Clostridium botulinum*. There are eight types and subtypes of *Cl. botulinum*, of which types A, B, and E are of most importance in human botulism; types C and D affect farm animals, mainly cattle, horses, chickens, and sheep.

In 1978, a major outbreak of botulism (type D) caused the deaths of many dairy cattle and small ruminants from farms in central and northern Israel (7). Spores of *Cl. botulinum* type D, as well as the toxin itself, spread to many farms from two central animal feed plants by recycled poultry manure that included remains of avian cadavers

[1]Dr. John Wortabet (1827–1908), an Armenian-Lebanese physician who worked in the St. John Hospital in Beirut and lectured in the medical college there during the second half of the 19th century diagnosed trichinosis in humans in south Lebanon during a massive outbreak in the village of El-Chiam in November 1880—more than 115 years ago. His exemplary observations about the outbreak, which involved 262 cases, including six deaths, were published in Lancet (6). A second outbreak, in the Northern Golan, was reported by Dr. Wortabet in Lancet (4 August 1883). In this outbreak, 40 inhabitants of the village Ein-Kinya, were infected after consuming raw pork from a wild boar. However, in this outbreak, no deaths were recorded. His footnote, at the end of the report, is still timely: "From personal observation and experience I have found the use of pork in Syria decidedly unhealthy. The wild boar in winter is a delicacy, but unless previously examined with the microscope, according to German law, or cooked more thoroughly than is usually done, its use cannot be free from the danger of communicating trichinae to man." Data about Dr. Wortabet courtesy of Drs. Mertyn Malkinson and Arieh Sheskin.

and was not properly sterilized. Many farms are suspected to have been contaminated by the spores through the centrally produced and countrywide distributed infected feed. Consequently, annual botulism vaccinations of cattle and of other vulnerable animals with a vaccine, including toxoids of *Cl. botulinum* types C and D adsorbed onto aluminum hydroxide gel, are conducted in most parts of Israel. Sporadic cases involving unvaccinated animals (mainly young cows) occur every year, along with an exceptionally large number of outbreaks, (23) recorded in 1995, found by the Kimron Veterinary Institute to be caused by botulism type D. In all these cases, poultry manure was the suspected source of intoxication.

Although botulism types C and D are not generally regarded pathogenic to humans, it has recently been decided to prohibit the release of meat from ruminants with suspected cases for human consumption.

Echinococcosis

Echinococcosis, also called hydatid disease, is caused in humans by ingestion of eggs of the cestode Echinococcus, of which the most predominant and epidemiologically significant is *Echinococcus granulosus*. The adult tapeworm is carried by dogs and other carnivores. The intermediate hosts in which the cystic forming larval stage develops include various food animals and humans. The infection cycle is maintained by the availability of infected organs to carnivore hosts. Data from slaughterhouses show that the countrywide infection rate per 1,000 animals in 1995 was 0.9 for cattle and 39.9 for sheep (8).

In 1981, human hydatid disease was declared notifiable by the Ministry of Health. During 1991 to 1995, 38 human cases were officially recorded in the country, but the actual number is much higher. For example, El-On et al. (9) conducted epidemiologic studies in 1988 in the town of Yirka, a semirural Druze community of 8,200 persons in northern Israel, and found a cumulative percentage of confirmed present or recent past hydatid infections of 12 (1.6%) of 758, leading to an extrapolated rate of 1,583 per 100,000 persons, which is comparable with that in hydatidosis-endemic areas worldwide. This remarkable situation could be explained by four combined factors: 1) High infection rates in sheep and goats (±10%); 2) high infection rates in owned dogs (14.2%); 3) widespread practice of illegal slaughter of sheep and goats, performed without veterinary inspection and followed by uncontrolled disposal of the infected slaughter offal; and 4) a large number of hunting dogs kept at the homes of many of the Druze people, who traditionally hunt.

The prevalence of hydatid disease in neighboring Moslem villages was lower, mainly because of the smaller number of dogs kept at homes and the general aversion in the Moslem community to any contact with dogs.

Israel's Measures to Prevent the Introduction of BSE

BSE, a fatal neurologic disease of adult cattle first discovered in the United Kingdom in 1986, is one of the transmissible spongiform encephalopathies caused by unconventional agents extremely resistant to heat and chemical treatments.

Israel has adopted the following rigorous steps (taken since the very first announcement of the new disease in the United Kingdom) to prevent the introduction of BSE and to safeguard early detection of eventual occurrence of the disease in the local livestock:

1. State veterinarians, farm animal practitioners, slaughterhouse veterinarians, and cattle breeders associations were notified and updated by a) articles and updates in the monthly Veterinary Bulletins (more than 350 items since December, 1987; b) workshops and lectures, central and regional, some of them with participation of British experts and demonstration of British videotaped clinical BSE cases; circulars, including detailed descriptions of clinical BSE epidemiology and symptoms.

2. The import of meat and bone meals, derived from ruminants from the United Kingdom was banned since December 1988, and from all other countries since July 1990.

3. BSE was declared an officially notifiable disease in 1992.

4. A pathologist from the Kimron Veterinary Institute was trained in contemporary diagnostic techniques at the Central Veterinary Laboratory, Weybridge, United Kingdom (October 1992).

5. Since 1993, bovine brains presented to the Kimron Veterinary Institute after any CNS-related symptoms, are histopathologically examined for BSE changes. During 1993–1996, 583 brains (1993, 72; 1994, 134; 1995, 167; 1996, 210) were examined, all found negative. Most of

the brains were of bovines older than 2 years. This sample size exceeds the number of brains to be examined annually to detect BSE on a country level, as recently recommended by the Office International des Epizootics. The recommendation was to examine a minimum of 50 brains annually if the national cattle population, 209 months of age or older, is up to 500,000 and 91 brains if the cattle population is 1,000,000. The Israeli cattle population of said age group is less than 250,000.

6. In April 1996, an interministerial BSE expert advisory panel was established to recommend new/updated steps for BSE prevention/monitoring.Detailed recommendations were presented in July 1996, most of which are already adopted.

7. The panel paid special attention to the import conditions for meat and offals. This issue is related to the documented presence in Israel, of a considerable population-cluster that may be exceptionally vulnerable to transmissible spongiform encephalopathies. Accumulated scientific evidence indicates that this population, composed mainly of Jewish persons of Libyan and Tunisian origin, demonstrates a genetically linked susceptibility to CJD, probably related to a codon 200 mutation (10). This is expressed by an annual CJD incidence of nearly 100 per million, compared to less than 1 per million in the general population (E. Kahana, pers. comm.). Probable enhanced vulnerability of these persons, if exposed to the BSE agent, cannot be ruled out.

8. Another recommendation referred to adding immunohistochemistry and immunoblotting techniques to the routinely used conservative histopathologic examination of BSE and further increasing the number of examined bovines.

9. CJD was declared a notifiable disease in humans by the Ministry of Health in June 1996.

10. Since August 1996, the feeding of farm animals, including poultry and fish, with meat and bone meals derived from mammals has been officially banned.

11. Imported meals from poultry or fish origin are systematically examined at the port of entry to confirm the obtained written certification regarding the absence of mammalian ingredients.

The emergence of zoonotic diseases in Israel may be due to unique epizootiologic, climatic, and ecologic conditions in the regions as well as specific conditions within the country.

Nomadism, prevalence of disease carriers and their vectors, close proximity of ultramodern farms housing susceptible livestock of exotic breeds or their crosses to traditionally maintained flocks of disease-endemic breeds, and dense overpopulated protected wildlife are the major contributing factors to emerging zoonoses. But the cardinal factors predominant in the Middle East are the need for improved veterinary infrastructure, direct information exchange, and cooperation between the countries of the region.

Out of the seven diseases we reviewed, at least four, brucellosis, rabies, leishmaniasis, and echinococcosis, can be effectively controlled only if cooperation and animal health information systems are established on a regional level.

The assistance of the international community to the establishment of regional veterinary cooperation in the Middle East is of great importance.

Acknowledgements

Thanks to Dr. Van Ham of the Department of Epidemiology, Veterinary Services and Animal Health, Beit Dagan, Israel, for the maps used in this article.

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1978;35:93-9.
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Electronic Media and Emerging Zoonoses

As indicated by the term "emerging," the principal challenge in following emerging diseases is their extremely dynamic epidemiology. Changes in these diseases or the pathogens causing them cannot be followed through standard texts and journals. The traditional mode of reporting an outbreak in a peer-reviewed journal several months after it occurred or in a textbook one or more years later is not appropriate. Weekly publications of the World Health Organization and reports from national ministries of health are more reliable but are still limited by delays in data acquisition, processing, editing, and production. To avoid such delays, some weekly publications are now being distributed electronically.

In response to the need for quick dissemination of information, several electronic sites specifically tailored to information in the fields of infectious diseases, emerging diseases, and zoonoses have become available. Some have subspecialized to a single disease, animal, vector, or pathogen. Most combine zoonotic and nonzoonotic diseases (e.g., mosquito-transmitted diseases) or emerging and nonemerging diseases. Even though they provide quality control, better sites urge users to verify the source of individual reports. Moreover, as the number of electronic sites has grown, data have become more and more diffuse, and users are unable to access and collate materials that appear over time.

Computer programs can facilitate data access by storing and indexing reports according to country, pathogen, vector, and animal reservoir. The University of Tel Aviv has developed a computer software program for information, diagnosis, and disease simulation that incorporates the status of infectious diseases (zoonotic and others) in all countries. This program includes information published in print and electronically. The program can generate a ranked differential diagnosis for rash and fever following a tick bite in Uganda or present the biology, bacteriology, epidemiology, therapy, clinical features, historical background, and current status of tularemia in Norway. The program—Global Infectious Diseases and Epidemiology Network (GIDEON, CY Informatics, Ltd, Ramat Hasharon, Israel)—also contains modules for the identification and characterization of pathogens and the pharmacology and usage of antiinfective drugs and vaccines. The program incorporates 319 infectious diseases; however, even though the GIDEON program is updated through quarterly diskettes, its information is not adequate during disease outbreaks. In this case, therefore, electronic communication is complementing, rather than replacing, traditional communication media.

Various communication media have advantages and deficiencies. Data presented in a textbook, on the Internet, or in a software program may have unlimited scope. In contrast, data presented in journals and government reports are generally narrow, focused, and targeted to a specific readership. Major journals and texts benefit from peer review and documentation of sources. The availability of electronic media and software is limited by access to hardware, user training and sophistication, and in some situations, adequate electricity and telephone services.

Even though electronic communication has become an inevitable and irreplaceable adjunct to the field of emerging diseases, many problems remain in the areas of data access, credibility, standardization, and ease of use.

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The Reemergence of *Aedes aegypti* in Arizona

To the Editor: *Aedes aegypti*, primarily an urban, tropical mosquito, is a competent vector of dengue and yellow fever viruses. In the early 1900s, *Ae. aegypti* was found in every country in the Western Hemisphere except Canada. In the United States, repeated attempts to eradicate it have failed, and the mosquito is now well established in the southern states, from Texas to South Carolina, and more recently in Maryland and New Jersey (1,2).

Although the arid landscape of southern Arizona is an unlikely habitat for *Ae. aegypti*, these mosquitoes were identified in the cities of Tucson between 1931 and 1946 (3,4) and Yuma in 1951 (5). Elsewhere in the western United States, *Ae. aegypti* has been conspicuously absent, except for periodic reports of populations in New Mexico during this same period (3,6).

Beginning in 1969, the Arizona Department of Health Services initiated an arbovirus surveillance program involving state and local officials in routine monthly mosquito sampling between May and October of each year. Until 1994, no *Ae. aegypti* specimens had been identified in Arizona through routine surveillance, which involved adult collection with CO_2 and New Jersey light traps and larval dipping surveys, or through other mosquito research (7,8).

In August 1994, a University of Arizona entomology professor reported finding several *Ae. aegypti* in his Tucson backyard. Followup surveys in September and October 1994 by state and county health officials identified a number of *Ae. aegypti* in that same neighborhood as well as in central Tucson. During September 1995, additional specimens were collected in Nogales and again in Tucson. The adult mosquitoes were collected with CO_2 traps in various locations in these two cities. In Tucson, trapping from four of five sites yielded 85 adult *Ae. aegypti* (8.5/trapnight). In Nogales, trapping from two of four sites yielded 122 adults (12.2/trapnight). Trapping done earlier in 1995 at these sites yielded no *Ae. aegypti* adults.

Between 1994 and 1995, *Ae. aegypti* were trapped exclusively after the monsoon season (late July to early September), when late summer precipitation allowed for sufficient breeding conditions in backyards. However, in late March 1996, the Arizona Department of Health Services responded to a report of "ankle-biting" mosquitoes in central Tucson. Subsequently, two adult *Ae. aegypti* were trapped in the complainant's home. Since then, adult *Ae. aegypti* have been found in several new areas in and around Tucson (0-10/trapnight). Adult specimens have also been found for the first time in the Arizona-Mexico border towns of Douglas (<1/trapnight) and Naco (<1/trapnight; an additional 17 adults were collected by aspiration).

It is not certain whether *Ae. aegypti* mosquitoes were newly introduced in southern Arizona, or if they have been present at low, undetectable levels until favorable weather conditions allowed the population to proliferate. However, trapping data from the last three decades suggest the former.

Oviposition trapping has been the method of choice for *Ae. aegypti* surveillance (9). In Arizona hay infusion-enhanced oviposition traps were used (10). Because of the climate, initial attempts to use oviposition traps for *Ae. aegypti* surveillance were unsuccessful: the containers did not maintain enough water long enough for the hay infusion to attract egg-laying. Future oviposition trapping attempts will use variations on this trap, such as more infusion medium and/or larger containers, and will focus on careful trap placement. These changes may yield a more appropriate *Ae. aegypti* trap for use in the arid deserts of Arizona.

The establishment of populations of *Ae. aegypti* in Arizona is of particular concern to the local health services because of the presence of more than 400 laboratory-confirmed cases of dengue fever in the bordering Mexican state of Sonora in 1996 (R. Navarro Coronado, pers. comm.). While no cases of endemic dengue have been reported in Arizona, two imported cases were identified in 1994. Records show that between 1941 and 1946 nine cases of dengue fever were reported in Arizona's residents, eight of which were from the Tucson and Nogales areas. No exposure or travel history for those cases is available. Imported cases of yellow fever were reported southeast of Tucson in the late nineteenth century. The mere presence of infected patients allowed for possible endemic disease transmission, because of the simultaneous presence of *Ae. aegypti* populations. If, or when, new cases of dengue are identified in Arizona residents, this same predicament will again exist. *Ae. aegypti* surveillance throughout

southern Arizona will be expanded in the coming years, and surveillance will continue for new dengue cases, imported or otherwise.

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To the Editor: The dispatch by Izurieta et al. (Emerg Infect Dis 1997;1:65-8) reporting exudative pharyngitis possibly due to *Corynebacterium pseudodiphtheriticum* was very interesting, especially with the resurgence of diphtheria in the former Soviet Union. However, I was somewhat surprised at the treatment received by the 4-year-old patient whose case is reported. Erythromycin is an effective antibiotic in diphtheria, but it is secondary in importance to diphtheria antitoxin.

The presence of a thick grayish white adherent pseudomembrane, adenopathy and cervical swelling, and low grade fever should certainly provoke a high index of suspicion of diphtheria, especially in a child who has not received pediatric immunization. The diagnosis of diphtheria is primarily made presumptively on clinical grounds and confirmed by the recovery of toxigenic *Corynebacterium diphtheriae* by the laboratory.

Antitoxin treatment cannot wait for laboratory confirmation. Prompt administration of antitoxin is important because diphtheria toxin binds rapidly and irreversibly to tissue sites. Delay in initiating antitoxin treatment is associated with increased incidence of myocarditis, paralysis, and death. Also, it would have been good practice to have placed this child in isolation until the diagnosis was established by the laboratory. The primary care physician in this case is indeed fortunate that the patient did not have diphtheria; the results could have been tragic.

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Reply to P.D. Ellner: We agree that diphtheria antitoxin should be administered promptly on the basis of a presumptive clinical diagnosis of respiratory diphtheria. Because laboratory confirma-tion may be delayed, the decision to treat with antitoxin and the dose of antitoxin must be based on the site and size of the diphtheritic membrane, the degree of toxicity, and the duration of illness (1,2).

Respiratory diphtheria is rare in the United States. From 1980 to 1995, only 41 cases were reported (zero to five cases in any given year) (3). With this low incidence, the likelihood that a patient with membranous pharyngitis has respiratory diphtheria is low. In addition, membranous pharyngitis could be associated with infections by other organisms such as streptococci, Epstein Barr virus, *Candida albicans*, *Borrelia vincenti*, *Herpes simplex* virus, *Arcanobacterium hemoliticum*, nontoxigenic *Corynebacterium diphtheriae*, and *Corynebacterium pseudodiphtheriticum* as in the case we reported (4-10).

The diagnosis and clinical management of exudative pharyngitis with a pseudomembrane in a country where diphtheria is extremely rare

represent a dilemma for the practitioner. In weighing the benefits and risks of diphtheria antitoxin treatment, it is prudent to err on the side of using antitoxin.

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Meeting Summaries

The First International Workshop on Molecular Epidemiology and Evolutionary Genetics of Pathogenic Microorganisms

Under the auspices of the Centers for Disease Control and Prevention (CDC), ORSTOM (the national French agency for scientific research in developing countries), and CNRS (the national French agency for basic research), the First International Workshop on Molecular Epidemiology and Evolutionary Genetics of Pathogenic Microorganisms was held in Atlanta, from June 16 to 19, 1996. The workshop was cosponsored by the National Institutes of Health (NIH), the Burroughs Wellcome Fund, the National Foundation for CDC, Boehringer Mannheim, the French Ministry of Foreign Affairs, and Emory University. Five hundred participants (health care providers, public health professionals, and laboratory scientists) from 25 countries attended the 3-day workshop, whose purpose was to exchange information on the use of molecular tools and approaches in areas of molecular epidemiology and evolutionary genetics in studies of emerging, reemerging, and endemic diseases. The workshop provided an opportunity for CDC, NIH, the World Health Organization, the Walter Reed Army Institute of Research, the Kenya Medical Research Institute, and ORSTOM to present jointly their perspectives on meeting the challenges of emerging infectious disease.

During the workshop, public health and laboratory science-based presentations on parasitic, fungal, bacterial, and viral diseases identified information gaps in the areas of disease and pathogen detection; laboratory-based presentations focused on the use of molecular tools and approaches in pathogen identification and evolution; and other presentations focused on specialized themes, such as the definition of a strain, tools and approaches in molecular epidemiology, emerging infections, concomitant infections, insect disease vectors, opportunistic infections, and tropical parasites.

Many of the challenges of dealing with emerging and reemerging pathogens are common to parasitologists, virologists, bacteriologists, and mycologists. Many pathogens cannot be maintained or propagated in culture or in animal models often because the biology and physiology of these pathogens are not known. This difficulty highlights the advantage of moving directly to molecular probes, polymerase chain reaction amplification, and sequence-based identification for substantiating epidemiologic relationships. Infectious disease clinicians and epidemiologists are faced with whether the disease under investigation is caused by a recently acquired infection or a recrudescent infection and whether an infection is caused by multiple species/strains. In addition, host and pathogen genetic factors that influence susceptibility and pathogenesis and environmental factors that influence transmission of pathogens are critical in the assessment of risk factors for acquiring infections. Molecular approaches to identifying emerging, reemerging, and endemic pathogens were described as most likely to yield the tools needed by epidemiologists to assess the source and risk factors, thus allowing the formulation of needed prevention and control guidelines.

Various approaches and tools now used in detecting pathogens and in studying evolution were examined, and molecular biology and evolutionary genetics applications in the following areas were discussed: 1) diagnosis of known pathogens and development of rapid means to identify unknown pathogens; 2) strain characterization for epidemiologic tracking; 3) ecologic and biologic factors that influence emergence of pathogens; 4) reassessment of taxonomy using molecular biologic data; 5) evaluation of the impact of genetic diversity of microorganisms on vaccine, drug, and insecticide efficacies; 6) gene flow in natural populations of vectors and pathogens; and 7) the role of vectors in the evolution of pathogens. Regardless of the organism under study, a unified approach in evolutionary genetics and population biology was recommended.

Two other topics related to emerging infections were also examined. The first concerned the risk for infection of human recipients of xenogeneic agents through xenotransplantation (and the subsequent transmission of these pathogens to the general population). The second concerned the role of immune activation caused by chronic infections (parasitic and bacterial) and immunization (pneumococcal and influenza) in HIV-infected persons in promoting the replication of HIV and associated progression of disease manifestations. The former is a concern in areas of sub-Saharan Africa and Asia where HIV coexists

with parasitic (e.g., malaria and schistosomiasis) and bacterial (e.g., tuberculosis) infections; the latter is a concern in the United States, where pneumococcal and influenza vaccinations are recommended for HIV-infected persons. Field-based, prospective, and longitudinal studies are needed for a complete picture of the extent of interaction between vaccination and pathogen-induced immune activation, HIV replication, and associated rapid progression to AIDS.

The need for a global partnership to facilitate a more rapid identification of infectious agents in a manner that discriminates among closely related strains and species and uses genetic information to study evolution, emergence, and dispersal of infectious agents was emphasized. To address emerging infectious disease threats, CDC has a strategic plan that emphasizes surveillance and applied research for a strong public health-based defense against infectious disease. A goal of this plan is the integration of laboratory science and epidemiology to develop and use tools to detect and promptly identify emerging and reemerging pathogens and investigate factors that influence their emergence. To promote international collaborations and interaction between clinicians, epidemiologists, and laboratory scientists, CDC, ORSTOM, and CNRS will cosponsor the 2nd International Workshop on Molecular Epidemiology and Evolutionary Genetics of Pathogenic Microorganisms at ORSTOM, Montpellier, France from May 26 to 28, 1997.

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Simian Virus 40 (SV40), a Possible Human Polyomavirus (Workshop Held at NIH)

During the past 4 years, polymerase chain reaction (PCR) assays have detected DNA sequences related to SV40 (an oncogenic simian polyomavirus) in a variety of human tissues, especially choroid plexus tumors, ependymomas, mesotheliomas, and osteosarcomas (1-7). These findings were supported by the isolation of infectious SV40 from a choroid plexus tumor (8). Although another paper reported the failure to detect SV40 DNA in mesotheliomas (9), these studies have reawakened interest in inadvertent human exposure to SV40 in the late 1950s and early 1960s when polio and adenovirus vaccines prepared in rhesus monkey cells containing SV40 were used (10,11). In response to the implications of detecting SV40 DNA in human tumors, the Food and Drug Administration, National Institutes of Health, National Vaccine Program Office, and Centers for Disease Control and Prevention sponsored a workshop on SV40 on January 27-28, 1997 at the National Institutes of Health to examine the possibility that SV40 is an infectious agent in humans.

The workshop first reviewed the biology of SV40 and the human polyomaviruses JC and BK and the data associating SV40 DNA with human tumors. In addition to tumors, SV40 DNA sequences have been detected in human pituitary gland tissue, peripheral blood mononuclear cells, and seminal fluids from healthy persons (3,5,7). Two laboratories were unable to detect SV40 DNA by PCR assays in human tissue, including mesothelioma; researchers noted the ability of the PCR primers used in these assays to amplify DNA sequences from JC and BK viruses as well as from SV40 and discussed whether each set of primers in the PCR reaction requires specific conditions to amplify virus-specific DNA. Furthermore, preliminary data suggested that primers considered to be SV40-specific could, under certain conditions, amplify what appeared to be host DNA sequences. Two laboratories demonstrated that the sensitivity of different PCR primers to detect SV40 DNA was 1-10 to 10-1,000 SV40 genomes. These discussions emphasized the need for caution in interpreting PCR data and the need for standardized, quantitative PCR assay procedures.

National Institute for Biological Standards Control scientists described the use of PCR assays to search for SV40 DNA in current and early lots of polio vaccines and concluded that polio vaccines used in the United Kingdom in 1971 to 1996 did not contain SV40 DNA, while early vaccines prepared in rhesus monkey cells contained easily detectable amounts of SV40 DNA. To evaluate the relationship between exposure to SV40 in the early polio vaccines and the development of tumors (choroid plexus tumors, ependymomas, mesotheliomas, and osteosarcomas), scientists described epidemiologic surveys

that used tumor registries in two countries (two in the United States, one in Sweden). The surveys compared tumor incidence data in persons who could have been exposed to SV40 in polio vaccines with those who, because of their date of birth, could not have been exposed directly; no discernible relationship between exposure to SV40 and development of tumors was found. The surveys also found no association between exposure to SV40-contaminated polio vaccines and the incidence of tumors of the brain and ovaries. These results support the findings of most of the earlier epidemiologic studies (10,11) and help mitigate public health concerns about the use of SV40-contaminated polio vaccines.

Whether SV40 is a human infectious agent that might play a role in human neoplastic disease was also discussed. If SV40 DNA sequences are present in choroid plexus tumors in children born many years after vaccines were SV40 free (8), the possibility that SV40 is present in the population must be considered. Researchers reviewed data on SV40 antibodies in sera taken before 1954 (12) and in sera from persons in remote regions (13,14) not exposed to SV40-contaminated polio vaccines; the data suggest that SV40 might have been present in humans before the polio vaccines were introduced in 1954 (11). Because of cross-reactivity between BK, JC, and SV40 antibodies (15) and the lack of standardized serologic assays to identify SV40 specificity of antibodies present in single samples of human serum, it is difficult to determine whether SV40 was present in humans before the population was exposed to SV40 in the early polio and adenovirus vaccines. Thus, determining whether SV40 is an infectious agent in humans and whether humans were exposed before the polio vaccine was introduced requires further study.

Two preliminary studies showed that SV40 T proteins are expressed in some cells in mesotheliomas and these proteins can bind to both the p53 and Rb cell-cycle control proteins. The SV40 T protein-p53 and SV40 T protein-RB protein interactions are thought to contribute to neoplastic transformation; however, the role of these interactions in SV40-induced neoplastic transformation is unresolved. Further attempts to assess the link between SV40 DNA sequences and neoplastic processes in humans will require more conclusive data about whether SV40 DNA sequences are present in tumors, whether this viral DNA is integrated or extrachromosomal, and whether it is expressed. Two new experimental approaches were suggested to assess the ability of SV40 to contribute to neoplastic development in humans: prospective studies on the presence of SV40 in mothers and other family members of children with choroid plexus tumors and studies comparing the function of what may be an SV40-associated defect in the p53-independent cell-cycle control gene SEN6 in SV40-transformed human cell with cells from tumors containing SV40 DNA sequences.

The sponsors of the workshop were reassured by the independent epidemiologic surveys in the United States and Sweden that the incidence of neoplastic diseases in persons exposed to SV40 in viral vaccines has not increased. However, the sponsors should take the following steps to resolve questions raised by human exposure to SV40 in viral vaccines prepared in rhesus monkey cells in the 1950s and 1960s: 1) Form working groups to a) analyze the sensitivity and specificity of PCR reactions for detecting SV40 DNA in human tissues and develop standardized conditions to ensure confidence in the data generated by such reactions; b) develop methods for assessing the specificity of human polyomavirus neutralizing antibodies in plaque neutralization assays and consider other assays that can measure antibodies to virus-specific epitopes on the virions of polyomavirus; and c) develop ways to search for SV40 in the environment. 2) Encourage additional attempts to isolate SV40 from human tissues and increase the number of completely sequenced SV40 chromosomes obtained from SV40 field isolates. 3) Develop standardized reagents and make them available to laboratories who wish to assess the sensitivity and reliability of their PCR assay for detecting SV40 DNA. 4) Identify reagents such as archived tumor specimens, serum specimens and databases useful for epidemiologic evaluations, and any other specimens critical for evaluating when SV40 or SV40-like viruses entered the population.

For information about transcripts and audio and video recordings of the workshop, contact the Food and Drug Administration Freedom of Information Staff HFI-35, Rm. 12A-16, 5600 Fishers Lane, Rockville, MD 20857; phone: 310-443-1813. The proceedings of the workshop will be published in Developments in Biological Standardization.

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Conference on Foodborne Pathogens: Implications and Control

More than 400 food protection and public health professionals from 18 countries, including microbiologists, epidemiologists, physicians, and health policy makers in industry, academia, and government, attended the Conference on Foodborne Pathogens: Implications and Control. The conference participants examined the response of the food industry and its related public health/food safety regulatory agencies to the emergence of new microbiologic threats and to the reemergence of known pathogens in previously unimplicated foods. The 3-day conference was held in Alexandria, Virginia, USA, March 24-26, 1997. It was organized by the International Life Sciences Institute North American (ILSI N.A.), the Centers for Disease Control and Prevention, the U.S. Department of Agriculture, and the U.S. Food and Drug Administration, in cooperation with the Food and Agriculture Organization and the Pan American Health Organization.

The specific goals of the conference were to identify factors that foster the emergence/reemergence and dissemination of foodborne microbial hazards, explore scientific and food safety strategies to identify and address these hazards, determine future research needs, and review the lessons learned and knowledge gained concerning the emergence and dissemination of food-related microbial threats to health.

The rapid emergence and dissemination of microbial foodborne pathogens and human diseases is affected by factors related to the pathogens themselves, their hosts, and the food production and consumption environment. The conference explored the role of the rapid mutation of foodborne pathogens such as *Escherichia* and *Salmonella*; the increasing numbers of susceptible persons; the effect of current livestock production practices, produce handling and food processing practices, and aquaculture; and changes in consumer lifestyles and food preferences.

Identifying and anticipating new foodborne microbial hazards require concerted efforts. The changing epidemiology of foodborne disease calls for improved surveillance including rapid subtyping methods, cluster identification, and collaborative epidemiologic investigation (including case-control studies). Also examined was the

need for better integrated, coordinated, and standardized animal disease surveillance and health monitoring programs. Several speakers stressed the importance of risk assessment (a component of overall risk analysis that combines science and policy) as a decision support tool and the need to effectively communicate risk to consumers. Because microbes do not respect national borders, they need to be addressed at a global level through strengthened infrastructure and standardized trade that will ensure the health of consumers.

The new problems of foodborne disease require new control and prevention strategies (as well as further research) to ensure that food in both domestic and international trade is safe. The development of the Hazard Analysis Critical Control Point (HACCP) process was presented as a first step toward an analytic process for identifying hazards and their points of control. Other research needs in the area of foodborne pathogen control were also examined. Topics included a need for multidisciplinary teams that can provide "just in time" research; for basic research to explain factors associated with food production and processing that contribute to new foodborne microbial threats; for prompt evaluation and implementation of innovative preservation methods (e.g., food irradiation) to meet consumer demand for fresh foods; for a centralized system accessible electronically, with information on pathogenic organisms in a standardized format; for the use of emerging molecular methods (e.g., DNA hybridization and polymerase chain reaction) to examine emerging viral and parasitic foodborne disease organisms; and for models to predict the probability of a particular microbial event (e.g., growth and death), which may be useful in the design of HACCP programs and in defining processes, formulations, and storage conditions to yield foods with acceptable shelf life and safety characteristics.

Lessons learned from outbreaks in the last 15 years contribute to developing strategies for the mobilization of resources to respond rapidly to emerging foodborne microbial hazards. Retrospective analyses of data from cases of *E. coli* O157 infections identified risk factors, variations in treatment, and estimates of the incidence of hemolytic uremic syndrome. Unusual foods have been associated with outbreaks of *Clostridium botulinum*, including potatoes baked in aluminum foil, bean dip, cheese sauce, and mascarpone cheese; nontoxigenic clostridia could emerge as a new pathogen with the transfer of botulism toxin genes. The multistate outbreak of *Salmonella* serotype *enteritidis* underscored the value of molecular subtyping and public health action based on epidemiologic data in identifying outbreak cases when dispersed in a larger group of unrelated infections. Finally, epidemiologic data were presented from a multistate outbreak of *Cyclospora* infection associated with consumption of raspberries from Guatemala. These examples emphasized that the future of foodborne disease epidemiology will involve new technology and greater coordination among local, state, and federal public health and regulatory agencies.

Papers from this conference will be published in the Emerging Infectious Diseases journal.

International Conference on Emerging Infectious Diseases in the Pacific Rim, Bangkok, Thailand

Approximately 200 participants gathered in Bangkok, Thailand, March 6-8, 1997, to discuss issues related to emerging infectious diseases in the Pacific Rim. The meeting was organized under the auspices of the U.S.-Japan Cooperative Medical Science Program. Scientists from the United States, Japan, the host country, and 15 other nations of the region, as well as from the World Health Organization (WHO) attended. The meeting focused on research topics relevant to emerging diseases and discussed surveillance and disease prevention. Formal presentations focused on themes of special interest to the region: enterohemorrhagic *Escherichia coli* (EHEC), dengue and dengue hemorrhagic fever (DHF), and the growing problem of antimicrobial resistance. Summaries of the WHO global and regional plans to address emerging infectious diseases were presented along with summaries from participating countries of their national plans and problems relevant to these diseases.

The session on EHEC included presentations on the status of this important pathogen in the United States, Japan, Australia, and Thailand, as well as a summary of recent efforts to develop better strategies to detect, treat, and prevent EHEC illness. Presentations on dengue included

a discussion of atypical infections and brief mention of new results regarding the stability of dried whole blood samples for serologic examination and the use of insecticide-impregnated screens to control vector mosquitoes; the prospective clinical study of DHF under way in Bangkok, carried out as a multicenter collaborative study involving Thai, United States, and Japanese scientists, was described. Presentations on nosocomial and community-acquired resistant infections, acute respiratory infections, and tuberculosis underlined the growing problem of antimicrobial resistance.

Several themes emerged from country reports: the growing importance of dengue fever/DHF and Japanese encephalitis in many countries of the region; increasing problems with diarrheal diseases and other food or waterborne diseases, including cholera; antimicrobial resistance and the need for assistance in laboratory culturing and sensitivity testing; the need for regional surveillance to better define the current patterns of antimicrobial resistance and for the establishment of regional quality control and proficiency testing as one aspect of the regional response; frustration with existing surveillance systems and need for assistance in developing improved surveillance tools and easier information sharing; the need for improved laboratory support, especially the regional availability of high quality diagnostic reagents and development of regional reference facilities; and the desire for a regional approach to addressing emerging infectious diseases.

The meeting concluded with recognition of the need for both greater research in the areas of the epidemiology, diagnosis, treatment, and prevention of EHEC and other Shiga-toxin producing organisms; further studies on DHF, including pathogenesis, clinical intervention, viral genetic variability, and genomic analysis; vaccine development; and improved vector control and fundamental strengthening of public health practices to address emerging infectious diseases including improved laboratory capacity, better surveillance programs, easier and more open communications and information sharing, and assistance in outbreak responses. Participants highlighted the need for greater training opportunities for scientists of the region and for development of regional reference facilities and centers of excellence. The meeting did not cover human immunodeficiency virus and AIDS, although there was clear recognition of its importance within the region.

James W. LeDuc
Centers for Disease Control and Prevention,
Atlanta, Georgia, USA

International Conference on Emerging Zoonotic Infectious Diseases, Taipei, Taiwan

The International Conference on Emerging Zoonotic Infectious Diseases, cosponsored by the Taiwan Departments of Health and Defense and the Centers for Disease Control and Prevention, was held March 1-4, 1997 in Taipei, Taiwan. The conference brought together scientists from Australia, France, the United States, and Taiwan and highlighted local work on dengue, Japanese encephalitis, plague, and rodentborne hantaviral infections.

The opening session outlined current efforts in the United States and internationally to improve and coordinate surveillance, laboratory diagnosis, and research of emerging infectious diseases. An example of a disease (yellow fever) whose threat has not been realized was described and reassessed in the context of globalization and other factors favoring and mitigating against the virus' dissemination. Although the possibility of epidemic yellow fever in Asia is small, it is important to reduce the disease at its sources in Africa and South America to further minimize this possibility. Ongoing efforts to elucidate the pathogenesis of dengue hemorrhagic fever, a growing problem in Taiwan and a leading cause of childhood illness and death in Asia and the tropics were summarized. Recent studies in Thai children have defined early clinical immunologic markers that differentiate febrile patients who contract dengue hemorrhagic fever from those with self-limited dengue fever; these findings suggest potential approaches to early recognition and specific intervention.

A session on viral hemorrhagic fevers reviewed recent Ebola virus outbreaks and the

discovery of a rapidly growing number of arena- and hantaviruses, their phylogeny and associations, and their specific rodent hosts. The virtual explosion of viruses identified in rodent reservoirs has left studies of their biologic, clinical, and epidemiologic correlates lagging; many of the newly discovered agents are orphan viruses. A report of local rodent surveys showed the presence of several hantaviruses in numerous species in Taiwan; human disease has not been recognized but epidemiologic studies are planned to define the spectrum and incidence of human infection. Approaches toward producing recombinant hantavirus vaccines and efforts to produce naked DNA vaccines for related vectorborne infections were reviewed.

Summaries of the recent emergence of dengue and dengue hemorrhagic fever globally and on Taiwan led to a series of talks on dengue vaccine development. Various approaches were discussed, including candidate live attenuated vaccines, purified inactivated and recombinant subunit antigens, and infectious clone-derived viruses and their engineered chimeras. A similar session focused on Japanese encephalitis (JE), its changing ecology and epidemiology on Taiwan and regionally in Australia, the molecular taxonomy of JE viruses, and recent developments in producing much needed rapid diagnostic kits. The cellular and molecular basis of JE pathogenesis was addressed in a series of reports on the protective role of *bcl-2* in viral-induced apoptotic death, viral inhibitory activity of cell derived NO_2, and viral genetic determinants of virulence and attenuation. Alternatives to the only internationally accepted JE vaccine, the relatively reactogenic and expensive inactivated mouse brain-derived vaccine, were discussed, including the live-attenuated SA14-14-2 vaccine produced in China, a Vero cell-derived inactivated vaccine under development in Taiwan, and a chimeric JE vaccine engineered upon a yellow fever 17D virus infectious clone.

The final session concerned plague; it described the history and current status of plague globally and on Taiwan; reviewed new developments in the molecular taxonomy of *Yersinia pestis;* compared the performance characteristics of various serologic and PCR-based diagnostic tests; and described plague pathogenesis and vaccine development. F1 and V antigens were defined as important virulence factors in mouse and primate parenteral and aerosol challenge models. Preliminary studies indicate their promise as constituents of a recombinant subunit vaccine.

Theodore Tsai
Centers for Disease Control and Prevention
Ft. Collins, Colorado, USA

The 4th International Conference on Hantaviruses, Atlanta, Georgia March 5-7, 1998

The Centers for Disease Control and Prevention in Atlanta and cosponsors will host the 4th International Conference on Hantaviruses to allow exchange of scientific information on hantaviruses in the areas of epidemiology, clinical management, ecology, molecular biology, laboratory diagnostics, pathogenesis, drugs, and vaccine development.

The meeting will host plenary sessions with invited speakers as well as oral and poster sessions based on accepted abstracts.

Deadline for abstract submission is October 31, 1997. For more information, call 404-639-1510.

International Conference on Emerging Infectious Diseases, Atlanta, Georgia, March 8-12, 1998

Preliminary Information and Call for Abstracts

The Centers for Disease Control and Prevention (and other cosponsors) will convene a conference to 1) encourage the exchange of scientific and public health information on global emerging infectious disease issues, 2) highlight programs and activities that address emerging infectious disease threats, 3) identify program gaps, 4) increase emerging infectious disease awareness in the public health and scientific communities, and 5) enhance partnerships in addressing emerging infectious diseases.

The meeting will host plenary sessions and symposia with invited speakers as well as oral and poster sessions based on accepted abstracts. Major topics will include current work on the surveillance, epidemiology, research, and prevention of emerging infectious diseases as well as on emergency preparedness and response. Abstracts should address new, reemerging, or drug resistant infectious diseases that affect human health, e.g., foodborne, tropical, sexually transmitted, and respiratory diseases; diseases transmitted by animals and arthropods or acquired in health care settings; diseases in infants and children, immunodeficient persons, and minority and other populations at risk; and diseases related to blood safety and xenotransplantation.

Conference attendance will be limited to 2,500 participants. Deadline for abstract submission is October 31, 1997. Proceedings of the conference will be published in the Emerging Infectious Diseases journal.

For additional information on registration and abstract submission send an e-mail message to meetinginfo@asmusa.org or call 202-942-9248.

Emerging Infectious Diseases Laboratory Fellowship Program: Recruitment Begins for a Third Class of Fellows

The Emerging Infectious Diseases Advanced Laboratory Training Fellowship, a 1-year program designed for bachelors or master level scientists, emphasizes the practical application of technologies, methods, and practices related to emerging infectious diseases. Fellows participate in a core curriculum session at the Centers for Disease Control and Prevention (CDC)/Atlanta to gain a general understanding of the public health laboratory system and how it relates to infectious disease surveillance, prevention, research, and control. Fellows are placed within federal and state public health laboratories to receive advanced infectious disease laboratory-related training tailored to each fellow's areas of interest, high-priority laboratory personnel needs, and host laboratory capabilities.

The Emerging Infectious Diseases Post-Doctoral Laboratory Research Fellowship, a 2-year program designed for doctoral level (Ph.D., M.D., D.V.M.) scientists, awards fellowships for the conduct of research or development in infectious diseases areas relevant to public health. This program's fellows also participate in the core curriculum session at CDC/Atlanta and are then placed within federal and state public health laboratories to conduct approved research.

For further information and application materials, contact EID Laboratory Fellowship Program, ASTPHLD, 1211 Connecticut Avenue, N.W., Suite 608, Washington, D.C. 20036, phone: 202-822-5227, fax: 202-887-5098. Fellowship application deadline: June 12, 1997.

Emerging Infections: Clinical and Pathologic Update II

This year, the Armed Forces Institute of Pathology course on infectious disease (November 8-11, 1997) will be held in collaboration with Emory University School of Medicine and the Centers for Disease Control and Prevention. The course, which will be held at the Emory University Conference Center, will be directed by Drs. Ann Marie Nelson and C. Robert Horsburgh, Jr. and will focus on newly emerging and reemerging diseases (including yellow fever, dengue hemorrhagic fever, leptospirosis, AIDS, bovine spongiform encephalopathy, cholera, diphtheria, tuberculosis, *Mycobacterium avium* complex, chancroid, meningitis, *Escherichia coli* O157:H7, fungal infections, malaria, babebiosis, filariasis, and emerging infections in captive wildlife). The course will also cover antibiotic resistance and the role of zoonotic infections and will feature a roundtable discussion of emerging infectious disease issues. The epidemiology and clinical features, as well as the pathology and pathogenesis of each disease, will be presented by experts in emerging infectious disease. An optional slide review session of 10 hours is available the day following the lecture series. (Approximately 30 CME credits)

For further information, contact the Department of Education Services, 14th and Alaska Ave., NW, Washington D.C. 20306-6000;

telephone (202) 782-5021, toll free (800) 577-3749 (U.S. only); fax (202) 782-7164 DSN 662-5021.

Rabies Conference

The 8th Annual Rabies Conference in the Americas will take place in Kingston, Ontario, Canada, November 2-6, 1997. For more information, contact Dr. Christopher Nunan, Ontario Ministry of Natural Resources, 300 Water Street, P.O. Box 7000, Peterbourough, Ontario, Canada, K9J8M5; telephone (705) 775-1554; e-mail: nunanc@gov.on.ca or Dr. Rolly Tinline, Director, GIS Center, Queens University, Kingston, Ontario, Canada, K7L3N6; telephone (613) 545-6039; e-mail: tinliner@qucdn.queensu.ca.

Erratum: Vol. 3, No. 1

In the article "Exudative Pharyngitis Possibly Due to *Corynebacterium pseudodiptheriticum*, a New Challenge in the Differential Diagnosis of Diphtheria," by H.S. Izurieta, P.M. Strebel, T. Youngblood, D.G. Hollis, and T. Popovic on page 68, the following were omitted from the list of references:

45. Feery BJ, Forsell P, Gulasekharam J. Streptococcal sore throat in general practice—a controlled study. Med J Aust 1976;1:898-91.
46. Clarridge JE. When, why and how far should coryneforms be identified? Clin Microbiol Newslett 1986;8:32-4.

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. A Spanish version of the first two volumes is available electronically from the National University of La Plata, Faculty of Veterinary Science, Argentina (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" (*JAMA* 1993:269[17]:2282-6).

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 adaption 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.