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1354 Biologic Agents and Disease Emergence
P. Potter
Industrial hygiene specialists from the National Institute for Occupational Safety and Health (NIOSH) visited hospitals and medical centers throughout Taiwan. They assisted with designing and evaluating ventilation modifications for infection control, developed guidelines for converting hospital rooms into SARS patient isolation rooms, prepared designs for the rapid conversion of a vacated military facility into a SARS screening and observation facility, assessed environmental aspects of dedicated SARS hospitals, and worked in concert with the Taiwanese to develop hospital ventilation guidelines. We describe the environmental findings and observations from this response, including the rapid reconfiguration of medical facilities during a national health emergency, and discuss environmental challenges should SARS or a SARS-like virus emerge again.

The outbreak of severe acute respiratory syndrome (SARS) placed unprecedented demands on healthcare practitioners, healthcare institutions, and public works personnel worldwide. Taiwan reported the third largest number of SARS infections and deaths, followed by Hong Kong and mainland China (1). At the request of the Taiwan Department of Health, the Centers for Disease Control and Prevention (CDC) sent staff to Taiwan that included epidemiologists, infectious disease experts, and environmental and logistical specialists. Industrial hygienists were requested to investigate and help develop guidance for hospitals about patient isolation rooms, personal protective equipment, general infection control, and hospital health and safety.

From April 29, 2003, through June 13, 2003, four industrial hygienists from the National Institute for Occupational Safety and Health (NIOSH) conducted environmental assessments of 32 hospitals and medical centers throughout Taiwan. NIOSH staff were deployed serially (a team of two initially and later, two persons at two different times) within larger teams stationed first in Taipei and then in Kaohsiung. CDC personnel worked together with Taiwanese scientists from the Taiwan Department of Health (DOH), Taiwan Center for Disease Control, National Taiwan University, College of Public Health (NTUCPH), and the Taiwan Institute of Occupational Safety and Health (Taiwan IOSH). During the peak of the SARS epidemic, CDC environmental support focused on immediate steps to isolate SARS patients, protect healthcare workers and other personnel during fever screening and patient care, and provide advice on disinfection, direct contact, and airborne precautions. As the epidemic waned, efforts turned to assessing the implementation of infection control practices, strategies for handling future SARS patients, facility designs for effective patient isolation and fever screening stations, personal protective equipment practices, and training of healthcare workers. Thirty-two hospitals or medical centers that were either accepting and treating SARS patients or were under consideration for use as dedicated SARS treatment facilities in anticipation of a more widely disseminated epidemic were visited.

We describe an uncharacteristic industrial hygiene and public health response that occurred under conditions of a national health emergency. The circumstances, an evolving epidemic occurring in a worldwide atmosphere of tremendous uncertainty, elicited a unique response, which went beyond the traditional industrial hygiene investigative model. The requirement that the response teams deploy to the field on short notice, swiftly conduct multiple site investigations, and provide expedient recommendations on
multiple topics in various healthcare environments, increased the challenges of this assignment. Although environmental findings and observations from hospital site visits and the rapid reconfiguration of medical facilities into dedicated SARS patient facilities are described, they are not necessarily prescriptive. Global health emergencies demand quick action, which was the case for the SARS epidemic. We describe the environmental challenges that could occur should SARS or a SARS-like virus emerge again. Prescriptive environmental guidelines are available elsewhere (2). Comprehensive ventilation engineering or facility evaluations of every hospital or healthcare facility were not possible to obtain.

**Chronology and Methods**

Two staff members arrived April 29, 2003, and met with CDC team members to coordinate response roles. Meetings were arranged with Taiwanese government officials and scientists, including Taiwan DOH, Taiwan Centers for Disease Control, NTUCPH, and Taiwan IOSH. The need was agreed on for controls to isolate SARS patients and ensure protection of healthcare workers and other personnel exposed to patients suspected or known to be infected with SARS. A four-phase environmental approach was developed, which included the following: 1) conduct environmental needs assessments for healthcare facilities; 2) conduct environmental field assessments for healthcare facilities; 3) develop written environmental guidelines for healthcare facilities; and 4) conduct environmental audits of selected healthcare facilities.

**Environmental Needs Assessment for Healthcare Facilities**

In collaboration with Taiwanese scientists, a needs assessment for healthcare facilities was conducted to understand the most important environmental issues for controlling the spread of SARS in hospitals. Additional airborne infection isolation rooms (AIIRs) were needed on a temporary and a permanent basis. Developing environmental guidelines for design and evaluation of such rooms was a priority. Training healthcare workers in appropriate use of personal protective equipment and infection control practices was also an immediate need at the hospital level.

**Environmental Field Assessments of Healthcare Facilities**

Field assessments of healthcare facilities were conducted to better understand standard design and ventilation parameters and how existing AIIRS were configured. The Taiwan Center for Disease Control selected the facilities to represent a cross-section of national, municipal, military, and private hospitals. Environmental field teams typically included NIOSH specialists, a Taiwan Center for Disease Control physician, an occupational health specialist, a Taiwan IOSH engineer, and a doctoral student from NTUCPH. Discussions were held with senior hospital management to understand general hospital configurations, total patient and AIIR capacity, infection control practices, and to determine if SARS patients were being treated at the facility. Ventilation designs were discussed with facilities staff, ventilation drawings were reviewed, and AIIRS, if present, were observed. Discussions with staff during the site visit allowed the team to evaluate environmental issues regarding the expedient addition of isolation rooms, issues affecting infection control, and the selection and use of appropriate personal protective equipment. Table 1 describes the first 10 hospitals and 2 fever clinics that were visited from April 29, 2003, through May 13, 2003, in and around Taipei and Kaoshiung, Taiwan.

**Environmental Guidelines for Healthcare Facilities**

A Taiwan IOSH engineer, 27 Taiwanese public health and ventilation specialists, and a NIOSH industrial hygiene specialists developed Guidelines for the Integrity and Inspection of SARS Isolation Wards. The information was to be used to construct permanent infrastructure and included the appropriate layout of a SARS treatment room and ward, ventilation design for sufficient negative pressure in AIIRs, and filtration and treatment of exhausted air. This document also included measures to enhance infection control and protect healthcare workers during maintenance of the isolation room and ventilation system. The document was written in Chinese and was posted on Web sites of the Taiwan Center for Disease Control and IOSH and distributed to healthcare facilities (http://www.iosh.gov.tw/eversion/sarse.htm). The Taiwan Center for Disease Control also used this document in training sessions for healthcare managers and workers throughout Taiwan.

Because of an immediate need for simple ventilation modifications to reconfigure existing patient rooms to serve as AIIRs, NIOSH staff developed a list of possible modifications to create and confirm negative pressure in typical patient rooms. Applications varied by facility and included increasing exhaust air volume with the addition of assist fans, ways to reduce room leakage and confirm negative pressure, such as using flutter strips on doors or pressure gauges on walls. More complex modifications that used self-contained air exchange, HEPA filtration, and ventilation units were also included.

**Environmental Audits of Selected Healthcare Facilities**

Audits evaluated how well the SARS isolation ward guidelines had been implemented. Hospital managers were contacted, and walkthrough surveys were arranged for
Table 1. Environmental findings from hospitals and medical centers in Taipei, Taiwan*<sup>a</sup>

<table>
<thead>
<tr>
<th>Hospitals</th>
<th>IRs</th>
<th>Single pass</th>
<th>HEPA</th>
<th>UV</th>
<th>Pressure monitors</th>
<th>BP review</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Medical center (largest healthcare facility category in Taiwan). Affiliated with Taiwan University School of Public Health. Two visits made by CDC team.</td>
</tr>
<tr>
<td></td>
<td>27&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Two visits made by CDC team. Three IRs were constructed within 1 week for the ER.</td>
</tr>
<tr>
<td>T-2</td>
<td>9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>No IRs. Instead windows in SARS patient rooms kept open. Suggested closing windows and adjusting thermostat and fan settings in patient rooms to increase negative pressure.</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Suburban hospital, scheduled to receive SARS patients.</td>
</tr>
<tr>
<td>T-3</td>
<td>0</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Suburban hospital where non-SARS patients from Ho-Ping Hospital (facility closed during the SARS outbreak) would be transferred.</td>
</tr>
<tr>
<td>T-4</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Rural hospital approximately 2 hours from Taipei City.</td>
</tr>
<tr>
<td>T-5</td>
<td>108–120&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Formerly closed military hospital, this facility was under conversion to designated SARS hospital.</td>
</tr>
<tr>
<td>T-6</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Medical center and only private hospital of the group visited. The newly installed single-pass ventilation system with HEPA/UV filtration was excellent.</td>
</tr>
<tr>
<td>T-7</td>
<td>10&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Under construction in paved parking areas adjacent to the hospital.</td>
</tr>
<tr>
<td>T-8</td>
<td>56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Under conversion to a designated SARS hospital.</td>
</tr>
<tr>
<td>T-9</td>
<td>77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Under conversion to a designated SARS hospital.</td>
</tr>
<tr>
<td>T-10</td>
<td>77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Under conversion to a designated SARS hospital.</td>
</tr>
<tr>
<td>Fever clinics</td>
<td>*</td>
<td>Y</td>
<td>*</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Under construction in paved parking areas adjacent to the hospital.</td>
</tr>
</tbody>
</table>

*All hospitals listed strongly suggested (or required) wearing filtering face-piece respirators when entering the hospital. Persons entering these facilities were screened for fever before entering the facility (using infrared skin or tympanic membrane sensors) and dispensed sanitizing gels or disposable hand-cleaning wipes.

<sup>a</sup>IRs, isolation rooms; AHU, air handling units; HEPA, high efficiency particle aerosol; UV, ultraviolet germicidal irradiation lamps; BP, blue print or engineering designs plans available for review; Y, yes; N, no; CDC, Centers for Disease Control and Prevention; SARS, severe acute respiratory syndrome.

<sup>b</sup>IRs available during initial visit (numbers include IRs in all areas of the hospital).

<sup>c</sup>IRs planned for completion (within weeks).

<sup>d</sup>IRs planned ranged from 2 to 6 per location. Ventilation in IRs ranged from simple (standard bathroom exhaust fans, without HEPA or UV treatment of exhaust air) to well-designed single-pass exhaust air systems with HEPA/UV treatment of exhaust air. No IRs present at time of visit. Hospital used standard patient rooms for SARS patients, providing 100% supply air, with exterior windows remaining open. Pressure differentials between patient rooms and remainder of floor where minimal hospital evaluation not possible. Determination made on the number or adequacy of IRs available. The NIOSH/CDC team recommended that this hospital not be used for SARS patients based on lack of information regarding ventilation system. Fever clinics included tented areas or small buildings (generally under construction) outside hospital ERs used to screen for fever and other symptoms to identify possible SARS-infected patients before entering the hospital.

investigators. The general objectives were to determine the adequacy of AIIRs, provide guidance for construction or conversion of existing patient rooms into AIIRs, provide technical assistance on ventilation and other controls (e.g., isolation, procedures, and training), evaluate appropriate use of personal protective equipment, and provide guidance on various environmental considerations for infection control.

Kaohsiung County, Tainan, and Chai-Yi

Twelve hospitals and medical centers in southern Taiwan were visited May 17–25, 2003. Meetings were held with facilities and ventilation engineers, hospital administrators, and medical and nursing staff to understand the number, type, and location of AIIRs. Mechanical drawings, blueprints, and ventilation testing reports were reviewed, and walkthrough surveys were conducted. Visible smoke testing was performed to evaluate pressurization between nurses’ stations and SARS patients’ wards and for as many individual patient rooms as possible. When possible, air handling units, HEPA filters, outside air intakes, and rooftop exhaust ductwork were inspected. Infection control procedures and personal protective equipment use were reviewed, including availability, staff knowledge regarding proper use, and implementation.

Standard versus simple negative-pressure isolation rooms were observed. Typically, standard isolation rooms had an anteroom for use by healthcare workers to put on and take off personal protective equipment, a digital or analog pressure manometer mounted outside the door, hard rather than suspended ceilings, and walls that extended floor-to-ceiling. Headwall and utility penetrations were sealed to reduce leakage, maintain negative pressure, and control airflow. Constant volume air-handling units were commonly used and configured to operate in the single pass mode. Most air-handling units could be tested and balanced to maintain negative pressure of 2.5 to 20 pascal (0.008–0.08 inches of water). Exhaust for standard AIIRs

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<sup>b</sup>IRs, isolation rooms; AHU, air handling units; HEPA, high efficiency particle aerosol; UV, ultraviolet germicidal irradiation lamps; BP, blue print or engineering designs plans available for review; Y, yes; N, no; CDC, Centers for Disease Control and Prevention; SARS, severe acute respiratory syndrome.

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were often configured with HEPA filtration. Some facilities installed ultraviolet germicidal irradiation (UVGI) lamps in HEPA filter banks, although the efficacy of using UVGI for control of SARS-associated coronavirus was uncertain.

Most of the simple isolation rooms had been designed for isolating patients with infectious diseases such as scabies. These rooms lacked an anteroom, generally had window-mounted air-conditioning units, and small (1,000–2,000 ft³/min) window-mounted vane axial fans for negative pressure. Wall penetrations were typically not sealed, manometers were generally not present, and ceilings were not hard. Unfiltered room air was exhausted outdoors through windows.

Aggressive infection control measures were evident in all hospitals. Hand sanitation stations with automated dispensers were abundant, especially on nurses’ wards, at elevator landings, and at every hospital entrance. Infection control staff dispensed sanitizing gels and cloth or disposable hand-cleaning wipes. Infection control personnel were also stationed at hospital entrances and screened for fever by measuring forehead skin temperatures. One hospital installed forward-looking infrared scanning cameras that displayed temperatures on TV monitors next to nurses’ stations. Visitors or staff with fever were denied entrance and sent to fever clinics outside the hospital for medical follow-up. Healthcare workers, hospital staff, and visitors all wore filtering face-piece surgical masks or respirators of varying brands and efficiencies (N95 to N100). Most hospitals cordoned a gurney pathway from ambulance entrances to an elevator landing, where a designated and preprogrammed elevator transported SARS patients to SARS wards. When meetings were conducted, all participants wore respirators or masks, and handshaking was minimized or discouraged.

Appropriate protective equipment (including eye protection, protective suits, aprons, gloves, head and foot coverings, and respirators) was widely available and healthcare workers were knowledgeable about their use. In many nurses’ stations, posters describing standard operating procedures for SARS protective equipment were present. Environmental findings were discussed at a closing meeting, and written reports were later sent to each hospital. Table 2 summarizes general environmental aspects of 10 hospitals investigated in southern Taiwan and provides examples of recommendations provided to these hospitals.

### Kaohsiung SARS Screening and Observation Facilities

Two proposals for the construction of specialized SARS screening and isolation facilities in southern Taiwan were reviewed by NIOSH and Taiwanese environmental specialists. One proposal considered configuring arrays of shipping containers (widely available in this port city) into

<table>
<thead>
<tr>
<th>Hospital</th>
<th>IRs</th>
<th>Single pass</th>
<th>HEPA</th>
<th>UV</th>
<th>Pressure monitors</th>
<th>BP review</th>
<th>Negative pressure in IRs?</th>
<th>Recommendations and notes</th>
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<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>S</td>
<td>Y</td>
<td>N</td>
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<td>N</td>
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<td>4</td>
<td>S</td>
<td>Y</td>
<td>N</td>
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<td>3</td>
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<td>2</td>
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<td>20</td>
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<td>Y</td>
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<td>J</td>
<td>8</td>
<td>S</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td></td>
</tr>
</tbody>
</table>

*IR: isolation rooms; AHU: air handling units; UV: ultraviolet germicidal irradiation; BP: blueprint or engineering designs plans available for review; S: standard isolation room; s: simple isolation room; Y: yes; N: no.

*Pressure monitors were either digital or analog manometers installed outside patient room.

*Repair collapsed rooftop exhaust stack.

*Inspect isolation rooms for leakage; four rooms not negatively pressurized.

*Ensure outdoor air intakes are not in proximity to exhaust fans for simple isolation rooms.

*32% isolation rooms were not negatively pressurized, no ICU, not recommended for SARS patients.


*Ventilation system needs balancing/modification (negative pressure varied from –1.4 to –22 pascal). Extend rooftop exhaust stacks, establish standard operating procedures for personal protective equipment use, require handwashing for all hospital contractors.

*Modify 2-way switches in simple isolation rooms so that fans cannot operate in reverse, replace wooden doorknobs with metal on SARS patient ward.

*Modify 2-way switches in simple isolation rooms so that fans cannot operate in reverse, position patient beds with head of bed near source of room exhaust for increased isolation, seal wall, window, and ceiling penetrations in simple isolation rooms.

*Modify 2-way switches in simple isolation rooms so that fans cannot operate in reverse, seal windows in simple isolation rooms to enhance negative pressure.
patient AIIRs linked in hub-and-spoke fashion by a central nurses’ station. This proposal was not recommended because of uncontrolled solar loads on the containers and feasibility issues for patient emergency medical treatment procedures. The second proposal was to convert vacated military barracks into a SARS patient–screening facility. An ambitious timeline required converting an open bay barracks into 20 individual patient isolation rooms and ultimately converting another barracks of similar design for a total of 40 beds. Demolition and reconstruction were to be completed and a functional facility available within 48 hours. A meeting was held May 22, 2003, to tour the site, sketch a preliminary design, and provide verbal recommendations for a proposed redesign of the open bays into an 18-bed facility, configured into simple patient isolation rooms. Demolition began immediately. The responders provided a detailed guidance document the following day, which outlined the following areas: facility design, construction, and renovation specifications for patient rooms; ventilation specifications, including hood designs to improve axial exhaust fans; fire, safety, and environmental guidelines; infection control practices for patients and healthcare workers; placement of sanitary facilities; and patient and staff traffic flow through the facility.

This facility was dedicated on May 28, 2003, substantially increasing the number of AIIRs available in southern Taiwan. The facility could also function as a quarantine station if needed. The floor plan of this converted barracks building is shown in Figure 1.

Kaohsiung County, Taidong, Taichung, Hualien, and Taipei

The Taiwan DOH issued a directive on April 23, 2003, mandating the establishment of 11 dedicated SARS hospitals and medical centers geographically dispersed throughout Taiwan (Table 3). General care hospitals were directed to treat mild SARS patients (those not on ventilators and with substantial pulmonary capacity) and patients undergoing rehabilitation; patients who needed ventilation were sent to medical centers with intensive care units. SARS-designated hospitals ceased providing general patient care and began complying with the AIIR construction schedule, including reengineering or installation of new ventilation equipment. After the design phase, hospitals completed the conversion (start of construction to patient acceptance) on an average of 13 days, resulting in a total of 698 negative-pressure AIIRs constructed by the completion of the nationwide project.

From May 31 through June 10, 2003, a total of 11 dedicated SARS hospitals were evaluated to assess AIIRs and wards, infection control practices, healthcare worker and patient entrance and egress pathways, protective equipment practices, and healthcare worker training (Figure 2).

As part of the arrangement by the Taiwan Center for Disease Control for the hospital site visits, hospital management were given a questionnaire that requested design criteria, number of isolation rooms, personal protective equipment requirements, and ventilation specifications. Evaluations began with an opening conference with administrative, engineering, infection control, and healthcare worker staff. Hospital objectives and the status of the hospital modifications were discussed, blueprints were reviewed, ventilation system and AIIR design were discussed, healthcare worker training was reviewed, and the personal protective equipment protocol was observed. A walkthrough of the patient and healthcare worker pathway was conducted, including an inspection of the various isolation gradients (nurses’ stations, change rooms, isolation ward corridor, anterooms, and AIIRs). Visible smoke was used to evaluate the pressurization of AIIRs and to assess airflow patterns both within the IR and the ventilation zones and the ventilation system (supply air location, exhaust discharge, HEPA filters, and UVGI) was inspected.
Maintenance practices, establishment of an infection control department, location of hand-washing stations, room pressure monitors, and availability of personal protective equipment were reviewed. Closing meetings were held with hospital management and healthcare worker staff to review findings and recommendations and followed up with written reports.

The Taiwan Industrial Technology Research Institute, Taiwan IOSH, and Taiwan Center for Disease Control provided general facility design specifications for these hospitals and other general care facilities treating SARS patients. Most hospitals contracted with architectural and engineering firms to manage design and reconstruction. A national hospital steering committee was formed to guide this effort. Design criteria included specifications from existing CDC tuberculosis guidelines (3), the American Society of Heating, Refrigerating and Air-Conditioning Engineers Design Manual for Hospitals and Clinics (4), and the American Institute of Architects Guidelines for Hospitals and Health Care Facilities (5).

Massive infrastructure modifications were necessary to achieve the desired objectives of increased capacity to triage and treat seriously ill patients and at the same time protect healthcare workers. Examples include the reconfiguration of the entrance and egress corridors for healthcare workers and patients to ensure complete physical and airflow separation, establish multistep, interlocked ventilation zones for healthcare workers to put on the required personal protective equipment, as well as a similarly tiered degowning procedures with final shower-out. Patients were received in a designated buffer area (in some cases containing automatic-spray cleaning systems to sanitize between patients) with dedicated elevators and corridors for patient flow. All negative-pressure isolation rooms were designed with single-pass (100% outside air with no recirculation) dedicated exhaust systems. Exhaust air in all hospitals was treated with HEPA filtration and UVGI before discharge. Visual indicators at the IR door and a remote indicator panel in the nurses’ station monitored isolation room pressure. Isolation design included a pressure gradient from the clean (e.g., nurses’ station or change room) to the less clean (patient room), including buffer zones to achieve the desired conditions (Figure 3). Designated laundry and medical waste corridors were established, and aggressive cleaning regimens were implemented to ensure frequent sanitation of all areas, including twice daily cleaning of patient rooms and autoclaving of waste before its removal from the facility. Personal protective equipment requirements varied somewhat among hospitals, but typically healthcare workers wore an N95 or

Table 3. Designated SARS Hospitals, Taiwan

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Hospital type</th>
<th>Location</th>
<th>Start 2003</th>
<th>Completion 2003</th>
<th>Days</th>
<th>No. isolation rooms</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSH-1</td>
<td>Referral</td>
<td>Northern Taiwan</td>
<td>5/8</td>
<td>5/19</td>
<td>11</td>
<td>102 Patient rooms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9 ICU beds</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 Operation room</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 Dialysis room</td>
</tr>
<tr>
<td>DSH-2</td>
<td>General care</td>
<td>Northern Taiwan</td>
<td>5/7</td>
<td>5/20</td>
<td>13</td>
<td>92</td>
</tr>
<tr>
<td>DSH-3</td>
<td>General care</td>
<td>Northern Taiwan</td>
<td>5/28</td>
<td>6/30</td>
<td>32</td>
<td>77 Patient rooms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(119 beds)</td>
</tr>
<tr>
<td>DSH-4</td>
<td>Referral</td>
<td>Central Taiwan</td>
<td>5/21</td>
<td>6/6</td>
<td>15</td>
<td>40 Patient rooms</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 ICU beds</td>
</tr>
<tr>
<td>DSH-5</td>
<td>General care</td>
<td>Central Taiwan</td>
<td>5/24</td>
<td>6/7</td>
<td>13</td>
<td>42</td>
</tr>
<tr>
<td>DSH-6</td>
<td>General care</td>
<td>Southern Taiwan</td>
<td>5/24</td>
<td>6/3</td>
<td>9</td>
<td>53</td>
</tr>
<tr>
<td>DSH-7</td>
<td>General care</td>
<td>Southern Taiwan</td>
<td>5/23</td>
<td>6/1</td>
<td>8</td>
<td>83</td>
</tr>
<tr>
<td>DSH-8</td>
<td>Referral</td>
<td>Southern Taiwan</td>
<td>5/22</td>
<td>6/4</td>
<td>12</td>
<td>72 Patient rooms</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>(6 ICU beds)</td>
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<td></td>
<td></td>
<td></td>
<td>1 Operation room</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>1 Dialysis room</td>
</tr>
<tr>
<td>DSH-9</td>
<td>General care</td>
<td>Southern Taiwan</td>
<td>5/23</td>
<td>6/6</td>
<td>13</td>
<td>77</td>
</tr>
<tr>
<td>DSH-10</td>
<td>General care</td>
<td>Eastern Taiwan</td>
<td>5/27</td>
<td>6/1</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>DSH-11</td>
<td>General care</td>
<td>Eastern Taiwan</td>
<td>5/27</td>
<td>6/1</td>
<td>4</td>
<td>32</td>
</tr>
</tbody>
</table>

SARS, severe acute respiratory syndrome; DSH, dedicated SARS hospital; ICU, intensive care unit.

Figure 2. Hospital worker in full personal protective equipment disinfects ambulance and hospital at Song Shan Hospital after delivery of a suspected severe acute respiratory syndrome patient, June 2003. Photograph courtesy of Max Kiefer, Centers for Disease Control and Prevention, Atlanta, GA.
N100 respirator, protective suit, double or triple disposable gloves, shoe covers, outer gown, hair cover or hood, and face shield, goggles, or both (Figure 4). To help healthcare workers alleviate heat stress from the encumbering personal protective equipment, work shifts in some hospitals were reduced to 3 or 4 hours. All hospitals had an established infection control department and an infection control plan. A summary of the key design features of the 11 SARS-dedicated hospitals is listed in Table 4.

Although the major reconfiguration design goals were the same for all hospitals, structural realities and other practical considerations resulted in differences in final configuration. Design changes during construction were also necessary to overcome unforeseen engineering obstacles. Examples of differences include the following: 2 of 11 hospitals did not have elevator capability for healthcare workers to access isolation wards, 5 of 11 hospitals did not have positive-pressure nurses’ stations in the isolation ward, not all hospitals had anterooms, and 3 of 11 hospitals did not have positive-pressure nurses’ stations in the isolation ward, not all hospitals had anterooms, and 3 of 11 hospitals did not have complete patient and healthcare worker pathway separation. Other differences included the number of UVGI units in the ventilation system, whether the UVGI was located in front of or behind the HEPA or pre-filter, the type (electronic or mechanical) and location of room pressure monitors, the redundancy strategy (dual exhaust, dual filter, or both), and whether the ventilation system received testing and balancing after installation.

Conclusions

From a facility, personnel, environmental and occupational health perspective, the response to SARS in Taiwan had a profound impact on the healthcare system of the nation. The Taiwanese government responded in a swift and comprehensive manner to contain the outbreak.

Although major gaps in knowledge existed regarding this first emerged infectious disease of the 21st century, decisions involving massive resource commitments had to be made quickly and decisively. Hospitals and medical centers islandwide renovated their facilities rapidly or constructed new patient treatment facilities to contain and treat known or suspected SARS patients. Healthcare workers learned to use personal protective equipment in a far more judicious and extensive manner than they were accustomed to. Large-scale retraining and reassignment of thousands of healthcare personnel was also required.

When SARS or a SARS-like pandemic recurs, industrial hygiene specialists will be faced with similar circumstances and should anticipate that they will be forced to respond in a highly charged environment of considerable scientific uncertainty. While the standard industrial hygiene rubrics of anticipation, recognition, evaluation, and control remain useful, more inventive approaches to risk and hazard assessment will be necessary and will test responders’ capabilities and tenacity. Some of the challenges these environmental specialists encountered during the SARS response included the following: developing expedient guidelines for engineering and administrative controls for workers and workplaces; developing personal protective equipment use guidelines for healthcare workers and the general public, including questions regarding the feasibility of disinfection and reuse and of disposable respirators; developing and providing training on personal protective equipment use by workers, especially healthcare workers; evaluating hospital isolation rooms and ventilation systems, including containment of window air-conditioner condensate from SARS patient rooms; working with hospital infection control and facilities personnel to develop alternative triage facilities, such as fever screening clinics; advising facilities design personnel on hospital

![Figure 3. Hospital workers in Kaohsiung, Taiwan, listen to a summary of findings from walkthrough survey and pressurization testing on a severe acute respiratory syndrome patient ward.](image3)

![Figure 4. A Center for Disease Control Taiwan investigator is screened for fever before entering a healthcare facility in Kaohsiung.](image4)
reconfiguration to improve patient transport and patient isolation; developing, applying, and interpreting results from unvalidated and novel environmental sampling techniques; creating effective risk communication tools for workers and the general public; advising local officials on issues of isolation, quarantine, and other public safety concerns, including obtuse inquiries such as the utility of disinfecting septic systems contaminated with SARS-CoV, and evaluating the feasibility of novel patient containment and treatment facilities, and the use of unproven, yet theoretically reasonable control technologies in an emergency situation.

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We thank Daniel B. Jernigan for his excellent leadership and guidance of the CDC team during the initial weeks of the Taiwan SARS response; Susan A. Maloney for her timely advice and support of the hospital evaluation project; Ta-Yuan Chang for his role in the initial visits to hospitals in and around Taipei, Taiwan, as well as the future training of others to perform environmental sampling for the SARS virus; Chang-Chuan Chan for facilitating the environmental evaluation of healthcare facilities and his scientific input to the development of the four-phased approach described in this article; William Wong, Peter B. Bloland, and Sarah Y. Park for their contributions, leadership, and support; Howard W.-H. Hsiao for his support conducting hospital site visits, with translations in the field, and for his overall sense of equanimity; Frank Chin-Hsun Su for his enthusiasm, support, and energy during the design and construction of the Kaohsiung SARS Screening and Observation Facility; and Ching-Chu Chiang for his outstanding support and kindness.

Mr. Esswein is a commissioned officer in the U.S. Public Health Service assigned as a senior industrial hygienist with the Denver Field Office, NIOSH, CDC. His research interests include indoor environmental quality, occupational hygiene in developing countries, and development of dermal exposure assessment and removal techniques for the presence of lead.

References


Address for correspondence: Eric J. Esswein, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Denver Field Office, Denver, CO 80225-0226, USA; fax: 303-236-6072; email: eje1@cdc.gov
In the past decade, fluctuations in numbers of imported malaria cases have been seen in Canada. In 1997 to 1998, malaria case numbers more than doubled before returning to normal. This increase was not seen in any other industrialized country. The Canadian federal malaria surveillance system collects insufficient data to interpret these fluctuations. Using local (sentinel), provincial, federal, and international malaria surveillance data, we evaluate and interpret these fluctuations. Several epidemics are described. With an ever-increasing immigrant and refugee population of tropical origin, improved surveillance will be necessary to guide public health prevention policy and practice. The Canadian experience is likely to be generalizable to other industrialized countries where malaria is a reportable disease within a passive surveillance system.

Malaria has been a reportable communicable disease in Canada since 1929, when a surveillance system for communicable diseases was first developed. Although no longer endemic in Canada, malaria has remained an important imported disease, principally in immigrants and travelers (1–3). Rarely, it has been transmitted in blood products (4). Published reports document delays in clinical and laboratory diagnoses of malaria and lack of understanding of malaria prophylaxis and fever management in travelers (3). The Canadian infectious disease surveillance system has reported an average of 538 malaria cases per year since 1990, and Statistics Canada reported an average of one death per year (5,6, Carole Scott [Division of Disease Surveillance, Health Canada], pers. comm.). The present federal surveillance system reports the age and sex of a patient and does not document malaria death rate, malaria species, nor the likely country of acquisition. While malaria-related deaths may be few, that any exist is a matter of concern. The continued incidence of malaria cases and deaths in Canada suggests that the malaria surveillance system should be strengthened and used more proactively to help identify appropriate preventive measures.

All 10 provincial and 3 territorial health authorities in Canada are required by law to report diagnoses of malaria and other selected diseases to federal authorities at Health Canada (2). Summary reports of these diseases are published by both levels of reporting in provincial and territorial news bulletins and by Health Canada in the Canada Communicable Diseases Report. In several instances over the past decade, malaria incidence in Canada as a whole, or in individual provinces, reached epidemic levels (7). Why some were not immediately identified and why no comprehensive analysis has been published as part of government surveillance systems are questions that will be addressed. Failing to recognize these epidemics has limited the ability of public health officials to assess and intervene appropriately to control the illness and death associated with imported malaria in Canada.

This study evaluated and summarized data collected over the past 22 years by local, provincial, and federal malaria surveillance systems, from Canadian federal immigration and refugee data resources and from international tourist resources, to identify and explore the causes of malaria epidemics. In addition, geographic patterns and Plasmodium spp. profiles of malaria are examined. This analysis led us to conclude that changes are needed in both the surveillance reporting instruments and how these surveillance results are analyzed and used.

Methods

The databases used for the present analysis include 22 years of records from a local malaria reference center in Montreal, Canada (the McGill University Centre for Tropical Diseases [TDC]) and up to 13 years of quality assurance and notifiable disease surveillance databases of the provincial and federal governments of Canada, France, India, Switzerland, the United States, and the United Kingdom. TDC is a clinical and laboratory facility that...
provides care to 800 to 1,100 new patients per year (approximately 60% primary cases and 40% consult cases), drawn mainly from the Montreal region. The TDC database from 1981 to 2002 has allowed previous detailed reviews of changing patterns of malaria in its patient populations (8–10). Malaria-relevant data captured include category of traveler (tourist, immigrant, refugee, expatriate, missionary, and volunteer), countries visited, and malaria species. A diagnosis of malaria is made if parasites are noted on a blood smear (thin, thick, or buffy coat) or if, in the last 5 years, the patient had a positive result on a malaria antigen-capture test (e.g., Macromed [Nova Century Scientific, Inc., Burlington, Ontario, Canada], ICT Malaria P.f. [ICT Diagnostics, Brookvale, New South Wales, Australia], or OptiMAL [Flow Inc., Portland, OR]). While active surveillance studies during this period included polymerase chain reaction (PCR) as a screening tool, PCR-positive cases were not included in any of the passive surveillance statistics unless they were also independently confirmed by either malaria antigen capture or smear.

Provincial reportable disease databases have included, in the past 10 years, patient characteristics such as age, sex, and malaria species, but not the likely country of acquisition. Because 90% of all malaria cases in Canada were reported by the Provinces of British Columbia (Monica Naus [British Columbia Centre for Disease Control], pers. comm.), Ontario (Lorraine Schiedel [Ontario Ministry of Health and Long-Term Care], pers. comm.), and Québec (Colette Colin [Ministère de la santé et des services sociaux, Québec], pers. comm.), the present analysis focuses on their data, primarily for 1990–2002 (11). Quality assurance data for the province of Québec (1994–2002) were provided by TDC and the Laboratoire de Santé Publique du Québec. The federal government’s notifiable disease database from 1990 to 2002 is a compilation of selected information from individual provincial databases and includes patient age and sex for each report but no malaria species or country of acquisition (Carole Scott [Division of Disease Surveillance, Health Canada], pers. comm.).

International malaria surveillance data (1990–2002) were acquired from the World Health Organization (WHO) Regional Office for South East Asia (Rakish Mani Rastogi, pers. comm.), the WHO Regional Office for Europe (12), and the United States (13–24). Malaria rates for all countries were based on population data of the U.S. Census Bureau (25).

Trends in Canadian immigration and refugee data for the years 1990–2002 were provided by Citizen and Immigration, Canada (Karen Tremblett [Medical Services Branch, Citizen and Immigration Canada], pers. comm.), data on language by Statistics Canada (26), and travel patterns of Canadians to the tropics by the World Tourism Organization, Madrid (27).

Results

TDC Database

Overall, 553 clinical cases of malaria were seen at TDC from 1981 to 2002, with some fluctuation over time but an overall gradual increase (Figure 1). In these 553 cases, 562 microscopy diagnoses were made; *Plasmodium falciparum* 295 (52%), *P. vivax* 218 (39%), *P. ovale* 26 (5%), *P. malariae* 16 (3%), and unknown species 7 (1%). Nine (2%) of the clinical cases were mixed infections, involving *P. falciparum* with either *P. malariae* or *P. vivax*. Seven patients were seen two or three times with relapses of *P. vivax* (recurrence >2 months later). The relative frequency of species changed over time, with a gradual increase in the proportion of *P. falciparum* cases from 20% to 30% in the early 1980s to 60% to 70% in the 1990s and to 70% to 80% in the present decade (Figure 2). Over this 22-year period, only one fatality occurred (3).

Sixty-one countries were identified as the most likely sources of the malaria exposure. Sub-Saharan Africa was the region where most patients contracted malaria, 353 case-patients (65%), followed by south Asia (23%), Southeast Asia (6%), Central America (5%), and South America (1%). However, India, with 110 cases (20%), was the single most frequent source country. Tourists (29%), immigrants or refugees (29%), and foreign workers (24%) represented the categories most frequently reported. A shift over time occurred in the importance of sub-Saharan Africa as a source of malaria cases. In the 1980s, 50% of malaria infections were acquired in Africa; in the 90s, 70%; and, since 2000, 85%. Patients of all categories were more likely to contract *P. falciparum* in Africa (74.3%) while it has been an uncommon species in south Asia (5.8%). The increase in *P. falciparum* cases over time correlated with...
the increase in the total number of malaria cases contracted in Africa; *P. falciparum* represented ≈30% of all cases in the early 1980s and increased to 70% in the late 1990s. From 1981 to 2002, 96% of malaria infections acquired in South Asia were non-falciparum malarias, while only 29% of infections from Africa were non-falciparum. None of the 553 cases of malaria originated from China, Malaysia, Saudi Arabia, Peru, or Venezuela, which are frequent travel destinations of Québécois. Other common travel destinations contributed little to the 20-year malaria case total (e.g., Philippines [1 case], South Africa [1], Costa Rica [2], Mexico [2], and Dominican Republic [3]). Malaria cases from Africa from 1992 to 2000 came predominantly (69%) from the French-speaking African countries, notably higher than the proportion of U.S. travelers (18%) who acquired malaria in these countries (13–24).

Two “epidemics” were observed at TDC during this period (Figures 1 and 2). The first was in 1986 to 1990 and resulted from increasing numbers of *P. falciparum* infections from Africa, thought to be due to increasing chloroquine-resistant *P. falciparum* in chloroquine-prophylaxed travelers (28), and the second was in 1999 through 2000, resulting from increased numbers of *P. falciparum* infections associated with the arrival in Québec of large numbers of refugees from Tanzanian refugee camps (29). Both epidemics were recognized and reported in the literature soon after their appearance.

**Federal and Provincial Databases**

A review of the Federal Health Canada databases for the incidence of malaria in Canada, from 1990 through 2002, documents a range from 364 to 1,029 cases per year, with an average of 538 cases per year during the period (or an average of 1.8 cases per 100,000 population per year) (6) (Carole Scott [Division of Disease Surveillance, Health Canada], pers. comm.).

While all ages were affected, patients were mostly adults from 20 to 59 years of age. A similar pattern of malaria incidence was observed in males and females. British Columbia had the highest rate per 100,000 (3.6 ± 2.8) over this period, followed by Ontario (2.2 ± 0.98), and Québec (1.3 ± 0.67) (Figure 3). However, the highest cumulative numbers for the 12-year period were reported from Ontario (N = 3,222), followed by British Columbia (N = 1,763), and Québec (N = 1,246). The Canadian data suggest that an epidemic occurred from 1995 to 1997, reflecting higher than average numbers of malaria cases in these years from British Columbia, Ontario, and to a lesser extent, from Québec (Figure 3). This epidemic was almost entirely due to increased *P. vivax* being reported in these provinces (Figure 4). From 1990 to 1999, two events occurred in Québec that did not occur in other Canadian provinces. In 1994, a quality assurance program for the province was initiated by TDC, in collaboration with the Laboratoire de Santé Publique du Québec. This three-pronged program provided: 1) a free, rapid turnaround confirmation service for positive or equivocal malaria diagnoses from any laboratory in Québec, 2) a biannual malaria-training course for clinical laboratory technologists, and 3) a voluntary proficiency testing program for Québec hospital laboratories, in which once or twice a year they are sent unknown positive and negative smears for identification and receive extensive feedback. From the inception of the quality assurance program, a parallel increase was seen in numbers of specimens being sent to the reference laboratory and to the Québec surveillance program (Figure 5). This fourfold increase represented an epidemic attributable to improved diagnosis and reporting. The second event in Québec was another epidemic, in this case of falciparum malaria, observed in 2000 to 2001 and associated with a large influx of refugees from Tanzanian refugee camps (Figure 4) (29).
International Malaria Surveillance

National surveillance systems for malaria are far from universal, and compliance with national surveillance instruments, when measured, is low. The stability of the degree of underreporting over time has not been evaluated. Despite these limitations, trends in malaria incidence over time in different countries can provide useful information. From 1995 to 1997, when parts of Canada were having malaria epidemics, similar but smaller changes in malaria rates were observed in the United States and United Kingdom (Figure 6). An examination of the geographic origin of malaria cases reported in the United States in the mid-1990s showed a more than twofold increase in malaria cases imported from India in 1995 through 1997, with an abrupt drop in these cases in 1998 (12–23). During this same period, a similar epidemic of *P. vivax* malaria occurred in certain states in India known to have important immigration and travel links with North America (Figure 7). During the 1990s, France had a 60% increase in malaria in the latter part of the decade (31), reportedly caused by African travel, and Denmark experienced an increase of 68%; Germany, Italy, Spain, Sweden, the Netherlands, and Belgium, however, had stable rates during this time (12,31). None of these countries had the increase in rates of malaria seen in Canada and, to a lesser degree, in the United States from 1995 to 1997.

Discussion

Malaria importations into Canada can occur by either immigration or travel, and changing malaria attack rates in the countries of exposure are likely to influence the incidence of imported disease. Changes in Canadian immigration and refugee patterns from 1990 to 2002 are notable for a threefold increase in annual immigrant numbers from the Indian subcontinent and relatively stable numbers from sub-Saharan Africa. Neither combined nor separate provincial immigration and refugee patterns explain the important swings in annual Canadian malaria rates.

While the geographic origins of immigrants and refugees do not immediately explain the epidemic changes in *P. vivax* malaria seen in the mid-1990s, their nonrandom aggregation in certain provinces allows additional insights. African immigrants and refugees have settled all across Canada in every province in numbers that paralleled the province’s population. Immigrants and refugees from the Indian subcontinent did not: 84% settled in Ontario and British Columbia, the provinces with the most pronounced *P. vivax* epidemics. Canadian travelers to malaria-endemic areas have gradually but steadily increased during the past 15 years, most notably with a threefold increase to Southeast Asia and Central and South America, a twofold increase to the important malarial region of south Asia, and a smaller increase to Africa. Travel patterns did not offer an explanation for either the *P. vivax* epidemics in British
Columbia and Ontario in the late 1990s or the *P. falciparum* epidemic in Québec from 2000 to 2001. The World Tourism Organization data do not break down Canadian travel by traveler’s province of origin; however, comparing U.S. malaria surveillance data with TDC surveillance data, both of which track the likely country of origin of a malaria case, Québec travelers acquire most African malaria in French-speaking African countries (69%), a minor source of malaria for Americans (18%). English-speaking Ontario and British Columbia likely have more “American” travel patterns than francophone Québécois. However, no fluctuations were seen in rates of travel to either East or West Africa or to the Indian subcontinent, the major source of Canada-acquired *P. vivax* malaria, which would explain the impressive change in Canadian malaria reporting from 1995 through 1997.

The two surveillance sources of India and the United States were also reviewed for malaria incidence trends. American malaria surveillance includes the likely country of origin of a malaria case. An obvious increase in *P. vivax* cases from India was seen in the United States, from 150 cases to 371 and down to 123, during 1995 to 1997. This increase paralleled the epidemic peak seen in Canada, primarily in Ontario and British Columbia. In India, an epidemic of *P. vivax* malaria occurred during this same period (1995–1997) in the Punjabi states of Punjab and Haryana (Figure 7). With negligible changes in travel destination or immigration numbers to explain the 1995–1997 epidemic in Canada, the explanation is probably an increased *P. vivax* attack rate in Canadians traveling to the Punjab, where a *P. vivax* epidemic occurred and ended at the same time as the Canadian epidemic.

Canadian notifiable diseases surveillance data generated by local, provincial, and federal sources provided evidence for the occurrence of two as-yet unreported malaria epidemics in Canada in the last decade. One was a *P. vivax* epidemic, the epicenter of which was almost certainly in the Punjab, India. The second was a *P. falciparum* epidemic in Québec related to an increased influx of Central African refugees from Tanzanian refugee camps. At the time, neither of these epidemics was brought to the attention of health practitioners in travel clinics through publication or other standard channels. Consequently, possible explanations and potential interventions were not discussed.

Trends in immigration do not explain the malaria incidence changes seen in Canada. These trends differ for each province both in terms of country of origin and numbers. However, the major fluctuations in federal and provincial malaria rates from 1990 to 2002, and, in particular, during the epidemic years, were not found to be directly linked to provincial immigration numbers or to the travel destinations of Canadians in general. Unfortunately, no mechanism records the destinations of travelers from specific provinces. Ontario and British Columbia are home to 86% of the Punjabi-speaking Canadian population. If provincial travel destination data were available, it would likely show that these provinces were the source of most Canadian travelers to the Indian Punjab (27).

Working back from individual case data in each province seems to be the most accurate way to identify countries where large numbers of imported malaria may originate. Country of likely origin of the malaria should be indicated on all requisitions for malaria laboratory diagnosis, and this information and the malaria species should be reported to provincial and then federal surveillance bodies. The fact that the 1995–1997 epidemic was primarily due to *P. vivax*, the predominant malaria species in India, and that it occurred at the same time as the *P. vivax* epidemic in the Indian Punjabi states of Punjab and Haryana, is strong evidence to conclude that the Canadian epidemic was an extension of the Punjab epidemic. This association is
supported by the abrupt halt of both Canadian and Punjabi epidemics in the same year.

The surveillance process for notifiable diseases in Canada and in other countries where malaria is now an imported disease should be reviewed. Specific conditions, such as the frequency of analysis of surveillance data, need to be discussed and agreed on by collectors of these data at each level of government. Without a firm plan in place for analysis and dissemination of results, the validity, not to mention the utility of the entire surveillance system, is placed in jeopardy. One approach could be the American emerging infections programs, a link between public health, academic, and clinical communities (32).

For surveillance data to be useful and cost-effective, it must be both available in a timely fashion and interpretable. Local surveillance systems have obvious benefits when increased water- and foodborne infections or vaccine-preventable diseases lead to quick public health action. Malaria surveillance differs in two major ways from these classical scenarios. Malaria is an imported disease, and no immediate intervention (e.g., vaccine, chemical disinfectant, and handwashing) will affect an epidemic. As with sexually transmitted infections, the control of a malaria epidemic in Canadian travelers requires public education. In the United States, both malaria speciation and country of likely acquisition of the malaria case are part of surveillance. Such information, if part of the Canadian system, would allow rapid appreciation of the etiology of epidemics such as those reviewed here, which would potentially lead to appropriate public health response.

Dr. MacLean is professor of medicine and director of the McGill University Centre for Tropical Diseases. His research interests are parasitic disease outbreak investigation (trichinosis, Metorchis infections, and malaria) and the development of diagnostic tests for the clinical parasitology laboratory.

References


Address for correspondence: J. Dick MacLean, McGill University Centre for Tropical Diseases, Montreal General Hospital, 1650 Cedar Ave, Room D7-153, Montreal, Québec, Canada H3G 1A4; fax: 514-933-9385; email: dick.maclean@mcgill.ca
Estimating Time and Size of Bioterror Attack

Johan Walden* and Edward H. Kaplan*†

In the event of a bioterror attack, rapidly estimating the size and time of attack enables short-run forecasts of the number of persons who will be symptomatic and require medical care. We present a Bayesian approach to this problem for use in real time and illustrate it with data from a simulated anthrax attack. The method is simple enough to be implemented in a spreadsheet.

In the event of a bioterror attack, once the biologic agent has been determined, rapidly estimating the size and time of attack enables a forecast of the number of persons who will be symptomatic and will require medical attention over the days (and perhaps weeks) after the attack. Such a forecast could play a key role in determining the response effort required, e.g., surge capacity planning at hospitals, distributing vaccines or antimicrobial agents to the population, as appropriate (1,2). We refer to early knowledge of the size and time of an attack as situational awareness.

We present a Bayesian approach to the real-time estimation of the size and time of a bioterror attack, from case report data, that is simple enough to implement in a spreadsheet. The model assumes a single-source outbreak caused by a bioterror attack at a particular point in time. Although the model assumes that the infectious agent is not contagious, the analysis still holds for contagious agents until secondary infections have progressed to symptomatic cases. Thus, our model should prove valuable within the first incubation period after an attack has been detected for a contagious agent and for longer time periods in the event of a noncontagious agent. However, in the event of multiple attacks at different points in time or an attack with a rapidly progressing contagious agent, the problem becomes more difficult and similar to the use of back-calculation to recover the incidence of infection over time from symptomatic case reporting (3).

The key assumptions in our analysis are that the biological agent used has been identified and that the probability distribution of the incubation time from infection through symptoms is known. The incubation time distribution for anthrax has been estimated by Brookmeyer and colleagues on the basis of the Swerdlowsk outbreak (4); data describing the incubation distribution for smallpox are summarized by Fenner et al. (5). Although smallpox is a contagious infection, historically the incubation time from infection through onset of symptoms is 7–17 days (5), a fact that renders our model applicable to smallpox for roughly 2 weeks after an outbreak or 1 week after the first observed cases (which is the shortest time until one would expect to see cases resulting from second-generation infections).

Likely ranges for the incubation times of other plausible bioterror agents are available at the Centers for Disease Control and Prevention’s bioterror Web site (6), in addition to sources in the literature. If the only information available regarding the incubation time for some agent is a likely range, then one approach to creating a distribution for use with our model is to assume that the range corresponds to a probability coverage interval from a plausible incubation time distribution (such as the lognormal) and match the parameters of the distribution accordingly.

We assume that the attack is detected through the appearance of infected persons with symptoms, and that as cases are identified, patient interview yields the approximate time at which symptoms appeared, a process which avoids the need for explicit estimates of reporting delay. Corrected as such, case reports provide two types of information. The number of cases observed provides a lower bound on the size of the attack. The specific timing of case reports also conveys information that can be better understood when filtered through the agent-specific incubation time distribution.

The mathematical details of our approach are described in the Appendix. We define the time origin as the instant when the first case (and hence the attack) is detected (though the time origin can be reassigned if case investi-
gation indicates that a subsequently reported case had earlier symptoms). At the moment the attack is detected, consistent with Bayesian principles (7), we presume a prior probability distribution (henceforth, prior) governing the size of the attack. For any given attack size $n$, the time since the attack can be equated to the minimum of $n$ independent incubation times (since the attack is detected by the first symptomatic case). As additional cases accumulate over time, the likelihood of observing cases at specific times is computed with standard methods. Given the data observed, application of Bayes rule enables estimation of the posterior distribution of both the size and time of attack, from which summary statistics such as the mean, standard deviation, and probability intervals of the attack size and time are easily estimated. Short-run forecasts of future cases are also easily achieved within this framework. We have developed an Excel spreadsheet (Microsoft Corp., Redmond, WA) for implementing this procedure.

As an example, we simulated an anthrax attack that infects 100 persons using the incubation time distribution for anthrax estimated from the Swerdlovsk outbreak (4). We assumed a broad prior that assigns equal likelihoods to attacks of different orders of magnitude from 1 to 10,000 (Appendix). Thus, attacks infecting 1–10, 11–100, 101–1,000, and 1,001–10,000 persons each have the same 25% probability of occurrence. With this prior, absent any data other than the first case observed at time 0, the estimated mean attack size is approximately 1,090.

Absent intervention, the 100 victims in this simulated attack would appear as case-patients in accord with Figure 1 (open dots). Using the methods shown in the Appendix, we report estimates of the attack size and the time of the attack based on the cumulative number of cases observed at the end of day 5 of the outbreak (where the time origin corresponds to the occurrence of the first observed case) (Table). At the end of day 1, the estimated mean attack size equals 850 (with a 95% probability interval ranging from 60 to 3,300). However, estimates approach the true value of 100 over time. Similarly, the estimates for the time of the attack improve from 1.1 days before the first case (estimated after day 1 of the outbreak) to 1.8 days before the first case is observed; the true time of attack is 1.8 days before the first case observed in the simulated outbreak (Figure 1).

Figures 2 and 3 illustrate the posterior distributions of the initial attack size and time of attack at the end of 5 days (when a total of 23 cases have appeared). For example, Figure 2 suggests that while the expected attack size, given the data, equals 90, initial attacks as small as 50 or as large as 150 are also plausible. Similarly, the time of the attack could have been as recent as half a day before the first case was observed, or as early as 3.5 days before the first case appeared.

![Cumulative epidemic](image)

**Figure 1.** Simulated actual (open dots) and forecasted (solid curve) cumulative cases in an anthrax bioterror attack that infects 100 persons 1.8 days before the first symptomatic case is observed. The cases were simulated from a lognormal distribution with median 11 days and dispersion 2.04 days, which corresponds to the incubation time estimated for anthrax based on the Swerdlovsk outbreak (3).

Given estimates of the initial size and time of attack, one can forecast the occurrence of future cases over time, as shown in Figure 1 (solid curve), where the forecast is made on the basis of cases observed through the end of day 5 after the first case was observed. Such a short-range forecast could be helpful in determining the resources required to treat those infected in the attack, although once a widespread response to the attack is mounted (e.g., distribution of antimicrobial agents, in the case of anthrax), the forecasts lose their validity (8).

The key assumptions in our model are that the probability distribution of the incubation time from infection through development of symptoms is known and that attack victims can report the times of symptom onset (so we have not explicitly accounted for reporting delay). In an actual bioterror attack, determining the incubation time distribution itself might be necessary. For example, as shown recently by Brookmeyer et al. (9), the incubation time for anthrax is dose-dependent. Thus, exposure to anthrax powders with much greater spore concentrations than evident either in Swerdlovsk or the U.S. postal attacks could lead to shorter incubation distributions. While we are investigating statistical methods for this more general problem (J.T. Wu, unpub. data), having a relatively simple tool is still helpful when the probability distribution of

<table>
<thead>
<tr>
<th>Days past case no. 1</th>
<th>Total cases</th>
<th>Estimated attack size</th>
<th>Estimated day of attack (before case no. 1)</th>
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</tr>
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<tr>
<td>5</td>
<td>23</td>
<td>90</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*See Appendix for details.*
incubation times is presumed known and a single, point-source outbreak is suspected. Our model might also prove helpful for education and training exercises, in addition to use during an actual bioterror attack.

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Dr. Walden has a docentship in applied mathematics from Uppsala University and is currently affiliated with the International Center for Finance at the Yale School of Management. His research focuses on mathematical finance, but he is also interested in the mathematical modeling of social systems, including statistical methods for modeling public health policy issues.

Dr. Kaplan is the William N. and Marie A. Beach Professor of Management Sciences at the Yale School of Management and professor of public health at the Yale School of Medicine, where he directs the Methodology and Biostatistics Core of Yale’s Center for Interdisciplinary Research on AIDS. His interests include the application of operations research, statistics, and mathematical modeling to public health policy problems such as HIV prevention, and more recently, bioterror preparedness and response.

References


Address for correspondence: Edward Kaplan, Yale School of Management, and Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, CT 06520-8200, USA; fax: 203-432-9995; email: edward.kaplan@yale.edu

Appendix

We seek to estimate the initial attack size and the time of the attack from observed cases of infection in real time. The case report data are the (reporting-delay corrected) times at which cases have been reported. We intend this model to be applied once an attack has been discovered and assume that the agent is noncontagious (or in the case of a contagious agent, that no secondary transmission has occurred) and that any interventions mounted (such as vaccination or the administration of antimicrobial agents) have not yet had any effect on the early case reporting data. We define $t_j$ to be the time at which the $j$th case is observed and define the origin as the time at which the first case is observed (so $t_1 = 0$). The unknown time from the attack until the first case is observed is denoted by $A > 0$ (and thus the actual date of the attack is equal to $-A$), while the unknown number of persons infected in the attack is denoted by $N > 1$.

We treat $A$ and $N$ as random variables and assume that the attack is detected through the reporting of the first case at time $t_1 = 0$. At the time the attack is detected, we quantify our beliefs regarding the size of the attack by the prior probability distribution $p(n) = Pr\{N = n\}$. Let $X$ denote the symptom-free incubation time for the attack agent, with probability density $f(x)$ and survivor function $S(x) = Pr\{X > x\} = \int_{x}^{\infty} f(u)du$. 

![Figure 2. Posterior probability density of the attack size based on the data in Figure 1 observed through the end of day 5 after the first case appeared.](image)

![Figure 3. Posterior probability density of the time of attack based on the data in Figure 1 observed through the end of day 5 after the first case appeared.](image)
If \( n \) persons were actually infected in the attack, then the time from the attack until the first case is observed would equal the minimum of \( n \) independent incubation times \( X_j, j = 1,2,\ldots,n \), thus

\[
A = \min \{ X_j \}_{j=1}^n.
\]  

Consequently, the probability that \( a \) units of time would pass before the attack would be detected by the first case equals

\[
\Pr \{ A > a \} = \prod_{j=1}^n \Pr \{ X_j > a \} = S(a)^n,
\]

from which the conditional probability density function of \( A \) given an attack of size \( n \) follows as

\[
f(a | n) = nS(a)^{-1} f(a) \quad \text{for} \quad a > 0, n = 1,2,3,\ldots
\]  

Equation 2 implies that the joint prior distribution for the size and time of attack when the first case is observed is equal to

\[
f(a,n) = p(n)xS(a)^{-1} f(a) \quad \text{for} \quad a > 0, n = 1,2,3,\ldots
\]  

Now, suppose that by time \( \tau \) an additional \( k - 1 \) cases have been observed at times \( t_j \) for \( j = 2,3,\ldots,k \). Conditional upon an attack of size \( n \) having occurred at time \( -a \), the joint probability density of the data observed (that is, the likelihood function) is given by

\[
L(t | a,n,\tau) = \frac{(n-1)!}{(n-k)!} \left( \frac{S(\tau+a)}{S(a)} \right)^k \prod_{j=1}^k \left( \frac{f(a+t_j)}{S(a)} \right)
\]  

where \( t = t_2, t_3, t_4,\ldots, t_k \). Equation no. 4 is simply the conditional joint density of the first \( k - 1 \) order statistics observed from a sample of size \( n - 1 \), given that \( a \) time units had passed from the attack until the first case was observed at time \( 0 \), adjusted for the fact that the period of observation extends to time \( \tau \) (1). Unconditioning the likelihood in equation no. 4 by the prior in equation no. 3 yields the joint density

\[
f(a,n,\tau) = \frac{n!}{(n-k)!} S(\tau+a)^{-1} \prod_{j=1}^k f(a+t_j) \quad \text{for} \quad a > 0, n = k,k+1,k+2,\ldots
\]  

[5]

and application of Bayes rule yields the joint posterior distribution of the size and time of attack as

\[
f(a,n,\tau) \mid S(\tau+a)^{-1} \prod_{j=1}^k f(a+t_j) \quad \text{for} \quad a > 0, n = k,k+1,k+2,\ldots
\]  

\[S(\tau+a)^{-1} \prod_{j=1}^k f(a+t_j) \]  

and \( S(\tau+a)^{-1} \prod_{j=1}^k f(a+t_j) \) du

[6]

\[
\sum_{i=0}^n \int_0^\infty \frac{n!}{(n-i)!} S(\tau+a)^{-1} \prod_{j=1}^k f(a+t_j) \mathrm{du}
\]

The posterior distributions of \( A \) and \( N \) are then easily obtained from equation no. 6 by summing (over \( n \)) or integrating (over \( a \)).

To obtain a short-run forecast of future cases, note that conditional upon an attack of size \( n \) that occurred a time units before detection, the expected number of cases that will occur by some future time \( \tau^* \) equals \( n[1 - S(a + \tau^*)] \). Unconditioning over equation no. 6 yields a simple short-run forecast of the number of future cases expected given all of the data observed to date. An even simpler approximation is obtained by substituting the posterior expected values of \( N \) and \( A \) in the expression above for the expected number of future cases; we used this approach in producing the forecast shown in Figure 1.

In our examples, we assume that, a priori, the logarithm of the attack size \( N \) is uniformly distributed between 0 and the logarithm of 10,000, and we approximate this distribution in a spreadsheet with 500 mass points equally spaced on the natural logarithmic scale. This procedure assigns equal probabilities to four different orders of magnitude, that is, attacks that infect 1–10, 11–100, 101–1,000, or 1,001–10,000 persons are each assigned the same 25% probability of attack. The expected prior attack size associated with this distribution approximately equals 9,999/\ln(10,000) = 1,090.

Our examples also assume that the incubation time from infection through onset of symptoms is distributed in accord with a lognormal distribution with a mean of 11 days and a standard deviation of 2.04 days. This is the distribution fit to the data from the anthrax outbreak in Swedlovsk (2). For numerical computations, this distribution is also approximated discretely within the spreadsheet.

The joint prior distribution of \( N \) and \( A \) under the stated assumptions is shown in Appendix Figure 1, available online at http://www.cdc.gov/ncidod/eid/vol10no07/03-0632-appG1.htm, Appendix Figure 2, available online at http://www.cdc.gov/ncidod/eid/vol10no07/03-0632-appG2.htm, displays the joint posterior distribution for \( N \) and \( A \) after a total of 23 cases have been observed by the end of 5 days after the first case was reported (Figure 1). Results similar to those from Figures 1 to 3 and Appendix Figure 2 are obtained if the attack size itself is assumed to be uniformly distributed from 0 to 10,000 for this example, but we believe the log-uniform prior is more sensible in that the primary a priori ignorance regards the order of magnitude of the attack size.

References


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As a transmissible infectious disease, severe acute respiratory syndrome (SARS) was successfully contained globally by instituting widespread quarantine measures. Although these measures were successful in terminating the outbreak in all areas of the world, the adverse effects of quarantine have not previously been determined in a systematic manner. In this hypothesis-generating study supported by a convenience sample drawn in close temporal proximity to the period of quarantine, we examined the psychological effects of quarantine on persons in Toronto, Canada. The 129 quarantined persons who responded to a Web-based survey exhibited a high prevalence of psychological distress. Symptoms of posttraumatic stress disorder (PTSD) and depression were observed in 28.9% and 31.2% of respondents, respectively. Longer durations of quarantine were associated with an increased prevalence of PTSD symptoms. Acquaintance with or direct exposure to someone with a diagnosis of SARS was also associated with PTSD and depressive symptoms.

S evere acute respiratory syndrome (SARS) was contained globally by widespread quarantine measures, measures that had not been invoked to contain an infectious disease in North America for >50 years (1–6). Although quarantine has periodically been used for centuries to contain and control the spread of infectious diseases such as cholera and the plague with some success (1,3,4,6–8), the history of invoking quarantine measures is tarnished by threats, generalized fear, lack of understanding, discrimination, economic hardships, and rebellion (1,3,4,6–8).

Quarantine separates persons potentially exposed to an infectious agent (and thus at risk for disease) from the general community. For the greater public good, quarantine may create heavy psychological, emotional, and financial problems for some persons. To be effective, quarantine demands not only that at-risk persons be isolated but also that they follow appropriate infection control measures within their place of quarantine. Reporting on SARS quarantine has focused on ways in which quarantine was implemented and compliance was achieved (1–4,6–8). Adverse effects on quarantined persons and the ways in which those quarantined can best be supported have not been evaluated. Moreover, little is known about adherence to infection-control measures by persons in quarantine.

Knowledge and understanding of the experiences of quarantined persons are critical to maximize infectious disease containment and minimize the negative effects on those quarantined, their families, and social networks. The objectives of our study were to assess the level of knowledge about quarantine and infection control measures of persons who were placed in quarantine, to explore ways by which these persons received information, to evaluate the level of adherence to public health recommendations, and to understand the psychological effect on quarantined persons during the recent SARS outbreaks in Toronto, Canada.

Methods

Description of Quarantine in Toronto

During the first and second SARS outbreaks in Toronto, >15,000 persons with an epidemiologic exposure to SARS were instructed to remain in voluntary quarantine (Health Canada, unpub. data). Data on the demographics of the quarantined population were collected but have not yet been analyzed (B. Henry, Toronto Public Health, pers. comm.). Quarantined persons were instructed not to leave their homes or have visitors. They were told to wash their hands frequently, to wear masks when in the same room as other household members, not to share personal items (e.g., towels, drinking cups, or cutlery), and to sleep in

*University of Toronto, Toronto, Ontario, Canada; †ASK Information Technologies, Toronto, Ontario, Canada; and ‡New York Academy of Medicine, New York, New York, USA
separate rooms. In addition, they were instructed to measure their temperature twice daily. If any symptoms of SARS developed, they were to call Toronto Public Health or Telehealth Ontario for instructions (5).

Study Population
All persons who were placed in quarantine during the SARS outbreaks in Toronto (at least 15,000 persons) were eligible for participation in this study. The survey was announced through media releases, including locally televised interviews with the principal investigators. Information on the study and invitations to participate were posted in local healthcare institutions, libraries, and supermarkets. Ethics approval was obtained from the research ethics board of the University Health Network, a teaching institution affiliated with the University of Toronto.

Survey Instrument
A Web-based survey composed of 152 multiple choice and short-answer questions was to be completed after participants ended their period of quarantine. It took approximately 20 minutes to complete. Questions explored included the following: 1) knowledge and understanding of the reasons for quarantine, (2) knowledge of and adherence to infection control directives, and (3) source of this knowledge.

The psychological impact of quarantine was evaluated with validated scales, including the Impact of Event Scale—Revised (IES-R) (9) and the Center for Epidemiologic Studies—Depression Scale (CES-D) (10). The IES-R is a self-report measure designed to assess current subjective distress resulting from a traumatic life event and is composed of 22 items, each with a Likert rating scale from 0 to 4. The maximum score is 88. In a study of journalists working in war zones, the mean IES-R score of posttraumatic stress disorder (PTSD) was 20. In these persons, the presence of PTSD symptoms, as measured by this scale, was correlated with diagnostic psychiatric interviews (11). The CES-D is a measure of depressive symptoms composed of 20 self-report items, each with a Likert rating scale from 0 to 3. The maximum score is 60 (10). A score of ≥16 has been shown to identify persons with depressive symptoms similar in severity to the levels observed among depressed patients (10,12,13). Open-ended questions provided respondents with the opportunity to relate the aspects of quarantine that were most difficult for them and allowed them to provide additional comments on their unique experiences.

Statistical Analysis
Means were calculated to summarize continuous variables. For categorical variables, group proportions were calculated. Student t tests were used to examine relationships between demographic variables and the psychological outcome variables, the scores on the IES-R and CES-D. A score of ≥20 on the IES-R was used to estimate the prevalence of PTSD symptoms (11). A score of ≥16 on the CES-D was used to estimate the prevalence of depressive symptoms (10,12,13).

Analysis of variance (ANOVA), chi-square, and the Cochran-Armitage test for trend were used to examine relationships between the IES-R and CES-D scores and the following independent variables: healthcare worker status, home or work quarantine, acquaintance of or direct exposure to someone with a diagnosis of SARS, combined annual household income, and the frequency with which persons placed in quarantine wore their masks. Linear regression for the trends between income categories and both PTSD and depressive symptoms was analyzed. The relationships between the IES-R and CES-D and whether persons in quarantine wore their masks all of the time versus never were examined by the Duncan-Waller K-ratio t tests. A p value of ≤0.05 was considered to be significant for all analyses.

Qualitative data were coded and analyzed to show emerging themes. The development and confirmation of the thematic coding structure is an iterative process involving two researchers in individual, recursive reading of the textual data and group meetings to discuss and test the emerging themes. Discrepancies were resolved by consulting specific instances in the data, discussing their relationship to established themes, and reaching consensus as a group (14).

Results
Demographics and Description of Quarantined Persons
The survey was completed by 129 of more than 15,000 eligible persons who were placed in quarantine (Figure). All respondents completed the survey at the end of quarantine with a minimum time from the end of quarantine to the completion of the survey of 2 days. The median time from the end of quarantine to completion of the survey was 36.0 days (interquartile range, 10–66 days). Sixty-eight percent of respondents were healthcare workers, 64% were 26–45 years of age, 58% were married, 72% had a college level of education or higher, and 48% had a combined household income of ≥$75,000 (Canadian dollars [CAD]).

The 129 respondents described 143 periods of quarantine with 90% of respondents being placed into quarantine only once; 66% of respondents were on home quarantine, while 34% were on work quarantine. The median duration of quarantine was 10 days (interquartile range, 8–10 days). Half of respondents knew someone who was hospitalized with SARS of whom 77% were colleagues; 10% knew someone who had died of SARS (Table 1).
Persons were notified of their need to go into quarantine from the following sources: their workplace (58%), the media (27%), their healthcare provider (7%), and public health officials (9%). Most (68%) understood that they were quarantined to prevent them from transmitting infection to others; 8.5% of respondents believed they were quarantined to protect themselves from infection; 15% did not believe they should have been placed into quarantine at all; and 8.5% provided more than one of these responses.

The source of notification for quarantine influenced understanding of the reason for quarantine. Those who were notified by the media or their workplace were more likely to understand the reason for quarantine than those who were notified by their healthcare provider or public health unit (p = 0.04). Healthcare workers were also more likely to understand the reason for quarantine compared with non–healthcare workers, 76.5% versus 52.5% (p = 0.007). Combined household income and level of education did not influence understanding of the reason for quarantine.

Information on Infection Control Measures

Persons received their information regarding infection control measures to be adhered to during their quarantine from the following sources: the media (54%), public health authorities (52%), occupational health department (33%), healthcare providers (29%), word-of-mouth (23%), hospital Web sites (21%), and other Web sites (40%)

Those who did not think they had been well-informed were angry that information on infection control measures and quarantine was inconsistent and incomplete, frustrated that employers (healthcare institutions) and public health officials were difficult to contact, disappointed that they did not receive the support they expected, and anxious about the lack of information on the modes of transmission and prognosis of SARS (see online Appendix at http://www.cdc.gov/ncidod/EID/vol10no7/03-0703.htm#app).

During the outbreaks, nearly 30% of respondents thought that they had received inadequate information about SARS. With respect to information regarding home infection control measures, 20% were not told with whom they could have contact; 29% did not receive specific instructions on when to change their masks; and 40%–50% did not receive instructions on the use and disinfection of personal items, including toothbrushes and cutlery; 77% were not given instructions regarding use and disinfection of the telephone. Healthcare worker status did not influence whether respondents thought they had received
adequate information regarding any of the listed home infection control measures, except regarding the frequency of mask changing: healthcare workers more frequently reported that they had received adequate information, 78.8% versus 60.5% (p = 0.03).

Adherence to Infection Control Measures

Eighty-five percent of quarantined persons wore a mask in the presence of household members; 58% remained inside their residence for the duration of their quarantine. Thirty-three percent of those quarantined did not monitor their temperatures as recommended; 26% self-monitored their temperatures less frequently than recommended, and 7% did not measure their temperatures at all. No differences between healthcare workers and nonhealthcare workers were found with respect to adherence to recommended infection control measures.

Psychological Impact of Quarantine

The mean IES-R score was 15.2±17.8, and the mean CES-D was 13.0±11.6. The IES-R score was ≥20 for 28.9%; the CES-D score was ≥16 in 31.2% of quarantined persons (Table 2). The mean IES-R scores were not different for persons on home or work quarantine, 14.1±18.8 versus 17.6±16.6 (p = 0.33); the mean CES-D scores were also not different between the groups, 12.0±12.0 versus 15.2±10.7 (p = 0.16).

The presence of PTSD symptoms was correlated with the presence of depressive symptoms (p < 0.0001, r = 0.78). Marital status did not offset the presence of PTSD symptoms, mean IES-R score of 14.5±16.6 for those who were unmarried versus 13.8±14.6 for those who were married (p = 0.82). Similarly, marital status did not influence the presence of depressive symptoms, with a mean CES-D score of 12.9±10.7 for those who were unmarried versus 12.5±11.4 for those who were married (p = 0.85).

A combined annual household income of CAD <40,000 versus CAD $40,000 to CAD $75,000 versus CAD >$75,000 was associated with increased PTSD symptoms (mean IES-R score of 24.2±20.6 versus 20.0±24.4 versus 11.8±11.6, respectively) (p = 0.03 for the three-way comparison). Linear regression testing for trend over income categories was also significant (p = 0.01). A combined annual household income of CAD <40,000 versus CAD $40,000 to CAD $75,000 versus CAD >$75,000 was also associated with increased depressive symptoms (mean CES-D score of 18.3±15.4 versus 15.5±13.2 versus 10.9±9.2, respectively) (p = 0.05 for the three-way comparison) (Table 2). Results of linear regression testing for trend over income categories were also significant (p = 0.01).

Neither age, level of education, healthcare worker status, living with other adult household members, nor having children was correlated with PTSD and depressive symptoms. The duration of quarantine was significantly related to increased PTSD symptoms, mean IES-R score of 23.7±27.2 for those in quarantine ≥10 days compared with 11.7±10.7 for those in quarantine <10 days (p < 0.05). Persons who were in quarantine for a longer duration showed a trend toward higher CES-D scores; however, this difference did not reach statistical significance (mean CES-D of 17.0±14.2 for those in quarantine ≥10 days versus 11.2±10.1 for those in quarantine <10 days [p = 0.07]). Acquaintance with or exposure to someone who was hospitalized with SARS was associated with a higher mean IES-R score, 18.6±20.2 versus 11.8±14.3 (p = 0.03) and a higher mean CES-D score, 15.5±12.1 versus 10.2±10.5 (p = 0.01). Overall, acquaintance with or exposure to someone who died of SARS was not correlated with PTSD or depressive symptoms (data not shown).

Persons were categorized as having worn their masks all of the time, including times when it was not

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Table 2. Prevalence of posttraumatic stress disorder and depressive symptoms according to patient demographics

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<th>Characteristic</th>
<th>No. (%) (N=129)</th>
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<td>&lt;$40,000</td>
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</tr>
<tr>
<td>&lt;10</td>
<td>11.2</td>
<td>10.1</td>
</tr>
<tr>
<td>≥10</td>
<td>17.0</td>
<td>14.2</td>
</tr>
<tr>
<td>IES-R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>11.7</td>
<td>10.7</td>
</tr>
<tr>
<td>≥10</td>
<td>23.7</td>
<td>27.2</td>
</tr>
</tbody>
</table>

*CES-D, Center for Epidemiologic Studies—Depression Scale (10); IES-R, Impact of Event Scale—Revised (9).

*By analysis of variance.
recommended, having worn their masks according to recommendations, or not having worn their masks at all. Those who wore their masks all of the time had higher mean IES-R scores (29.7±18.6 versus 14.1±17.9 versus 12.3±15.1, p = 0.003 for the three-way comparison) and higher mean CES-D scores (25.6±12.7 versus 12.2±11.1 versus 11.5±11.6, p = 0.002 for the three-way comparison). Those who wore their masks all of the time also had higher mean IES-R scores (p = 0.03) and higher mean CES-D scores (p = 0.002) than those who never wore their masks.

All respondents described a sense of isolation. The mandated lack of social and, especially, the lack of any physical contact with family members were identified as particularly difficult. Confinement within the home or between work and home, not being able to see friends, not being able to shop for basic necessities of everyday life, and not being able to purchase thermometers and prescribed medications enhanced their feeling of distance from the outside world. Infection control measures imposed not only the physical discomfort of having to wear a mask but also significantly contributed to the sense of isolation. In some, self-monitoring of temperature provoked considerable anxiety: “taking temperatures was mentally difficult” (respondent #27) and “taking my temperature made my heart feel like it was going to pound out of my chest each time” (respondent #62).

While most quarantined persons (60%) did not believe that they would contract SARS, 59% were worried that they would infect their family members. In contrast, only 28% were concerned that a quarantined family member would infect someone else in the home. Following quarantine, 51% of respondents had experiences that made them feel that people were reacting differently to them: avoiding them, 29%; not calling them, 7%; not inviting them to events, 8%; and not inviting their families to events, 7%.

**Discussion**

Persons placed in quarantine have their freedom restricted to contain transmissible diseases. This takes a considerable toll on the person. In relation to the recent global outbreak of SARS, considerable time has been spent discussing the specifics of quarantine and how to promote adherence to infection control measures. Little, if any, analysis has focused on the effect of quarantine on the well-being of the quarantined person. The objective of the study survey was to capture a range of experiences of quarantined persons to better understand their needs and concerns. This knowledge is critical if modern quarantine is to be an effective disease-containment strategy. To our knowledge, a consideration of the adverse effects of quarantine, including psychological effects, has not previously been systematically attempted.

Our results show that a substantial proportion of quarantined persons are distressed, as evidenced by the proportion that display symptoms of PTSD and depression as measured by validated scales. Although quarantined persons experienced symptoms suggestive of both PTSD and depression, the scales that were used to measure these symptoms are not sufficient to confirm these diagnoses. To confirm the diagnoses of PTSD and depression, structured diagnostic interviews are required. Because the survey was anonymous, this was not possible.

A score of ≥20 on the IES-R was used to estimate the prevalence of PTSD symptoms in our study population. This corresponds to the mean score measured on the IES-R in a study of journalists working in war zones that used diagnostic psychiatric interviews to confirm the presence of this disorder (11). Since most respondents to our survey were healthcare workers, we chose a work-related traumatic event for the comparison group. While other cut-off points may have been used to estimate the prevalence of PTSD symptoms in our population, the risk factors that we identified for increased PTSD symptoms, rather than the absolute prevalence of PTSD in our study participants, are the important findings of this study. This also applies to the risk factors that we identified for increased depressive symptoms in the respondents. Quarantined persons with risk factors for either PTSD or depressive symptoms may benefit from increased support from public health officials.

In this population, the presence of PTSD symptoms was highly correlated with the presence of depressive symptoms even though different clinical symptoms characterize the two disorders. Kessler’s National Comorbidity Study indicated a 48.2% occurrence of depression in patients with PTSD (15).

PTSD is an anxiety disorder characterized by avoiding stimuli associated with a traumatic event, reexperiencing the trauma, and hyperarousal, such as increased vigilance (16). This disorder may develop after exposure to traumatic events that involve a life-threatening component, and a person’s vulnerability to the development of PTSD can be increased if the trauma is perceived to be a personal assault (17). Increased length of time spent in quarantine was associated with increased symptoms of PTSD. This finding might suggest that quarantine itself, independent of acquaintance with or exposure to someone with SARS, may be perceived as a personalized trauma. The presence of more PTSD symptoms in persons with an acquaintance or exposure to someone with a diagnosis of SARS compared to persons who did not have this personal connection may indicate a greater perceived self-risk. The small number of respondents who were acquainted with or exposed to someone who died of SARS may explain the lack of correlation between this group and greater PTSD and
depressive symptoms (44 persons died of SARS in the
greater Toronto area).

This study also notes the trend toward increasing symp-
toms of both PTSD and depression as the combined annu-
al income of the respondent household fell from CAD
>$75,000 to CAD <$40,000. Quarantined persons with a
lower combined annual household income may require
additional levels of support. Since the survey was Web-
based and required that respondents have access to a com-
puter, the survey was likely answered by a more affluent
and educated subgroup of persons. Since respondents
with a lower combined annual household income experienced
increased symptoms of PTSD and depression, and since
those with lower combined annual household incomes
were not as likely to have access to a computer, the results
of this survey may underestimate the prevalence of psy-
chological distress in the overall group of quarantined per-
sons. Overall, most respondents did not report financial
hardship as a result of quarantine. This finding is likely
explained by the fact that >50% of the respondents report-
ed a combined annual household income of CAD
>$75,000.

As many as 50% of respondents felt that they had not
received adequate information regarding at least one
aspect of home infection control, and not all of the respon-
dents adhered to recommendations. Why some infection-
control measures were adhered to while others were not is
unclear. A combination of lack of knowledge, an incom-
plete understanding of the rationale for these measures,
and a lack of reinforcement from an overwhelmed public
health system were likely contributors to this problem.
Of particular interest, strictly adhering to infection control
measures, including wearing masks more frequently than
recommended, was associated with increased levels of dis-
tress. Whether persons with higher baseline levels of dis-
tress were more likely to strictly adhere to infection-
control measures or whether adherence to recommended
infection control strategies resulted in developing higher
levels of distress cannot be clarified without interviewing
the respondents. Regardless of the cause, this distress may
have been lessened with enhanced education and contin-
ued reinforcement of the rationale for these measures and
outreach efforts to optimize coping with the stressful
event.

This study has several limitations. The actual number
of respondents is low compared to the total number of per-
sons who were placed into quarantine and therefore may
not be representative of the entire group of quarantined
persons. However, lack of funding, confidentiality of pub-
lic health records, and an overloaded public health
response system limited sampling in this study.
Furthermore, a self-selection effect may have occurred
with those persons who were experiencing the greatest or
least levels of distress responding to the survey. In addi-
tion, respondents required access to a computer to respond,
which suggests that they may be more educated and have
higher socioeconomic status than the overall group who
were quarantined. They also had to be English speaking.
Recognizing these limitations, however, an anonymous
Web-based method was chosen because concerns about
persons’ confidentiality precluded us from access to their
public health records.

A Web-based format was chosen over random-digit
dialing for both cost considerations and time constraints.
The project was initiated and completed without a funding
source soon after the outbreak period at a time when con-
cerns about SARS were still a part of daily life in Toronto.
Obtaining as much information about the adverse effects
of quarantine as close to the event as possible was impor-
tant because a study conducted several months later would
have been subject to the limitations of substantial recall
bias. If this study were to be repeated, a study design
ensuring a more representative selection of the population
that used a combination of quantitative and qualitative
methods, including structured diagnostic interviews,
would be recommended to overcome these concerns. In
the event of future outbreaks, a matched control group of
persons who were not quarantined should be considered
because it would allow an assessment of the distress expe-
rienced by the community at large.

Finally, we determined only the prevalence of symp-
toms of PTSD and depression in our study population
because these were the predominant psychological dis-
tresses that were observed to be emerging in our SARS
patient population (W.L.G., pers. comm.). We also focused
on symptoms of PTSD and depression because we
believed that they would be the most likely to cause illness
and interfere with long-term functioning. Future studies
should assess persons for other psychological responses,
including fear, anger, guilt, and stigmatization. A standard-
ized survey instrument that considers the full spectrum of
psychological responses to quarantine should be devel-
oped. In the event of future outbreaks in which quarantine
measures are implemented, a standardized instrument
would enable a comparison between the psychological
responses to outbreaks of different infectious causes and
could be used to monitor symptoms over time.

Despite these limitations, the results of this survey
allow for the generation of hypotheses that require further
exploration. Our data show that quarantine can result in
considerable psychological distress in the forms of PTSD
and depressive symptoms. Public health officials, infec-
tious diseases physicians, and psychiatrists and psycholo-
gists need to be made aware of this issue. They must work
to define the factors that influence the success of quaran-
tine and infection control practices for both disease

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containment and community recovery and must be prepared to offer additional support to persons who are at increased risk for the adverse psychological and social consequences of quarantine.

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Dr. Hawryluck is an assistant professor of critical care/respirology at the University Health Network, University of Toronto. Her research interests include bioethical issues in medicine, SARS, decision-making, and communication regarding life-sustaining interventions.

References


Address for correspondence: Rima Styra, Toronto General Hospital, 200 Elizabeth Street, 8 Eaton North - Room 225, Toronto, Ontario, Canada MSG 2C4; fax: 416-340-4198; email: styra@attglobal.net

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The severe acute respiratory syndrome–associated coronavirus (SARS-CoV) is thought to be transmitted primarily through dispersal of droplets, but little is known about the load of SARS-CoV in oral droplets. We examined oral specimens, including throat wash and saliva, and found large amounts of SARS-CoV RNA in both throat wash (9.58 x 10^2 to 5.93 x 10^6 copies/mL) and saliva (7.08 x 10^3 to 6.38 x 10^8 copies/mL) from all specimens of 17 consecutive probable SARS case-patients, supporting the possibility of transmission through oral droplets. Immunofluorescence study showed replication of SARS-CoV in the cells derived from throat wash, demonstrating the possibility of developing a convenient antigen detection assay. This finding, with the high detection rate a median of 4 days after disease onset and before the development of lung lesions in four patients, suggests that throat wash and saliva should be included in sample collection guidelines for SARS diagnosis.

Severe acute respiratory syndrome (SARS) is an emerging infectious disease that spread rapidly from China to >30 countries, including Canada, Singapore, Vietnam, and Taiwan, in the first half of 2003 (1–5). In the latest update from the World Health Organization, the number of probable SARS cases is 8,096 (5). The etiologic agent of SARS has been identified as the novel SARS-associated coronavirus (SARS-CoV) (6–9). The disease is highly contagious and has the potential to cause a very large epidemic in the absence of control measures (10–11). Transmission appears to occur primarily through dispersal of droplets from the respiratory tract (12), generated when the patient talks, coughs, or sneezes (4,5,10–13). Although large amounts of SARS-CoV have been reported in sputum and nasal specimens, which may account for transmission during coughing and sneezing (7,14), little is known about the load of SARS-CoV in the oral cavity and how the virus is transmitted during talking. Since sneezing and rhinorrhea are not common symptoms of SARS, and cough with sputum is only seen in the later stage of infection (1–3,13), oral droplets generated during talking may represent an important route of transmission.

We examined specimens derived from the oropharynx and oral cavity, including throat wash and saliva, from 17 patients with probable SARS (15,16). Using a quantitative real-time reverse transcription–polymerase chain reaction (RT-PCR) assay and fractionation experiment, we investigated the load of SARS-CoV in these samples and different components of the throat wash.

**Materials and Methods**

**Patients**

From April 16, 2003, through May 1, 2003, during a 2-week period of the SARS outbreak in Taipei (16), 17 adult patients, who were admitted to the emergency department of the National Taiwan University Hospital and met the clinical case definitions for probable SARS (15), were included in this study. Physicians in the SARS Research Group of National Taiwan University Hospital made the
diagnosis for each patient after thorough evaluation of their travel or contact history; symptoms; laboratory data including lymphopenia, thrombocytopenia, and elevated levels of lactate dehydrogenase or creatine kinase; and pneumonic patch in the chest x-ray. The first day of fever was defined as day 1 of illness. The serologic test of an indirect immunofluorescence assay performed on serum specimens collected 28 days after onset confirmed SARS-CoV infection in 13 of the 17 patients. The other four patients had at least two positive real-time RT-PCR results. Therefore, all 17 cases with probable SARS in this study were confirmed by laboratory tests (15).

Sample Processing
With the patient’s consent, saliva and throat wash (by gargling 10 mL normal saline) were collected in an airborne isolation room, according to the guidelines for aerosol-generating procedures (17). All samples were transferred to the biosafety level 3 (BSL3) laboratory and stored at −80°C until use (18). After thawing, 5 mL of the throat wash was centrifuged at 1,500 x g for 15 min to separate the supernatant from the mucous-cell pellet. Four milliliters of the supernatant were collected as the throat wash supernatant. The remaining 1-mL portion that contained the mucous-cell pellet was treated with equal volume of N-acetyl-L-cysteine at room temperature for 25 min and centrifuged at 1,500 x g for 15 min to further separate the cell pellet from the supernatant, of which 1.12 mL was collected as the treated supernatant of throat wash. Instead of extensively washing the potentially contagious cell pellet, we kept the remaining 0.88 mL as the cellular fraction of throat wash. Equal amounts of the supernatant, treated supernatant, and cellular fractions were subjected to viral RNA extraction. An aliquot of the saliva, to which an equal volume of 1 x phosphate-buffered saline (PBS) was added, was also subjected to viral RNA extraction.

Isolation of Viral RNA
Viral RNA was isolated from aliquots of saliva and different fractions of throat wash from the 17 probable SARS patients and 12 healthy controls by using the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) in the BSL3 laboratory (18). Viral RNA was also isolated from culture supernatants of the SARS-CoV isolate, TW1 (19), human coronavirus 229E strain, and human enteric coronavirus Dallas 1 strain (American Type Culture Collection, Manassas, VA).

Quantitative Real-Time RT-PCR
The assay used forward and reverse primers and a fluorogenic probe of the SAR1S_AS Taqman assay design (Applied Biosystems, Foster City, CA). They matched to a region within a previously described region of the ORF1b (6,7), which is also completely conserved by different isolates of SARS-CoV (Figure 1A) (20,21). The sequences of the forward primer, reverse primers, and probe are 5'-CACACCCTTTCTACAGGTAGCT-3' (genome positions 15316 to 15338 of the Urbani strain) (20), 5'-GCCACACATGACCACATCTCATTAT-3' (positions 15380 to 15356) and 5'-ACGGTGTGCACACTCGGT-3' (positions 15355 to 15339), respectively. A 200-bp product covering this region was generated by using the primers (F1 and R1), the Superscript II one-step RT-PCR system (Invitrogen, San Diego, CA), and the RNA template derived from the SARS-CoV TW1 strain (19). The sequences of the primers F1 and R1 are 5' CAGAGCCATGCCTAACATGC-3' (genome positions 15239 to 15258) (20) and 5' GCATAAGCAGTTGTAGCATC-3' (positions 15439 to 15420), respectively. RT-PCR conditions were 52°C for 40 min and 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, and 68°C for 45 s. The
product was subsequently cloned into the TA cloning vector (Invitrogen, San Diego, CA) to generate the construct, ORF1b/pCRRII-TOPO (Figure 1B). The in vitro transcribed RNA was purified and quantified to determine the copy number of RNA as described previously (22). An aliquot (5 µL) of RNA isolated from the clinical sample and known amounts of the in vitro transcribed RNA (5 to 50 million copies) were subjected to real-time RT-PCR by using the SAR1S_AS primers, probe, and the Taqman one-step real-time RT-PCR master mix reagent kit (Applied Biosystems). The amplification conditions were 48°C for 30 min and 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. The ABI prism 7000 sequence detector was used to analyze the emitted fluorescence during amplification. A positive result is defined by the cycle number (CT value) required to reach the threshold as described previously (22). Precautions for PCR were followed to avoid contamination (23). Since 5 µL of 50 µL RNA eluates that were derived from 560 µL throat wash supernatant, was used in each reaction, the number of SARS-CoV RNA copies per reaction was divided by 560 µL throat wash, and that in the cell-associated component is 330 copies, corresponding to 2.5% of the total (Table 1).

SARS-CoV RNA in Components of Throat Wash

We used the following formula to calculate the copy numbers of SARS-CoV RNA in different components including the supernatant (S), the mucus-associated (M), and the cell-associated (C) components in the 5-mL throat wash, which was the starting volume in our fractionation experiment. The numbers of RNA copies in the S component equal the amount (copies/mL) in the supernatant times 5 (mL) (S = supernatant x 5 mL). Since treatment of the mucus-cell pellet with N-acetyl-L-cysteine presumably released SARS-CoV from the mucus and increased the volume twofold (from 1 mL to 2 mL), the copy numbers in the M component equal the amount in the treated supernatant (copies/mL) times 2 mL minus that from the originally untreated supernatant (1 mL) (M = treated supernatant x 2 mL – supernatant x 1 mL). The copy numbers in the C component equal the amount in the cellular fraction (copies/mL) times the volume of the cell pellet (C = cellular fraction x volume of cell pellet [in mL]). Taking patient ID17 as an example, S = 4,790 copies (958 x 5), M = 8,402 copies (4,680 x 2 – 958 x 1), and C = 330 copies (8,460 x 0.039). The amount of SARS-CoV RNA in the cell-free component, which equals the amount in the S component plus that in the M component, is 13,192 copies, corresponding to 97.5% of the total SARS-CoV RNA in 5 mL throat wash, and that in the cell-associated component is 330 copies, corresponding to 2.5% of the total (Table 1).

Table 1. Severe acute respiratory syndrome–associated coronavirus (SARS-CoV) RNA in cell-free and cell-associated components of throat wash from probable SARS cases

<table>
<thead>
<tr>
<th>Patient IDa</th>
<th>Sampling dayb</th>
<th>Cell-free component</th>
<th>Cell-associated component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>d2</td>
<td>848,000 (99.6)</td>
<td>2,982 (0.4)</td>
</tr>
<tr>
<td>2</td>
<td>d3</td>
<td>34,320 (99.7)</td>
<td>85 (0.3)</td>
</tr>
<tr>
<td>3</td>
<td>d3</td>
<td>10,680 (99.7)</td>
<td>34 (0.3)</td>
</tr>
<tr>
<td>4</td>
<td>d3</td>
<td>119,500 (98.8)</td>
<td>1,495 (1.2)</td>
</tr>
<tr>
<td>5</td>
<td>d3</td>
<td>18,160 (99.6)</td>
<td>72 (0.4)</td>
</tr>
<tr>
<td>6</td>
<td>d4</td>
<td>14,400 (95.4)</td>
<td>695 (4.6)</td>
</tr>
<tr>
<td>7</td>
<td>d4</td>
<td>631,280 (99.9)</td>
<td>416 (0.1)</td>
</tr>
<tr>
<td>8</td>
<td>d4</td>
<td>19,900 (99.4)</td>
<td>124 (0.6)</td>
</tr>
<tr>
<td>9</td>
<td>d4</td>
<td>17,800 (99.5)</td>
<td>91 (0.5)</td>
</tr>
<tr>
<td>10</td>
<td>d5</td>
<td>40,500 (99.9)</td>
<td>60 (0.1)</td>
</tr>
<tr>
<td>11</td>
<td>d5</td>
<td>3,152,000 (99.2)</td>
<td>25,146 (0.8)</td>
</tr>
<tr>
<td>12</td>
<td>d6</td>
<td>1,896,000 (99.4)</td>
<td>12,160 (0.6)</td>
</tr>
<tr>
<td>13</td>
<td>d6</td>
<td>11,100 (99.6)</td>
<td>40 (0.4)</td>
</tr>
<tr>
<td>14</td>
<td>d6</td>
<td>5,992 (99.4)</td>
<td>35 (0.6)</td>
</tr>
<tr>
<td>16</td>
<td>d8</td>
<td>33,400,000 (88.8)</td>
<td>4,222,000 (11.2)</td>
</tr>
<tr>
<td>17</td>
<td>d9</td>
<td>13,192 (97.5)</td>
<td>330 (2.5)</td>
</tr>
</tbody>
</table>

aPatients of probable SARS were diagnosed according to the World Health Organization clinical definitions (15).
bSampling day 2 (d2) is the second day of fever.

Immunofluorescence Assay

Aliquots of the cellular fraction of throat wash from patients and six healthy controls were fixed onto 12-well Teflon-coated slides and subjected to a previously described immunofluorescence assay (19). The first antibody was serum from a rabbit immunized with the recombinant nucleocapsid protein of the SARS-CoV (prepared by P.J. Chen), and the secondary antibody was the FITC-conjugated goat anti-rabbit immunoglobulin G (Pierce Biotechnology, Rockford, IL).

Statistical Analysis

Regression analysis was performed to examine the correlation between the amount in throat wash and saliva (soft-ware SPSS base 10.0, SPSS Inc., Chicago, IL).

Results

The demographic and clinical information of the 17 patients are summarized in Table 2. Viral RNA was extracted from saliva and supernatant of the throat wash and then subjected to a quantitative real-time RT-PCR assay by using the primers and probe within a highly conserved region of the ORF1b (Figure 1A) (20,21). Known amounts of the in vitro transcribed RNA covering this region were used as the standard for quantification. As shown in Figure 1B, a linear curve was observed as the
input RNA increased from 5 to 50 million copies per reaction. Positive signal was detected in the reactions containing RNA template derived from the SARS-CoV Taiwanese strain TW1 and not in those from 12 healthy controls and from two human coronaviruses (229E strain and human enteric coronavirus Dallas 1 strain) and not in the reaction containing no RNA (data not shown) (19,22).

The results of the real-time RT-PCR assay on the throat wash and saliva are summarized in Table 2. The sampling day of these patients varied from day 2 to day 9 after onset of fever, with a median of day 4. SARS-CoV RNA was readily detected in throat wash from all 17 patients. The amount of SARS-CoV RNA in the throat wash was 9.58 x 10^2 to 5.93 x 10^6 copies per mL (median 3.56 x 10^3 copies/mL). SARS-CoV RNA was also detected in saliva from all 14 available specimens. The amount of SARS-CoV RNA in the throat wash or saliva does not correlate with the sampling day (simple linear regression, coefficient of correlation r = 0.106 and 0.147, respectively), underscoring a more complex course of virus-host interaction. The amount of SARS-CoV RNA in the saliva was greater than that in the throat wash for every patient from whom both type of specimens were available. A linear relationship existed between the amounts of SARS-CoV in the saliva and throat wash (simple linear regression, r = 0.848, p < 0.005), which suggests, but does not prove, that they could originate from a common source in the respiratory tract.

To further investigate whether SARS-CoV is also present in the cellular component of the throat wash, we carried out the fractionation experiment and examined the amount of SARS-CoV RNA in different components. As shown in Table 1, SARS-CoV RNA was detected in the cell-associated component of the throat wash from all 16 specimens examined. The range of viral load was 34–422,000 copies per 5 mL throat wash. While 0.1%–11.2% of SARS-CoV RNA in the throat wash is present in the cell-associated component, a greater proportion of SARS-CoV RNA, 88.8% to 99.9%, is present in the cell-free component. This finding suggests that SARS-CoV is released very efficiently. The possibility that virus is released from the cells during thawing is unlikely, since the fractionation experiment performed for aliquots of some samples without prior freezing and thawing showed a similar result. For example, in ID7 the percentages of the cell-associated and cell-free components for an aliquot performed without freezing were 99.87% and 0.13%, respectively. These are similar to the results of 99.9% and 0.1% for another aliquot performed after freezing and thawing (Table 1).

Electron microscopic studies have shown SARS-CoV particles in the desquamated cells from bronchoalveolar lavage and lung tissues, both in the lower respiratory tract (6,8,24). Our detection of SARS-CoV RNA in the cell-

---

**Table 2. Clinical information and SARS-CoV RNA in throat wash and saliva from probable SARS case-patients**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Sampling day</th>
<th>Fever</th>
<th>Cough</th>
<th>Dyspnea</th>
<th>Chest x-ray</th>
<th>Throat wash (copies/mL)</th>
<th>Saliva (copies/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>M</td>
<td>d2</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>1.58 x 10^5</td>
<td>2.64 x 10^5</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>F</td>
<td>d3</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>4.69 x 10^7</td>
<td>1.12 x 10^7</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>F</td>
<td>d3</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>1.56 x 10^5</td>
<td>1.06 x 10^5</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>M</td>
<td>d3</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>2.39 x 10^6</td>
<td>8.22 x 10^6</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>M</td>
<td>d3</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>3.56 x 10^6</td>
<td>1.22 x 10^6</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>F</td>
<td>d4</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>2.88 x 10^4</td>
<td>7.08 x 10^3</td>
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<tr>
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<td>M</td>
<td>d4</td>
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<td>No</td>
<td>Yes</td>
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<td>9.05 x 10^4</td>
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<td>d8</td>
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<td>No</td>
<td>Yes</td>
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<tr>
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<td>F</td>
<td>d9</td>
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<td>No</td>
<td>Yes</td>
<td>9.58 x 10^4</td>
<td>2.80 x 10^4</td>
</tr>
</tbody>
</table>

*SARS-CoV, severe acute respiratory syndrome–associated coronavirus; M, male; F, female; NA = not available.

| Probable SARS was diagnosed according to the World Health Organization clinical definitions (15).

Sampling day 2 (d2) is the second day of fever.

Intubation for hypoxia was performed during the course of hospitalization.
associated component of the throat wash suggested that SARS-CoV also replicates in the upper respiratory tract. To further explore this possibility, we prepared spot slides from the cellular fraction of the throat wash and examined them with an indirect immunofluorescence assay by using a polyclonal serum from a rabbit immunized with a recombinant nucleocapsid protein of SARS-CoV. When used in the epithelial cells prepared from two randomly chosen specimens (ID11 and ID17), the postimmune serum, but not the preimmune serum, reacted with epithelial cells with a speckle pattern (Figure 2, C–D and F–G). The classification of these cells as epithelial cells was supported by the size and morphologic features of them under light microscope (Figure 2H). Only background signal was seen in the cells prepared from a healthy control (Figure 2E). These findings indicate that SARS-CoV can replicate in the epithelial cells of the upper respiratory tract, and such cells can be used in an antigen detection assay.

Discussion

We report large amounts of SARS-CoV RNA in the throat wash and saliva from probable SARS case-patients. This finding supports the possibility that SARS-CoV can be transmitted through oral droplets. Most coronaviruses are known to replicate in the epithelial cells of the respiratory or enteric tract. After budding into the pre-Golgi compartment, virus particles are released through an exocytosis-like process at the apical or basolateral surface, or both (25). Apical release is likely to facilitate the spread of virus in the respiratory or enteric tract, whereas basolateral release facilitates systemic spread. Our findings that SARS-CoV can be detected in cells derived from throat wash by the immunofluorescence assay and that most of the SARS-CoV in throat wash is present in the cell-free component suggest that after its replication in the epithelial cells, SARS-CoV is released efficiently and accumulates in the oropharynx and oral cavity, which may contribute to its transmission through oral droplets.

Practicing droplet and contact precautions prevents nosocomial transmission of SARS among healthcare workers (26). However, a cluster of SARS cases was reported among apparently protected healthcare workers during aerosol-generating procedures performed on SARS patients (27). This finding led to the controversial hypothesis of airborne transmission of SARS, in which very small particles (<5 µm) are spread in the air (17,27). A substantial proportion, 88.8% to 99.9%, of the SARS-CoV in the throat wash was present in cell-free form; this finding offers a mechanistic explanation of the possibility of airborne transmission of SARS.

Three types of specimens from the upper respiratory tract, nasopharyngeal aspirates, nasopharyngeal swab, and oropharyngeal swab have been recommended to detect SARS-CoV (28,29). However, RT-PCR performed on nasopharyngeal aspirates from SARS patients had positive rates of 32% at day 3, 50% at day 5, and 68% at day 14 (8,14). A recent study using nasopharyngeal aspirates reported a positive rate of 71% at a mean of 4.4 days (30). In this study, we reported that SARS-CoV RNA can be detected in both throat wash and saliva from all specimens examined at an average sampling day 4.8 (range day 2–9). Furthermore, specimens of throat wash from four of our study participants who came to our emergency department, a designated SARS screening site in Taipei during the SARS outbreak, were collected when radiographic evidence of pneumonia or respiratory distress syndrome had

![Figure 2](https://www.cdc.gov/eid/10/7/2004/1217/Figure2.jpg)
not been observed (Table 2). To our knowledge, this report is the first showing that SARS-CoV could be detected in probable SARS patients before lung lesions developed.

The high SARS-CoV detection rate in our study contrasts with those reported previously by using nasopharyngeal aspirates (8,14,30). One possibility is that more SARS-CoV are present in the oropharynx and oral cavity than in the nasopharynx. In spite of the differences in the dilutional factors (for example, 10 mL of normal saline in the throat wash, 1.5–2 mL in the nasopharyngeal aspirates, and none in the saliva), the amounts of SARS-CoV RNA in the throat wash and saliva in our study, 9.58 x 10^8 to 6.38 x 10^8 copies per mL, were in the same range as those previously reported for the nasopharyngeal specimens (10^3–10^8 copies/mL) (7,14). A mutually nonexclusive possibility is that more respiratory secretions, mucus, and cells can be removed from the respiratory tract through throat wash than through nasopharyngeal aspiration, nasopharyngeal swabs, and oropharyngeal swabs. Regardless of the reason, SARS-CoV was also detected in the throat wash of nine SARS patients in a previous report (6), which suggests the need to evaluate the benefit of collecting throat wash to diagnose SARS.

To our knowledge, this report is the first that demonstrates the possibility of devising a SARS-CoV antigen detection assay by using cells derived from throat wash. Since a small number of patients were examined, future study with more patients and controls is required to develop a useful diagnostic test. Technically, throat wash and saliva are easier to collect when compared with the collection of currently recommended respiratory specimens (28,29). In addition, they can be obtained without close contact between the patient and healthcare worker, and thus reduce the risk for infection of healthcare workers. Another commonly obtained sample is sputum; however, it is rarely available at the early stage of infection, when virtually no cough or only dry cough is present (1–3,13). These features, together with the high detection rate at early stage and before the development of lung lesions, suggest that throat wash and saliva are ideal specimens for early diagnosis of SARS and should be included in guidelines for sample collection for SARS diagnosis (28,29). Further studies with longitudinally collected throat wash and saliva specimens from a larger number of SARS patients would help determine the onset and duration of infectiveness, extent of infectiveness of some patients, such as superspreaders, and the response to antiviral agents.

Acknowledgments

We are indebted to all the medical personnel at the National Taiwan University Hospital for taking care of SARS patients during the outbreak in Taipei and members of the SARS Research Group of the National Taiwan University College of Medicine/National Taiwan University Hospital, including Ding-Shinn Chen, Yuan-Teh Lee, Hong-Nerng Ho, Chu-Min Teng, Ming-Fu Chang, Bor-Liang Chiang for discussion and coordination, Tun-Hou Lee at the Harvard School of Public Health for critical comments, and Chao-Min Lee for assistance.

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Dr. Wei-Kung Wang is an associate professor of virology at the Institute of Microbiology, College of Medicine, National Taiwan University. His research interests include study of viral load, immune activation markers, and the pathogenesis of viral infectious diseases, such as dengue virus and SARS-CoV.

References


Address for correspondence: Shan-Chwen Chang, Department of Internal Medicine, National Taiwan University Hospital, No. 7 Chung-Shan South Road, Taipei 100, Taiwan; fax: 8862-23971412; email: sc4030@ha.mc.ntu.edu.tw
We describe a method for comparing the ability of different alert threshold algorithms to detect malaria epidemics and use it with a dataset consisting of weekly malaria cases collected from health facilities in 10 districts of Ethiopia from 1990 to 2000. Four types of alert threshold algorithms are compared: weekly percentile, weekly mean with standard deviation (simple, moving average, and log-transformed case numbers), slide positivity proportion, and slope of weekly cases on log scale. To compare dissimilar alert types on a single scale, a curve was plotted for each type of alert, which showed potentially prevented cases versus number of alerts triggered over 10 years. Simple weekly percentile cutoffs appear to be as good as more complex algorithms for detecting malaria epidemics in Ethiopia. The comparative method developed here may be useful for testing other proposed alert thresholds and for application in other populations.

Accurate, well-validated systems to predict unusual increases in malaria cases are needed to enable timely action by public health officials to control such epidemics and mitigate their impact on human health. Such systems are particularly needed in epidemic-prone regions, such as the East African highlands. In such places, transmission is typically highly seasonal, with considerable variation from year to year, and immunity in the population is often incomplete. Consequently, epidemics, when they occur, often cause high illness and death rates, even in adults (1,2). The value of timely interventions—such as larviciding, residual house spraying, and mass drug administration—to control malaria epidemics has been documented (3), but much less evidence exists about how to identify appropriate times to take such action when resources are limited (4). Ideally, public health and vector control workers would have access to a system that provides alerts when substantial numbers of excess cases are expected, and such alerts should be sensitive (so that alerts are reliably generated when excess cases are imminent), specific (so that there are few false alarms or alerts that do not precede significant excess cases), and timely (so that, despite some inevitable delays between sounding the alert and completing interventions, adequate lead time exists to take actions that will reduce cases before they decline “naturally”).

A number of such systems have been proposed or implemented, but the comparative utility of these systems for applied public health purposes has not been rigorously established. For example, the World Health Organization has advocated the use of alerts when weekly cases exceed the 75th percentile of cases from the same week in previous years (5), and other methods, based on smoothing or parametric assumptions, have also been considered (6–8). Such methods, known as early detection systems because they detect epidemics once they have begun, can correctly identify periods that are defined by expert observers as epidemic, albeit with varying specificity. However, the ability of early detection systems to generate timely alerts that prospectively identify periods of ongoing excess transmission has not, to our knowledge, been evaluated. A detection algorithm is useful for identifying interventions only if it identifies epidemics at an early phase (9), and it (as opposed to prediction) will work only to the extent that epidemics persist (and indeed grow) over time. Thus, detecting unusual cases at one time point will be a reliable indicator that an epidemic is under way (and will be so for long enough that action taken after the warning can still have an effect).

Another approach, known as early warning, attempts to predict epidemics before unusual transmission activity begins, usually by the use of local weather or global climatic variables that are predictors of vector abundance and efficiency, and therefore of transmission potential (10–14). Such systems have the advantage of providing more advance warning than systems that rely on case counts, but climate- and weather-based systems require data not widely available to local malaria control officials in Africa in real time. Such systems also depend on relatively complex prediction algorithms that may be difficult to implement in the field. Studies of the forecasting ability of such systems are beginning to emerge (15); initial studies have focused...
on the sensitivity rather than on the specificity or timeliness of the alerts.

We describe a method for evaluating the public health value of a system to detect malaria epidemics. We use this method to evaluate several simple early detection systems for their ability to provide timely, sensitive, and specific alerts in a data series of weekly case counts from 10 locations in Ethiopia for approximately 10 years. The fundamental question we address is whether detecting excess cases for 2 weeks in a row, under a variety of working definitions of “excess,” can be the basis for a system that anticipates ongoing excess malaria cases in time for action to be taken.

Materials and Methods

Study Area and Data
We collected datasets consisting of weekly parasitologically confirmed malaria cases over an average of 10 years from health facilities in 10 districts of Ethiopia (online Appendix Figure 1; available from http://www.cdc.gov/ncidod/EID/vol10no7/03-0722_app.G1.htm). The data arise from passive surveillance systems in selected districts for the years 1990–2000. Original data collected on the basis of Ethiopian weeks (which range from 5 to 9 days) were normalized to obtain mean daily cases for each Ethiopian week, and normalized data were used for all analysis. Data are summarized in Table 1.

Epidemic Detection Algorithms To Be Tested
We investigated four classes of algorithms for triggering alert thresholds. In each case, an alert was triggered if the defined threshold was exceeded for 2 consecutive weeks. (This choice is intended to improve the specificity of the alert system for any given threshold.) If another alert was triggered within 6 months, it was ignored, on the assumption that intervening after the first alert would prevent another epidemic within the next 6 months. For the purposes of historically based thresholds (1 and 2 below), the thresholds for each year were calculated on the basis of all other years in the dataset for a given health facility, excluding the year under consideration.

Weekly Percentile
The threshold was defined as a given percentile of the case numbers obtained in the same week of all years other than the one under consideration. The use of percentile as alert threshold is straightforward, and the method is relatively insensitive to extreme observations.

Weekly Mean with Standard Deviation (SD)
We defined the threshold as the weekly mean plus a defined number of SDs. Mean and SD were calculated from case counts, smoothed case counts, or log-transformed case counts.

Slide Positivity Percentage
Some studies have indicated that the proportions of positive slides were significantly higher than the usual rate during epidemics (16,17), but whether the rise in proportion of positive slides occurs early enough to serve as a useful early detection system is not known. Slide positivity proportion was calculated from the number of blood slides tested and positive slides for malaria parasites.

Slope of Weekly Cases on Log Scale
We hypothesized that rapid multiplication of the number of normalized cases from week to week might signal onset of an epidemic. To test this hypothesis and the usefulness of detecting such changes as a predictor of epidemics, we defined a set of alert thresholds on the basis of the slope of the natural logarithm of the number of normalized cases. An advantage of the slide positivity and log slope methods over the others is that they can, in principle, be used to construct alert thresholds in the absence of retrospective data.

Comparison of Alert Thresholds
To circumvent the difficulties inherent in defining a “true” epidemic and to compare the properties of these

Table 1. Characteristics of the study areas

<table>
<thead>
<tr>
<th>District</th>
<th>Follow-up (y)</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
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thresholds on a scale that reflects the potential, operational uses of alert thresholds, we evaluated each alert threshold algorithm for the number of alerts triggered and the number of cases that could be anticipated and prevented ("potentially prevented cases") if that alert threshold were in place. Potentially prevented cases (PPC) for each alert were defined as a function of the number of cases in a defined window starting 2 weeks after each alert (to allow for time to implement control measures). The window of effectiveness was assumed to last either 8 or 24 weeks (to account for control measures whose effects are of different durations). Since no control measure would be expected to abrogate malaria cases completely, we considered two possibilities for the number of cases in each week of the window that could be prevented: 1) cases in excess of the seasonal mean and 2) cases in excess of the seasonal mean minus 1 SD. When the observed number of cases in a week is less than the seasonal mean or the seasonal mean minus the SD, PPC is set to a minimum value of zero for that week. Figure 1 depicts graphically how the PPC was calculated. For each value of each type of threshold at each health facility, the number of PPC was transformed into a proportion (percentage), by adding the number of PPC for the alerts obtained and dividing this sum by the sum of the number of potentially prevented cases, over all weeks in the dataset.

To compare the performance of dissimilar alert types on a single scale, a curve was plotted for each type of algorithm that showed mean percent of PPC (%PPC) over all districts versus average number of alerts triggered per year, with each point representing a particular threshold value. “Better” threshold types and values are those that potentially prevent higher numbers of malaria cases with smaller numbers of alerts.

Random, Annual, and Optimally Timed Alerts

To evaluate the improvement in timing of alerts provided by each of these algorithms, we calculated PPC for alerts chosen on random weeks during the sampling period. We also made comparisons to two alert-generating policies that could not have been implemented but are in some sense optimal in hindsight. First, we evaluated a policy of triggering one alert each year on the “optimal” week, i.e., the week with the maximum value of PPC. The value of PPC corresponding to the optimal week simulated an “optimally timed” policy of annual interventions; thus, it represents one alert every year. Second, we retrospectively went through data for each site to identify the optimal timing of alerts if one had perfect predictive ability; namely, we compared PPC for a single alert generated on every week of the dataset and chose the optimal week for one alert; then we went through the remaining weeks and chose the optimal week for a second alert, and so on. This system allowed us to plot an upper bound curve for the best choice of alert times, given a defined alert frequency.

Results

The dataset consists of a total of 687,903 microscopically collected malaria cases from a health facility in each of 10 districts over an average of 10 years. On average, each of the 10 health facilities treated 11–39 malaria cases daily and >300 cases per day during the peak transmission season (Table 1). In most districts, including Awasa, Zeway, Nazareth, Jimma, Diredawa, Debrezeit, and Wolayita, the number of cases showed clear seasonal fluctuation over time. Alaba, Bahirdar, and Hosana showed longer term variation, with an increasing trend in Alaba and more complex patterns in Hosana and Bahirdar. The number of cases in all districts shows a clear year-to-year variation.

The number of alerts triggered and %PPC obtained for each level of a threshold by type of algorithm varied in the 10 districts (online Appendix Figure 2). Number of alerts triggered and %PPC for a single alert threshold level are represented by a point. These points are summarized in Figure 2, which compares the performance of all algorithms on a single scale and explores the sensitivity of results to the choice of function for determining PPC [reducing cases to weekly mean, (a) and (b), or weekly mean minus 1 SD, (c) and (d)] and the choice of window of effectiveness [8 weeks, (a) and (c); 24 weeks, (b) and (d)]. All alert threshold algorithms potentially prevented a larger number of cases than random alerts, whose performance is shown as a straight line with cases increasing in proportion to the number of alerts.

The alert threshold algorithm based on percentile performed as well as or better than the other algorithms over the range of number of alerts triggered that we examined. For a given number of alerts triggered, it prevented a greater %PPC compared to other methods.
optimally timed alerts, the percentile algorithm performed well, within 10% to 20% of the best achievable performance. The slope on log scale algorithm performed slightly better than the random but much worse than the other algorithms.

Threshold algorithms defined as the weekly mean plus SDs based on different forms of the data (normalized case counts, smoothed case counts, or log-transformed case counts) performed similarly, except that the algorithms based on the smoothed cases and log-transformed cases triggered fewer alerts at a given threshold value compared to the algorithm based on normalized cases.

For highly specific threshold values (triggering relatively few alerts), the slide positivity proportion showed a lower %PPC than any other algorithm except the log slope. This pattern was reversed at more sensitive threshold values; slide positivity thresholds of ≤65% showed a higher %PPC than the other threshold methods for a given number of alerts per year.

The annual alert, which corresponds to intervening every year during a fixed optimal week (generally just before the high transmission season), prevented 28.4% of PPC. However, an equivalent %PPC was prevented by the weekly mean and percentile algorithms with only 0.5 alerts per year.

The preceding numbers refer to the weekly mean with 8-week window assessment (Figure 2a). Comparative performance of the different alert thresholds was insensitive to the length of the window and the choice of function to define potentially prevented cases (Figures 2a–d). In all cases, the percentile algorithm performed best overall, although the difference became smaller for the 24-week window.

In all alert threshold algorithms, the %PPC rises with increasing number of alerts and then levels off approximately at 0.4 to 0.6 alerts per year. The interrelationship between levels of percentile used, number of alerts triggered, and %PPC is presented in detail to illustrate the factors that would contribute to choosing a cost-effective threshold value. Table 2 shows that 85th percentile as a threshold level triggered 0.72 alerts per year with 31.9% of PPC; 80th and 75th percentiles, on the other hand, gave 0.79 and 0.9 alerts per year with 32.6% and 31.2% of PPC, respectively. For an additional 0.1–0.2 alerts per year, the gain is nil. Similarly, 70th percentile with approximately one alert every year resulted in even fewer potentially prevented cases (29.7% of PPC). Most of the possible maximum PPC can be achieved by using a weekly percentile alert threshold that can only trigger 0.4–0.6 alerts per year, and threshold based on 85th to 90th percentile trigger, on
are based on the duration of effects of common interven-
ventions. These window periods differ in effectiveness of performance of the different algorithms was tested by using alerts below the underlying mean. The sensitivity of the relative transmission periods on the assumption that such periods are the ones in which interventions are likely to prevent the most cases.

Given the wide variations in malaria transmission, no standard expectation exists about what proportion of cases can be averted with what intervention. With the assumption that the magnitude of the effect of an intervention would be related to the difference between the observed number of cases and size of the long-term seasonal mean and SD, we calculated PPC. In other words, we assumed that an intervention would lower the number of cases towards the underlying seasonal mean or, if very effective, to 1 SD below the underlying mean. The sensitivity of the relative performance of the different algorithms was tested by using different window periods (8 or 24 weeks) of effectiveness of possible intervention methods. These window periods are based on the duration of effects of common interventions, such as insecticide spraying, which have residual activity of 8 to 24 weeks (18–20), and other emergency malaria epidemic control measures such as mass drug administration that could lower the incidence of malaria within an 8- to 24-week range (21). Unlike the complex detection algorithms tested for other diseases (22–26), the algorithms compared in this study are simple to implement without the use of computers, which are currently unavailable to malaria control efforts in most parts of Africa.

At relatively smaller number of alerts triggered, threshold algorithms based on percentile anticipated the highest percentage of the potentially preventable malaria cases of all approaches. The percentile algorithm’s good performance relative to the optimally timed alerts indicates that it triggers alerts at the beginning of epidemics rather than in the middle of ongoing epidemics. Given the attractive characteristics of the percentile algorithm, a further ques-

Discussion
We have described a novel method for evaluating the performance of malaria early detection systems for their ability to trigger alerts of unusually high malaria case numbers with sufficient notice so that control measures can be implemented in time to have an effect on the epidemic. By defining the performance of an algorithm in terms of the potentially prevented cases falling in a given time window after the alerts are generated, we attempted to capture the public health value of an alert system, which is its ability to predict excess malaria cases. Given the same number of alerts triggered by different potential detection algorithms, the objective is to identify an alert threshold algorithm that triggers alerts at the beginning of unusually high transmission periods, on the assumption that such periods are the ones in which interventions are likely to prevent the most cases.

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We have described a novel method for evaluating the performance of malaria early detection systems for their ability to trigger alerts of unusually high malaria case numbers with sufficient notice so that control measures can be implemented in time to have an effect on the epidemic. By defining the performance of an algorithm in terms of the potentially prevented cases falling in a given time window after the alerts are generated, we attempted to capture the public health value of an alert system, which is its ability to predict excess malaria cases. Given the same number of alerts triggered by different potential detection algorithms, the objective is to identify an alert threshold algorithm that triggers alerts at the beginning of unusually high transmission periods, on the assumption that such periods are the ones in which interventions are likely to prevent the most cases.

Given the wide variations in malaria transmission, no standard expectation exists about what proportion of cases can be averted with what intervention. With the assumption that the magnitude of the effect of an intervention would be related to the difference between the observed number of cases and size of the long-term seasonal mean and SD, we calculated PPC. In other words, we assumed that an intervention would lower the number of cases towards the underlying seasonal mean or, if very effective, to 1 SD below the underlying mean. The sensitivity of the relative performance of the different algorithms was tested by using different window periods (8 or 24 weeks) of effectiveness of possible intervention methods. These window periods are based on the duration of effects of common interventions, such as insecticide spraying, which have residual activity of 8 to 24 weeks (18–20), and other emergency malaria epidemic control measures such as mass drug administration that could lower the incidence of malaria within an 8- to 24-week range (21). Unlike the complex detection algorithms tested for other diseases (22–26), the algorithms compared in this study are simple to implement without the use of computers, which are currently unavailable to malaria control efforts in most parts of Africa.

At relatively smaller number of alerts triggered, threshold algorithms based on percentile anticipated the highest percentage of the potentially preventable malaria cases of all approaches. The percentile algorithm’s good performance relative to the optimally timed alerts indicates that it triggers alerts at the beginning of epidemics rather than in the middle of ongoing epidemics. Given the attractive characteristics of the percentile algorithm, a further ques-

Table 2. Potentially preventable cases (PPC) by level of the seasonal percentile threshold in relation to number of alerts per year (8-week window)

<table>
<thead>
<tr>
<th>District</th>
<th>95th percentile</th>
<th>90th percentile</th>
<th>85th percentile</th>
<th>80th percentile</th>
<th>75th percentile</th>
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<tbody>
<tr>
<td></td>
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<td>No. alerts</td>
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<td>%PPC</td>
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<td>Alaba</td>
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<td>28.1</td>
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<td>0.55</td>
<td>27.9</td>
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<td>Debrezet</td>
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<td>19.8</td>
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<td>28.5</td>
<td>0.54</td>
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</tr>
<tr>
<td>Diredawa</td>
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<td>25.2</td>
<td>0.61</td>
<td>26.6</td>
<td>0.82</td>
<td>26.9</td>
</tr>
<tr>
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<td>24.9</td>
<td>0.78</td>
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<td>33.9</td>
<td>0.86</td>
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<td>0.54</td>
<td>33.9</td>
<td>0.86</td>
<td>30.5</td>
</tr>
<tr>
<td>Zeway</td>
<td>0.36</td>
<td>30.2</td>
<td>0.36</td>
<td>30.2</td>
<td>0.84</td>
<td>37.2</td>
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<td>Total</td>
<td>0.46</td>
<td>25.9</td>
<td>0.53</td>
<td>27.8</td>
<td>0.72</td>
<td>31.9</td>
</tr>
</tbody>
</table>

Figure 3. Percent of potentially preventable cases (PPC) obtained using weekly and monthly data with an 8-week window.
tion is what percentile level one should use. Beyond 0.4 to 0.6 alerts/year, the %PPC leveled off because most of the peaks with higher numbers of cases, possibly epidemic periods, were detected with fewer alerts by using 85th to 90th percentiles. The leveling off of %PPC occurs because we assume that an alert triggered at week t, which leads to application of intervention measures, will prevent another alert until week t + 24. In practical terms, an intervention initiated after an alert was triggered by a less-specific alert threshold during relatively lower transmission might provide little benefit for a community in reducing malaria transmission, especially if it consumed scarce resources that would then be unavailable during periods of higher transmission.

In situations in which cost is not an issue and yearly application of preventive measures is possible, slide positivity proportion could be recommended. It performed as well as or better than all other types of algorithms when all algorithms were set to trigger an average of one alert per year. During malaria epidemics, the slide positivity proportion becomes very high (16,17), and the rise in the proportion of positive slides may begin at the onset of the epidemic to give an early warning, as our data showed. The interannual variation in the time and intensity of the peak of malaria transmission impacts the effectiveness of the annual alert with interventions at a fixed week every year; using the slide positivity proportion would identify the right time for intervention. The limitation for using slide positivity proportion is that it requires evaluating the cut-off level in individual health facilities and revising the baseline with a change of health personnel because the baseline slide positivity proportion may vary due to differences in epidemiologic patterns of malaria and other causes of fever. Thus, although slide positivity proportion thresholds could be defined in the absence of historical data, our results suggest that such data would be required to calibrate the threshold properly for any given locality. The slope on log scale algorithm performed poorly because the largest proportional rate-of-change for the number of cases tended to occur during periods of very low case numbers (perhaps reflecting chance fluctuations).

Comparative performance of different alert thresholds was insensitive to the length of the window and the choice of function to define potentially prevented cases. This study indicated the use of weekly data rather than monthly data in constructing threshold methods and in follow-up prevented more cases, consistent with the World Health Organization’s recommendations (5).

A key limitation of our study was that the use of a long-term measure of disease frequency from a retrospective dataset assumes that the long-term trend did not change significantly and that the method of data collection remained the same. Factors such as change of laboratory technician affect the number of slides that are judged positive for malaria parasites. Such changes should be considered, and revising the threshold values frequently with the most recent data and standardized training of laboratory technicians are advisable. Moreover, existing interventions (which may, in some places, have been based in part on algorithms of the sort we considered) could also interfere with the trend. In this analysis, we did not exclude epidemic years from the data since, on the one hand, we do not have a standard definition of malaria epidemics and, on the other hand, all possible data points should be used to calculate measures of disease frequency and scatter to come up with potential threshold levels unless the data points were considered as outliers.

We deliberately chose to evaluate only simple, early detection algorithms, rather than more complex ones that might require climate or weather data or complicated statistical models. In the dataset we considered, the best of these simple algorithms performed quite well relative to the best possible algorithm, which suggests that they may be adequate for many purposes. In principle, the method we propose could easily be applied to evaluate more complex, early warning algorithms and to test whether their added complexity results in substantially better performance. It is an open question whether the same methods would work as well in localities (or for diseases) with different patterns of variation in incidence, for example, in those with less pronounced seasonal peaks in incidence.

In conclusion, we have shown that simple weekly percentile cutoffs appear to perform well for detecting malaria epidemics in Ethiopia. The ability to identify periods with a higher number of malaria cases by using an early detection method will enable the more rational application of malaria control methods. The comparative technique developed in this study may be useful for testing other proposed alert threshold methods and for application in other populations and other diseases.

Acknowledgments

We thank the Ministry of Health of Ethiopia for allowing us to access the information, and Andrew Spielman and Christina Mills for comments.

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Dr. Teklehaimanot worked as a medical director and zonal malaria control program officer in Ethiopia for 4 years. Since 2001, he has been enrolled in the doctoral program in epidemiology at the Harvard School of Public Health. His primary research interest is in early warning of malaria epidemics.
References


Address for correspondence: Hailay D. Teklehaimanot, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Ave, Boston, MA 02115, USA; fax: 617-566-7805; email: htekleha@hsph.harvard.edu
Central Africa was the epicenter of the HIV type 1 (HIV-1) pandemic. Understanding the early epidemic in the Democratic Republic of the Congo, formerly Zaire, could provide insight into how HIV evolved and assist vaccine design and intervention efforts. Using enzyme immunosorbent assays, we tested 3,988 serum samples collected in Kinshasa in the mid-1980s and confirmed seroreactivity by Western blot. Polymerase chain reaction of gag p17, env C2V3C3, and/or gp41; DNA sequencing; and genetic analyses were performed. Gene regions representing all the HIV-1 group M clades and unclassifiable sequences were found. From two or three short gene regions, 37% of the strains represented recombinant viruses, multiple infections, or both, which suggests that if whole genome sequences were available, most of these strains would have mosaic genomes. We propose that the HIV epidemic was well established in central Africa by the early 1980s and that some recombinant viruses most likely seeded the early global epidemic.

Almost 70% of all HIV type 1 (HIV-1) infections worldwide are found in sub-Saharan Africa, and central Africa is the only region where all HIV-1 groups and group M subtypes have been identified (1–3). This broad range of diversity suggested central Africa as the epicenter of the HIV-1 pandemic (1). Phylogenetic reconstruction has shown that HIV-1 appears in three distinct lineages, groups M, N, and O (4–7), and each is believed to have arisen through separate zoonotic infections with chimpanzee simian immunodeficiency virus strains in central Africa (8,9). HIV-1 group M viruses are primarily responsible for the current global epidemic, while group O infections are far fewer and generally found in west-central Africa. Rare strains of group N viruses have been identified in Cameroon, also located in western equatorial Africa. The group M viruses can be subdivided into 9 subtypes, A–D, F–H, J, and K, and at least 14 circulating recombinant forms (CRFs) (http://hiv-web.lanl.gov). These phylogenetic clusterings of HIV strains, subtypes, and CRFs are important records of the epidemic histories of HIV, and they highlight the differences in their geographic distributions and areas where they are endemic.

Some of the earliest characterized whole-genome HIV-1 sequences were collected in Zaire, currently called the Democratic Republic of the Congo (DRC), in the late 1970s and early 1980s. During this time, Kinshasa, the capital of Zaire, was the largest urban area in central Africa, with a population estimated at 2.5 million (10). From 1984 through 1991, the Zaire Department of Public Health conducted a long-term collaborative HIV research and surveillance program, Project SIDA, with the U.S. Department of Health and Human Services and the Institute of Tropical Medicine, Antwerp, Belgium. In 1991, Project SIDA was abruptly terminated because of civil unrest.

A low and stable HIV prevalence in Zaire/DRC from 1976–1997 (11,12) has been documented, despite social and political upheavals. A genetic survey of HIV strains performed in three regions of the DRC in 1997 demonstrated a high diversity of HIV-1 group M subtypes (1). Serum samples, available from Project SIDA, offered us the opportunity to characterize HIV strains collected in Kinshasa, Zaire, in 1984 and 1986 from employees at Mama Yemo Hospital (13,14). The results provide new insights into the dynamics of HIV infections in a low-prevalence area where multiple subtypes cocirculate, early in the global HIV-1 epidemic.

Methods

Specimens and Serologic Analyses

Serum samples acquired through Project SIDA from a 1984 and 1986 cross-sectional study of HIV infection among hospital employees at Mama Yemo Hospital in Kinshasa, Zaire were sent to the National Institutes of
Health (NIH) and stored at −20°C. A total of 3.988 serum samples were sent to the Centers for Disease Control and Prevention for serologic and genetic analysis. Specimens numbered 30,000 through 32,000 were collected in 1984. Samples numbered 33,000 through 35,585 were collected among the same population in 1986. The specimens tested represent a convenience sampling from the U.S. repository at NIH and did not include all or only the samples in the original study. The volumes of the specimens ranged from approximately 200 μL to 4 mL. All samples were tested in two separate whole viral-lysat enzyme immunosorbent assay (EIA) for antibodies to HIV-1 and HIV-2 (Genetic Systems, Redmond, WA). Samples with insufficient volumes of serum were excluded from the analysis. Serum specimens reactive by EIA were further tested by using an HIV-1/2 Western blot assay (Genelabs Diagnostics, Singapore, version 2.2).

RNA Extraction, RT, and PCR

Samples with sufficient volumes of serum for reverse transcription–polymerase chain reaction (RT-PCR) amplification with three different sets of primers were selected for further study. RNA was extracted from serum by either the NucliSens nucleic acid manual or automated protocols (Organon Teknika, Boxtel, the Netherlands). RT-PCR was performed by using the Promega RT kit (Promega, Madison, WI) following the manufacturer’s protocol. PCR reactions were placed in a GeneAMP 9600 thermocycler (Perkin-Elmer Cetus, Norwalk, CT) for 35–40 cycles as follows: 94°C for 30s, 55°C for 30s, and 72°C for 60s for all but env gp41, which was at 50°C for 30s and 72°C for 60s, with the final extension at 72°C for 5 min. Nested PCR products were electrophoresed in 1.5% agarose gels (Gibco, Grand Island, NY) and visualized with ethidium bromide staining. To avoid contamination, sample processing and pre-PCR set-up were performed in different rooms than post-PCR manipulations. All samples with discordant phylogenies in different gene regions were verified by having a different person reamplify and resequence the gene regions from a second, unopened vial of serum, if available, or from the previously used vial.

Primers

For nested PCR amplification of 380-bp env C2V3C3 fragments, two sets were used: JH44F and JH35MR (outside) and JH33F and JH48R (nested) (15), and MK369 and MK4616 (outside) and MK650 and CO601 (nested) (15). Primers for PCR amplification of a 475-bp gag p17 fragment were CL1028 and AB1033 (outside) and CL1029 and AB1032 (nested) (16); those used for a 460-bp fragment of env gp41 were GP40F1 and GP41R1 (outside) and GP46F2 and GP48R2 or GP47R2 (nested) (17).

Sequencing PCR Products

The PCR-amplified products were purified with Qiagen PCR purification kits (Qiagen Inc, Chatsworth, CA) and directly sequenced by using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems/Perkin Elmer, Foster City, CA) and both forward and reverse nested PCR primers. The sequencing reactions were purified by using the DyeEx Spin Kit (Qiagen–USA, Valencia, CA) and resolved on a 377 ABI Automated DNA Cycle Sequencer (Applied Biosystems).

Phylogenetic Analysis

Sequences were edited by using Sequencher Software v.3.1 (Gene Codes Corp., Madison, WI) and aligned with the Se-Al sequence alignment editor v.1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=seal). Sequences from DRC collected in 1997 (1) were truncated to align with our C2V3C3 sequences, and gaps and ambiguous positions were removed, resulting in an alignment of 304 nt sequences; the first 60 nt represented the C2 region. For the outgroup in our tree, a consensus sequence representing the most common nucleotide at each position for each subtype represented in the Zaire/DRC dataset was selected, and then an overall DRC consensus sequence (DRCcons) was inferred. No positions were undefined in any of the consensus sequences, and no unclassified or unresolved (subtype assignment) sequences were included in the consensus sequence calculation. The Modeltest program, v.3.1 (18) was used to test for a statistically justified model of DNA substitution. The model of evolutionary change used in the tree was Transversion Model (TVM) + g (0.8575), where g is the shape parameter of the γ distribution (heterogeneity among sites), and TVM is the model of substitution whereby A↔G = C↔T and the other four rates are unique. To search for the best tree with such a large dataset (280 taxa) using the DRCcons sequence as the outgroup, several relatively fast tree-building algorithms were employed: Neighbor (http://www.t10.lanl.gov/billb/neighbor/index.html), neighbor-joining (PAUP), BioNJ (PAUP), and Fitch (PAUP). Trees derived from these programs were input to the PAUP* (19) tree scores program to compare the generated log likelihood scores. The neighbor-joining tree program was selected for our phylogenetic analysis since it had the best log likelihood score, although it was not substantially better (Shimodaira-Hasegawa test) than the BioNJ or Neighbor trees.

Genetic Distance Analysis

Subtype and CRF designations were determined from the phylogenetic analysis, and sequences grouping in subtypes were input into the MEGA program version 2.1 (http://www.megasoftware.net) to calculate the means and
standard errors of the intrasubtype/intraCRF diversity, using the Kimura 2P distance model.

Results

All 3,988 serum samples were tested by EIA, and 209 seroreactive samples were further tested by Western blot: 140 (3.5%) were HIV-1 seropositive, and 69 (1.7%) had indeterminate Western blot patterns. Samples from infected persons who have not yet seroconverted can display indeterminate Western blot patterns. If we were to assume that all 69 persons with indeterminate Western blot results were seroconverting at the time of sample collection, then the upper limit for the frequency of HIV infections within these early samples was still low at 5.2%. No HIV-2 EIA-positive specimens were confirmed by HIV-2 Western blot.

Table 1. Comparison of intrasubtype/CRF distribution

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Nucleotide distance (%)</td>
</tr>
<tr>
<td>A</td>
<td>22</td>
<td>15.1</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>13.1</td>
</tr>
<tr>
<td>D</td>
<td>10</td>
<td>12.1</td>
</tr>
<tr>
<td>CRF01-AE</td>
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<td>9.6</td>
</tr>
<tr>
<td>G</td>
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<td>15.6</td>
</tr>
<tr>
<td>F1</td>
<td>3</td>
<td>10.0</td>
</tr>
<tr>
<td>H</td>
<td>2</td>
<td>18.7</td>
</tr>
<tr>
<td>J</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>K</td>
<td>1</td>
<td>–</td>
</tr>
</tbody>
</table>

CRF, circulating recombinant forms; DRC, Democratic Republic of Congo.

bSignificant difference (distances significance test = t test, frequency of subtypes significance test = Fisher exact test).
To determine an estimate of potentially “pure” subtypes, we analyzed 66 of our mid-1980s HIV strains from which we were able to amplify and sequence two or three gene regions: 53 (80.3%) viruses were amplified in all three gene regions (p17, C2V3C3, and gp41). Figure 2 shows the viruses with concordant phylogenies (possibly pure subtypes) in the sequenced gene regions as follows: A (27%), C (3%), D (18%), F1 (2%), G (8%), K (3%), and our unusual B/D virus (2%). The remaining 37% of the strains represented recombinant viruses; 32% of these appeared to be unique recombinants, except where indicated in Table 2, and 5% were CRF01_AE, a mosaic lineage containing predominantly subtype A and a unique env lineage called E. Not only was this circulating recombinant form present by the mid-1980s, but only env subtypes A, D, and G were more prevalent. Some of the discrepant gene regions possibly indicate the presence of dual/multiple infections, and the sets of specific gene region primers selectively amplified different strains. However, since our hospital worker population does not represent a high-risk group, the prevalence of infections in Kinshasa was low, and recombinant viruses are generally rapidly observed following dual infections (20), we henceforth refer to samples with discordant gene regions as recombinant viruses.

**Discussion**

Despite a low frequency of HIV infections in Zaire in the mid-1980s compared to the level of infections currently observed in Africa, we found a remarkably high diversity of HIV-1 strains, with gene regions representing all the group M clades as well as unclassifiable regions. Intr subtype diversity was already high among our samples, indicating that the HIV epidemic in Kinshasa was already mature by the mid-1980s. No significant change was seen in the frequency of group M subtypes between
our early Kinshasa strains and a set of DRC samples collected in 1997 (1), despite political and social upheavals from 1986 to 1997.

We hypothesized that if the HIV epidemic originated in central Africa, phylogenetic analysis of our 1984/1986 Kinshasa and 1997 DRC C2V3C3 sequences might provide a more detailed history of HIV-1 evolution. We found a spectrum of diversity both within and between currently recognized HIV-1 group M subtypes and sub-subtypes (Figure 1). Some lineages, thought to represent discrete subtypes (or sub-subtypes) within the current global epidemic, no longer appeared distinct when analyzed in the context of the large diversity within Zaire/DRC sequences, i.e., multiple, distinct C2V3C3 subtype A lineages; new lineages containing unclassifiable strains; a continuum of diversity within and between the F1 and F2 sub-subtypes; and deep, unclassifiable branches. These findings demonstrate that the HIV diversity in the mid-1980s in Kinshasa was far more complex than in strains currently found in other parts of the world.

Another finding was the placement of C2V3C3 sequences of CRF01-AE in the apical branches of a lineage containing subtype A and unclassifiable sequences as basal branches. CRF01-AE is generally believed to be a recombinant virus with \textit{gag} and \textit{pol} genes sharing a common ancestry with subtype A, while the \textit{env} and \textit{vpu} genes are derived from a currently unknown parental strain, subtype E. However, others hypothesize that the unique subtype E portion of envelope is not a result of recombination (21) but may be because a higher evolutionary rate in the \textit{env} and \textit{vpu} genes make it unrecognizable as subtype A. Our data may be the first demonstration of subtype E \textit{env} sequences grouping within a cluster of subtype A and previously unclassifiable sequences.

A combined Zaire/DRC consensus sequence used as an outgroup was very short and located near the center of the tree, close to the expected location of the hypothetical ancestor of the HIV-1 group M subtypes. This finding, along with the complexity of the combined phylogenetic tree, supports the theory that the group M subtypes evolved from a common ancestor, which may have originated in this part of Africa.

Since less than 1/10 of the genome was sequenced and analyzed in the present study, and recombination can occur anywhere along the genome, 37% recombinant viruses is very likely a significant underestimate of the actual frequency of chimeric strains cocirculating in Kinshasa by the mid-1980s. In fact, most HIV-1 strains may have contained recombinant genomes by that time. Unfortunately, our data do not allow us to discriminate whether this level of recombination through superinfection intensified the fitness of HIV strains at that time or merely contributed to viral diversity.

Although the precise ancestry of HIV-1 is still uncertain, it appears to have a zoonotic origin (8,9). Ecologic factors that would have allowed human exposure to a natural nonhuman primate host carrying a precursor virus to HIV were therefore instrumental in introducing the virus.

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Table 2. Subtypes of unique recombinant viruses shown in Figure 2.

<table>
<thead>
<tr>
<th>Viruses*</th>
<th>Specific gene region</th>
</tr>
</thead>
<tbody>
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<td></td>
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</tr>
<tr>
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</tr>
<tr>
<td>D</td>
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<td>G</td>
<td>A</td>
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<td>?</td>
<td>H</td>
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</tr>
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<td>C</td>
<td>C</td>
</tr>
<tr>
<td>A</td>
<td>–</td>
</tr>
<tr>
<td>F1</td>
<td>A</td>
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<td></td>
</tr>
<tr>
<td>A</td>
<td>?</td>
</tr>
<tr>
<td>n = 2</td>
<td>J</td>
</tr>
</tbody>
</table>

*Except where noted, each row represents a single virus.

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into humans. This fact suggests that zoonotic introductions of HIV may have occurred in isolated populations but went unnoticed as long as the recipients remained isolated (11) (Figure 3A). However, with increasing movements from rural areas to cities, which occurred in sub-Saharan Africa during the 1960s and 1970s, such isolation became, or was becoming, increasingly rare. The number of sub-Saharan African cities with >500,000 inhabitants rapidly increased from 3 in 1960 to 28 by 1980 (23). From 1965 to 1985, the proportion of the total population living in urban areas of central Africa rose from 21% to 35% and in western Africa from 17% to 29%.

Transmission of HIV would have amplified among high-risk populations, such as commercial sex workers, where HIV superinfections generating recombinant viruses could have resulted (Figure 3B). These recombinants could then have been spread to other, lower risk persons, such as men who visit female sex workers, their spouses, other heterosexual partners, and persons at highest risk for sexually transmitted diseases (24). Other transmission routes could also have helped create and spread recombinant viruses. For example, in 1986, studies from Kinshasa showed a strong association between receiving medical injections and HIV seropositivity among HIV-seropositive infants born to seronegative mothers (25), among hospitalized children 2–14 years of age (26), and among healthcare workers (27). Demand for blood transfusions was also high because of malaria-associated anemia, pregnancy-
related complications, and sickle-cell anemia (28,29); at this time, blood was generally not screened for HIV infection (30). Therefore, many factors associated with population migration and urbanization, patterns of sexual activity, and medical practices could have played roles in producing and spreading high numbers of recombinant viruses in Kinshasa by the mid-1980s.

When HIV subtypes were initially genetically characterized in the early 1990s, the first identified viruses were assumed to represent pure subtypes, and viruses found afterward were compared to these prototypic strains. However, our data demonstrate that substantial intersubtype recombination had already occurred at the time when HIV-1 viruses were initially classified (Figure 3C). With so many recombinant viruses present at the cusp of the global HIV epidemic, at least some of the recombinant viruses in central Africa were likely classified as pure subtypes after being exported from Africa and establishing regional epidemics in other parts of the world. This realization could affect our current understanding of the range of diversity within the HIV epidemic (http://hiv-web.lanl.gov/content/hiv-db/REVIEWS/PEETERS2000/Peeters.html); of immunogen selection for vaccine design (31,32), especially in light of superinfections involving multiple subtypes (33); and of mathematical models based on molecular clocks and nonrecombinant strains (33,34,35) for dating the introduction of HIV into humans.

Acknowledgments

We thank Andrew Leigh Brown for critical review of the manuscript and Kimberly Distel for editorial review.

Dr. Kalish is team leader of the Viral Evolution and Transmission Laboratory, HIV and Retrovirology Branch, Division of AIDS, STD, and TB Laboratory Research, National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention. Her research interests include the evolution and molecular epidemiology of HIV, the study of its unusual transmission migration and urbanization, patterns of sexual activity, and medical practices could have played roles in producing and spreading high numbers of recombinant viruses in Kinshasa by the mid-1980s.

References


Address for correspondence: Marcia Kalish, Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop G19, Atlanta, Georgia 30333, USA; fax: 404-639-3254; email: mkalish@cdc.gov
Complete DNA sequences of the small subunit ribosomal RNA (SSUrRNA) gene and partial sequences of three other loci were obtained from three variant-type and three classic-type Plasmodium ovale isolates from Southeast Asia and compared with GenBank-available data. Three different SSUrRNA sequences (Pov 1–3) were found in each variant-type isolate, and two different SSUrRNA sequences (Poc 1–2) in each classic-type isolate. Pov 1–3 were closer to sequences previously found in the Cameroon and MAL/MAI isolates, whereas Poc 1–2 were closer to sequences previously found in two clones of the Nigerian I/CDC strain. The 3’ half of Pov 1–3 was identical to the partial sequence of the SSUrRNA gene from the London School (LS) strain. Results support grouping P. ovale into two groups, the classic type (including the Nigerian I/CDC strain) and the variant type (Cameroon, MAL/MAI, and LS isolates).

The geographic range of the human malaria parasite Plasmodium ovale has been thought to be mostly limited to tropical Africa, the Middle East, Papua New Guinea, and Irian Jaya in Indonesia; it has rarely been described in other countries of Southeast Asia. More recently, however, with the aid of polymerase chain reaction (PCR)-based species identification and improved microscopic techniques, P. ovale infections have been frequently reported in Southeast Asia (1,2). P. ovale may represent an emerging cause of benign and relapsing tertian malaria in this region or, alternatively, may have been overlooked in previous surveys based on classic microscopy techniques (1). The widespread distribution of P. ovale in Southeast Asia affects the choice of appropriate drugs for malaria chemoprophylaxis in travelers, since most currently used regimens are not effective against the dormant liver stages of P. ovale and P. vivax, which may cause relapses several months after the primary infection (3).

During our previous molecular studies of P. ovale in southern Vietnam (4), we found two field isolates whose partial sequences at the block 9 region (5) of the small subunit ribosomal RNA (SSUrRNA) genes had a deletion of 2 nt (G-G) and a substitution of 1 nt (C to T), when compared with the classic type, the Nigerian I/CDC strain (6). These polymorphisms had practical implications, since they occurred in the target of a diagnostic oligonucleotide probe used by the commercially available microtiter-plate hybridization (MPH) method for malaria diagnosis (4). Soon after, the same sequence variation was reported in three cases imported from Africa into Japan (7); all patients had single infections with the variant P. ovale. Later, variant-type sequences were found in the Cameroon (8) and MAL/MAI isolates (L.K. Basco, unpub. data), as well as in isolates from other Southeast Asian countries such as Thailand, Laos, Myanmar, and Indonesia (9–12). Four features of sequence variation in P. ovale soon became clear: 1) both classic and variant-type parasites occurred in sympatry (i.e., they co-occurred in the same disease-endemic areas); 2) parasites with variant-type sequences did not differ morphologically from classic parasites; 3) variant-type parasites were present in both Asia and Africa; and 4) parasites with variant-type sequences tended to produce higher parasitemia levels and higher proportions of single-species infection, when compared with classic P. ovale infections acquired in the same region (2,11).

In contrast with P. falciparum and P. vivax, little is known about the patterns of genetic diversity in field isolates of P. ovale. So far, full sequences of the SSUrRNA gene have been analyzed for only three isolates, the Nigerian I/CDC strain (6) and two African isolates, Cameroon and MAL/MAI; partial sequences are also available only for four isolates, the London School of Hygiene and Tropical Medicine strain (LS train) and the Nigerian I/CDC strain (13), and two isolates from Papua New Guinea (14) and Ghana (C. Severini et al., unpub. data). The cysteine protease gene was sequenced only for...
the Nigerian I/CDC (15), whereas the Harding strain is the only source of available sequence for the cytochrome b (cyt b) gene (16). More recently, two types of sequences have been characterized for the ookinete surface protein genes, Pos 25, Pos 28-1, and Pos 28-2 in P. ovale isolates from Thailand (17,18). They correspond to the two types of SSUrRNA genes, Nigerian I/CDC and LS, which suggests that two sequence types might represent distinct variants or subspecies (13,18).

We have obtained sequence data of the SSUrRNA, cysteine protease, ookinete surface protein, and cyt b genes of P. ovale isolates from Myanmar and Indonesia and compared our data with GenBank-available sequences. Our analyses of both nuclear and mitochondrial genes provide further support to the division of P. ovale into at least two types.

Materials and Methods

Field P. ovale Isolates

All isolates were obtained during our recent field surveys in Myanmar and Indonesia (2,11,12). For molecular analysis of the variant and classic types, patients with single infections were selected. The variant isolates we analyzed were ST243 (Rakhine State) and MC53 (Tanintharyi Division), both from Myanmar, and M474 (Flores Island, eastern Indonesia). The three classic isolates of M3 (Shan State), M4 (Bago Division), and T134 (Mon State) were collected from Myanmar.

Isolation of Parasite DNA and Confirmation of P. ovale by Sequence Analysis

Parasite DNA templates were isolated from blood by using a DNA isolation Kit (High Pure PCR Template Preparation Kit, Boehringer Mannheim, Germany). Then the target sequences at the block 9 region used for PCR-based diagnosis were further analyzed to confirm the presence of the variant- or classic-type in P. ovale–positive samples. Amplified DNA products using the P1F-Up and specific reverse (PoR2) primers (Table 1) underwent direct sequencing, whereas the first PCR products were cloned into the pCR II plasmid from a TA Cloning Kit (Invitrogen, San Diego, CA). The target fragments of 12 positive clones from each sample were sequenced by using Big Dye Terminator sequencing kit on an ABI 310 sequencer (PE Applied Biosystems, Foster City, CA).

Table 1. Oligonucleotide primers used in this study

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<th>Sequences (5’→3’)</th>
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<td></td>
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<td></td>
<td>18S R</td>
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<td>Po8R1C</td>
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<td>Po1R1-in</td>
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<td></td>
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<tr>
<td></td>
<td>Po4R1-in</td>
<td>CAATATGAATTTCAATCC</td>
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Analysis of SSUrRNA, Cysteine Protease, and cyt b Genes

Primer sets used were shown in Table 1. For analysis of the SSUrRNA gene, PCR amplification was performed by using AmpliTaq Gold polymerase (PE Applied Biosystems) at 96°C for 10 min, 36 cycles at 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min, followed by one cycle at 72°C for 10 min.

For analysis of the cysteine protease and cyt b genes, PCR conditions were slightly modified from the original methods (15,16). The conditions used were one cycle at 96°C for 10 min, 36 cycles at 94°C for 30 s, 50°C for 1 min, and 72°C for 90 s, followed by one cycle at 72°C for 10 min. The amplified PCR products were cloned into the pCR II vector. Plasmid DNA was purified from the positive colonies and sequenced in both directions by using the primers described in Table 1 in combination with M13 sequencing primers. Any ambiguity and putative polymorphism was checked by additional amplification and sequencing. Sequences obtained were compared with those reported in databases.

Gene sequences used for the SSUrRNA genes were clone 9 and 26 of the Nigerian I/CDC strain (6), isolates from Cameroon (8), MAL/MAI (X99790), Papua New Guinea (14), Ghana (AJ250701), and two strains of the Nigerian I/CDC and the London School of Hygiene and Tropical Medicine (13). For the cysteine protease, P. ovale (data not shown), the sequence was unknown. Partial sequences from Cameroon isolate (Figure 1). However, whether all of them were A (sexual)-type genes or sequences included S (sexual)- or O (ookinete/oocyst)-type (>97% identity with Poc 1–2) genes was unknown. Hereafter, these sequences are referred to as Pov 1–3 for the variant-type and as Poc 1–2 for the classic-type.

The differences among Pov 1–3 were seen at the 5′ half (Figure 1). When compared with the four complete sequences in GenBank, the Cameroon and MAL/MAI isolates were grouped as variant-type (>99% identity with Pov 1–3 and <97% with Poc 1–2). Both African isolates also shared the same mutation at the block 9 region (nucleotide positions of 1158–1160). Particularly, the sequence found in Cameroon isolate resembled that of Pov 1 (only 4-bp difference).

The alignment of four partial sequences showed that, despite their same origin, the partial sequence of the Nigerian I/CDC (13) also showed 9-bp and 5-bp differences from those of clones 9 and 26, respectively. Among these isolates, the LS strain possessed the same sequence as the 3′ half of Pov 1–3, and thus it was grouped as variant type (<96% identity with Poc 1–2). The sequence of the Papua New Guinea isolate was more similar to that of clone 9 of the Nigerian I/CDC (≥98.8% identity with Poc 1 and <97% with Pov 1–3) than to that of clone 26 or Poc 2 (98.2%–98.4% identity). The Ghana isolate was also grouped as classic-type (>97% identity with the Nigerian I/CDC or Poc 1–2 and <92% with Pov 1–3). These results suggest that the Papua New Guinea, Ghana, and our classic isolates are members of the classic- (Nigerian I/CDC-) type group, whereas the Cameroon and MAL/MAI isolates, as well as our variant isolates, are all members of the variant- (LS-) type group.

Sequence Analysis of the Cysteine Protease and the Ookinete Surface Protein Genes

The analysis of 531 bp of the cysteine protease genes, when compared with the reported sequence of the Nigerian I/CDC, showed that variant isolates differed at 19 bp (3.6%) with eight nonsilent mutations and that classic-type isolates had an almost identical sequence, except for a single base at position 700 (nonsilent substitution from Pro to Ala) (Table 2). Because the same substitution is also seen in the variant P. ovale, P. falciparum, P. vivax, P. malariae, P. reichenowi, and P. cynomolgi (data not shown), this nucleotide may have been misread in the sequence of the Nigerian I/CDC strain; if so, sequences of classic-type iso-
lates are identical to those of the Nigerian I/CDC strain. At the amino acid level, the variant isolates showed 96.0% sequence identity with the classic isolates.

Tachibana et al. (17) have analyzed the ookinete surface protein genes in Thai isolates and reported that two (Nigerian I/CDC and LS) types of *P. ovale*, defined by the SSUrRNA genes, have distinct sequences. Our nearly complete sequences of *Pos 25*, *Pos 28-1*, and *Pos 28-2* in variant isolates were identical to those found in LS-type Thai isolates, while sequences in the classic isolates were identical to those of the Nigerian I/CDC-type (data not shown).

### Sequence Analysis of the cyt b Gene

The analysis of 1035 bp of the cyt b genes showed that variant- and classic-type *P. ovale* isolates differed from each other at 12 bp, with one nonsilent substitution (Table 3). Sequences of variant isolates differed from those reported for the Harding strain at 15 bp (1.4%), with two nonsilent substitutions. The SSUrRNA gene of the Harding strain was not reported yet, and whether this strain is of the classic or variant type is not known. From its cyt b sequence, it was expected that this strain may belong to the classic group, despite the differences between the

**Table 2. Cysteine protease genes in different *Plasmodium ovale* isolates**

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<th>501</th>
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<td><strong>A</strong></td>
<td><strong>T</strong></td>
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<td><strong>A</strong></td>
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<tr>
<td>Variant isolate</td>
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<td>G</td>
<td>G</td>
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<td>C</td>
<td>G</td>
<td>T</td>
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(N→D) (P→A) (T→K) (K→R) (H→D) (S→A) (E→A)

Nucleotide numbers in boldface indicate positions resulting in nonsilent mutations (parentheses).
based on their grouped as variant type and Nigerian I/CDC or classic type of two different sequences in nuclear genes of parasites of the cysteine protease gene all confirmed the occurrence face antigen gene, presented here and elsewhere (18), and the type isolates shared the same mutations at the block 9 in was confirmed in Vietnam (4) and Africa (7); all variant-LS. Later, the presence of LS-type or variant-type rated into two types or subspecies, Nigerian I/CDC and genes, Li et al. (13) suggested that Harding strain and describe for classic-type and variant-type

Discussion

By analyzing the 3′ half of the P. ovale SSUrRNA genes, Li et al. (13) suggested that P. ovale might be separated into two types or subspecies, Nigerian I/CDC and LS. Later, the presence of LS-type or variant-type P. ovale was confirmed in Vietnam (4) and Africa (7); all variant-type isolates shared the same mutations at the block 9 in the SSUrRNA gene. Sequence analyses of the ookinite surface antigen gene, presented here and elsewhere (18), and of the cysteine protease gene all confirmed the occurrence of two different sequences in nuclear genes of parasites grouped as variant type and Nigerian I/CDC or classic type based on their SSUrRNA gene sequence.

Whether the different sequences of SSUrRNA genes we describe for classic-type and variant-type P. ovale isolates correspond to A genes or include S or O genes is unclear (5,29). In P. falciparum (30) and P. vivax (5), extensive pairwise sequence diversity (>13% difference) has been reported between A and S or O genes. In both classic type and the variant isolates, however, SSUrRNA gene sequences were quite similar to each other (<4% difference), which suggests that they may all correspond to A genes. The occurrence of different A gene–like sequences may be a distinctive feature of P. ovale, indicating a possible field for future research.

Because of the strict sequence conservation of the mitochondrial cyt b gene in natural isolates of the human malaria parasites P. falciparum and P. vivax, the divergence we found between sequences from variant- and classic-type parasites are putatively of major importance in defining two genetically distinct types of P. ovale. Analyzing the SSUrRNA gene of the Harding strain and determining whether this strain belongs to the variant-type or classic-type group or a third, poorly characterized group would be of interest.

The prevalence and geographic distribution of P. ovale, the last human malaria parasite to be described, have elicited little interest until recently. We have previously shown that P. ovale is a widespread human pathogen in Southeast Asia (1,2); here we suggest that, in both Southeast Asia and Africa, at least two different types of P. ovale circulate in human hosts. This situation is reminiscent of that recently described for P. vivax, which may be divided into two types occurring respectively in the Old World and the New World (31). However, both variants of P. ovale (in contrast to those of P. vivax) occur in sympatry, which suggests that the genetic differentiation between them is not associated with geographic isolation. Moreover, the fact that human infections with variant-type P. ovale tend to be associated with higher levels of parasitemia, when compared with levels associated with classic-type parasites (2,4,11), may be the result of more dramatic biologic differences between these types, with possible clinical implications.

Acknowledgments

We thank P.T. Htoon, K. Lin, H. Kerong, M. Torii, O. Kaneko, and Y. Otsuka for their help.

This study was supported by the Grant-in-Aid for Scientific Research B2 (13576006, 15406014) and C (14570213) from the

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</tr>
</tbody>
</table>

Table 3. cyt b genes in different Plasmodium ovale isolates

Figure 2. A dendrogram based on cytochrome b sequences of Plasmodium species including P. ovale variant and classic isolates. Bootstrap values are provided as percentages over 1,000 replications.
Japan Society for Promotion of Science and by the Japanese Ministry of Health, Labor and Welfare (13C-5).

Dr. Win is a postdoctoral fellow at the Seattle Biomedical Research Institute, Seattle, Washington. Her current research interests include analysis of gene regulation in the ribosome of malaria parasites and assessment of specific targets of antimalarial drugs.

References


Address for correspondence: Fumihiko Kawamoto, Department of Social and Environmental Medicine, Institute of Scientific Research, Oita University Faculty of Medicine, Oita 879-5593, Japan; fax: +81-97-586-6741; email: hiko@med.oita-u.ac.jp
We report a case-control study of sporadic cryptosporidiosis with genotyping of isolates from case-patients. A postal questionnaire was completed by 427 patients and 427 controls. We obtained genotyping data on isolates from 191 patients; 115 were Cryptosporidium hominis, and 76 were C. parvum. When all cryptosporidiosis cases were analyzed, three variables were strongly associated with illness: travel outside the United Kingdom, contact with another person with diarrhea, and touching cattle. Eating ice cream and eating raw vegetables were both strongly negatively associated with illness. Helping a child <5 years of age to use the toilet and the number of glasses of tap water drunk at home each day were also independently positively associated with risk. Eating tomatoes was negatively associated. For C. hominis infections, the strongly significant risk factors were travel abroad and changing diapers of children <5 years of age. For C. parvum, eating raw vegetables and eating tomatoes were strongly negatively associated with illness; touching farm animals was associated with illness.

Cryptosporidiosis is due to infection by one or more species of the genus Cryptosporidium. Approximately 12 species are now recognized; two, C. hominis (previously known as C. parvum, genotype 1) and C. parvum (previously known as C. parvum, genotype 2), are the most important pathogens for humans (1). C. hominis is reported as being largely restricted to humans, and C. parvum is found in a wide range of animals (particularly cattle and sheep) as well as humans.

Most of what we know about the risk factors for Cryptosporidium infection was learned from outbreak investigations. Outbreaks have been associated with drinking water from public and private supplies, swimming in swimming pools, consumption of unpasteurized milk, and contact with farm animals, especially during farm visits. However, most cases of cryptosporidiosis are due to sporadic rather than outbreak-associated infections. Outbreaks represent <10% of all cases of Cryptosporidium infection (2,3), although a further proportion of cases will likely be associated with undetected outbreaks (4). Truly sporadic disease may not necessarily be due to the same causes (5). Only one substantive case-control study of sporadic cryptosporidiosis in the immune-competent population has been conducted in an industrialized nation (6). We are aware of only two other case-control studies of cryptosporidiosis in non–immune-compromised persons conducted in an industrialized nation (7,8); both studies were relatively small. One, from New Mexico, found an association with drinking water, although cases may possibly have represented an undetected outbreak (7). The other study, from Australia, found a borderline association with drinking bottled water. Concerns about the safety of the spring water on sale suggested that cases in this study may also have been part of an outbreak (8).

We report a large case-control study conducted in the North West Region of England and in Wales. The study was designed to investigate the etiology and epidemiology of sporadic cryptosporidiosis. The North West region has a history of several large waterborne outbreaks of cryptosporidiosis over the past decade; Wales has not had any reported waterborne outbreaks.

Methods
A case-control study was conducted in the North West of England and Wales from February 2001 to May 2002. The study received ethical approval from the Multi-centre Research Ethics Committee, relevant local Research Ethics Committees, and the Public Health Laboratory Service Research Ethics Committee. The principal hypotheses being tested in this study relate to the known epidemiology of outbreaks, namely, that sporadic cases of cryptosporidiosis are associated with drinking unboiled drinking water from public water supplies, swimming in a swimming pool, contact with animals, travel outside the
United Kingdom, and contact with other persons with diarrheal illness. These hypotheses were constructed before the study began and were based on a review of previous U.K. outbreaks.

**Recruitment of Case-Patients and Controls**

Participants were recruited through an enhanced program of surveillance of *Cryptosporidium* that had begun in the North West of England and Wales in December 2000. Details of confirmed cases identified by the enhanced surveillance were forwarded to Communicable Disease Surveillance Centre North West, through the consultant in Communicable Disease Control in each health authority.

Cases were defined as laboratory-confirmed *Cryptosporidium* in a resident of Wales or the North West region with diarrhea in the 2 weeks before a sample was taken, and which was not part of a formal outbreak investigation. All case-patients reported to Communicable Disease Surveillance Centre North West within 4 weeks of the date of report to the health authority were invited to take part in the study. Reports of patients in whom diarrhea had occurred more than 4 weeks previously were excluded because these patients may have had difficulty accurately recalling their activities before becoming ill.

A control was defined as a person who had not had diarrhea in the 2 weeks before completing a questionnaire. Controls were chosen to be within the same age band as the patients and within the same location; they were drawn from the same family physician’s practice or a neighboring practice. The age bands chosen were: <5 years of age, 5–16 years, and >16 years. Expecting control participation to be comparatively low, we attempted to recruit up to eight controls for each participating patient. We contacted the practice initially by mail. If no response was received, we contacted the practice manager by telephone. Each practice was approached only once in this way whether or not it had offered controls for our study and no matter how many cases had occurred in the practice. In many areas with high numbers of cases or low responses by practices, controls were not obtainable for many case-patients. Consequently, controls cannot be considered to be matched to cases. Rather, the control group was designed to be broadly comparable in age distribution to case-patients.

A total of 662 patients and 820 controls were invited to take part in the study. They received a questionnaire and an accompanying information leaflet by mail. If no response had been received after 2 weeks, a second questionnaire was sent. After this time, we assumed the person did not want to take part in the study.

Questionnaires were developed for both adult and child patients and controls. A person <16 years of age was defined as a child, and a person ≥16 years was defined as an adult. Finally, *Cryptosporidium* genotype data, held at the Cryptosporidium Reference Laboratory, was linked back to the recruited case-patients for epidemiologic analysis. Copies of the questionnaire can be obtained from the corresponding author.

**Genotyping**

At the start of the study, all laboratories in the North West and in Wales were asked to send *Cryptosporidium*-positive stools to the Public Health Laboratory Service Cryptosporidium Reference Unit in Swansea for typing. To confirm the identification of *Cryptosporidium* at this unit, fecal smears were stained by using a modified Ziehl-Neelsen stain (9) and inspected by bright-field microscopy or by using an auramine phenol method (10) and inspected by fluorescence microscopy. Before DNA extraction, oocysts were purified from the feces by using salt flotation (11). The *Cryptosporidium* genotype was investigated by using polymerase chain reaction–restriction fragment length polymorphism to identify polymorphisms within the *Cryptosporidium* oocyst wall protein and SSUrDNA gene loci (12). These two methods are the routine methods for genotyping *Cryptosporidium* at the U.K. Reference Laboratory.

**Data Analysis**

Data entry was performed by using EpiInfo (Version 6.04d, Centers for Disease Control and Prevention, Atlanta, GA). Initial analyses on the clinical severity and initial symptoms were conducted by using SPSS (SSPS Inc., Chicago, IL). Statistical modeling of risk factors was performed by the Public Health Laboratory Service Statistics Unit, using EpiInfo and GLIM (Generalised Linear Interactive Modelling [13]).

For etiologic analyses, each potential risk factor was considered singly by its odds ratio (OR) estimate (and 95% confidence interval [CI]). Continuity corrected chi-square tests or Fisher exact test was used when data were sparse. Dose-response rate was estimated by using chi-square tests for trends.

Variables that were positively or negatively associated with illness (p ≤ 0.2) were included in a logistic regression model. However, all the variables could not be added, as too many existed for the statistical package to handle. Thus, all positive and some negative factors were included in the initial model. The least significant variable was removed and another negative factor included. This process continued until all the protective factors had been included. Then a sequence of models was fitted; on each occasion, the least significant variable was dropped.

Whether a child ate soil was the first variable removed because it had the most missing data for a nonsignificant variable and its removal resulted in many more
observations being available for model estimation. Terms were assessed by comparing nested models by using likelihood ratio tests. Nonsignificant variables (p > 0.05) were removed one at a time from models, with the least significant ones being removed first. This process resulted in a final multivariable model, with most variables being significant or close to significant. The only variable that was retained whether or not it was significant was age.

Multivariable analyses were conducted separately on cases genotyped as either C. hominis and C. parvum. The set of variables for inclusion into initial multivariable models was determined by using all the data, as discussed above. Only cases with complete variables could be included in the final models.

Results
Completed questionnaires were received from a total of 427 patients (65% response rate) and 427 controls (52% response rate). By chance, the number of patients and controls was equal. Of the controls, 27 (6%) had had diarrhea in the 2 weeks before completing the questionnaire and were excluded from the analysis. Of the patients, 191 (45%) had had strains sent to the Cryptosporidium Reference Laboratory, and the genotype was therefore known; 115 were C. hominis and 76 were C. parvum.

The median age for recruited case-patients and controls was 12 years. By sex, 48% of cases and 48% of controls were male. The age distribution of patients and controls is shown in Table 1, which gives the average age for 5- or 10-year age bands. The median age for all patients (Figure, part A) showed a mean of 12.7 days and median of 11 days. For patients with C. hominis, the mean duration was 13.5 days (standard deviation [SD] 9.93, median 12.5) (Figure, part B). For C. parvum, mean duration was 11.33 days (SD 5.29, median 10.5) (Figure, part B). Levene’s Test for Equality of Variances showed that variance of duration for C. hominis was significantly lower than for C. hominis (F = 8.312, p = 0.005). However, the difference in median duration was not significant.

Table 2 shows the multivariable results for all cases, estimated from 552 observations. In this model, the health authority, travel outside the United Kingdom (OR 5.650, p < 0.001), contact with another person with diarrhea (OR 4.614, p < 0.001), and touching any cattle (OR 3.876, p = 0.003) were highly significantly positively associated with risk. Toileting contact with a child <5 years of age (OR 1.851, p = 0.025) and the number of glasses of unboiled water drunk at home (OR 1.135 per glass,
p = 0.019) were also positively associated. Eating ice cream (OR 0.472, p = 0.001), raw vegetables (OR 0.532, p = 0.004), and eating tomatoes (OR 0.616, p = 0.035) were negatively associated.

Table 3 shows the final model for cases of *C. hominis*, estimated from 433 observations. Health authority of residence, travel outside the United Kingdom (OR 6.841, p < 0.001) and diaper-changing contact (OR 3.991, p < 0.001) were strongly associated with infection. Sitting or sleeping on the ground (OR 0.241, p = 0.027), the number of persons 5–15 years of age living with the person (OR 0.639 per person, p = 0.037), eating fresh fruit (OR 0.222, p = 0.027), and the likelihood of washing fresh fruit and vegetables before eating (p = 0.022) were negatively associated with risk.

The model in Table 4 shows the results for cases of *C. parvum*, estimated from 392 observations. Eating raw vegetables (OR 0.222, p = 0.001) and tomatoes (OR 0.317, p = 0.005) was negatively associated with illness; touching any farm animal (OR 2.653, p = 0.028) was associated with illness.

Discussion

Our study is the first prospective epidemiologic study of sporadic cryptosporidiosis that has investigated independent risk factors for *C. hominis* and *C. parvum* infections. No significant differences were found between initial symptoms, severity of illness, or duration of hospital stay in persons infected with either *C. hominis* or *C. parvum* infections. No significant differences were found between median duration for *C. hominis* and *C. parvum*; however, the variation of duration for *C. hominis* was significantly higher than for *C. parvum* infections. This finding suggests that *C. hominis* infections may be less predictable in terms of duration and more prone to extremes than *C. parvum*.

The main risk factors identified—travel abroad, contact with a patient, and touching cattle—are broadly similar to those identified by Robertson and colleagues (6). Strongly significant negative factors were eating ice cream and eating raw vegetables. Factors significant at the 0.05 level were toileting contact with a child <5 years of age and number of glasses of unboiled water drunk at home. Eating tomatoes was negatively associated.

Health authority of residence was strongly significant in all models. However, given that we found that the ability to recruit controls differed between health authorities, much of this difference may be artifactual. Nevertheless, health authority of residence was retained in all models in the event that other risk factors varied in relation to locality of residence. The issue of geographic variation in cryptosporidiosis will be included in a subsequent report.

With regard to the main hypotheses under investigation, travel outside the United Kingdom, contact with other people with diarrhea, and contact with animals were all strongly associated with *Cryptosporidium* infection. Robertson et al. (6) also identified travel outside Australia as a risk factor. However, they suggested that OR may be inflated because of ascertainment bias of patients, which applies to our study as well. A general practitioner may be more likely to request a fecal sample from a patient with diarrhea who has traveled abroad. In addition, previous research indicates that most laboratories in the North West of England and Wales routinely screen for *Cryptosporidium* oocysts if the patient is known to have traveled outside the United Kingdom (14). When analysis is restricted to cases where the species was known, travel outside the United Kingdom was significant for *C. hominis* infection but not for *C. parvum*. The relationship between *C. hominis* infection and overseas travel has been noted previously (15,16).

The risk for infection increased significantly upon contact with cattle when all patients were compared to controls, and for *C. parvum* alone but not for *C. hominis* alone. Previous research has associated farm animal contact with

Figure. A, duration of illness, all patients. B, duration of illness for *Cryptosporidium hominis* and *C. parvum* patients.
outbreaks of Cryptosporidium; moreover, calf contact and lamb contact have been identified as risk factors for sporadic infection (6). Several outbreaks have also been associated with farm visits within the United Kingdom. The risk for contact with other farm animals was not significant. The association with C. parvum but not C. hominis is also consistent with previous findings (15,16). Our study was conducted during an epidemic of foot and mouth disease, when access to the countryside and contact with farm animals were severely restricted for a large period (17), a fact that makes the cattle association even more dramatic. No significant association was found between ownership

| Table 2. Final multivariable model, all data** |
|----------|------------|----------------|------|
| Variables | Adjusted OR | 95% CI         | p value |
| Health authority | | | |
| A | 1.000 | | 0.004 |
| B | 0.125 | 0.041–0.382 | 0.004 |
| C | n.e. | n.e. | |
| D | 0.482 | 0.166–1.398 | |
| E | 1.610 | 0.247–10.49 | 0.025 |
| F | 0.225 | 0.080–0.635 | |
| G | 0.326 | 0.068–1.552 | |
| H | 0.921 | 0.261–3.250 | |
| I | n.e. | n.e. | |
| J | 0.310 | 0.117–0.822 | |
| K | 316.600 | 0–∞ | 0.001 |
| L | 0.175 | 0.012–2.566 | |
| M | 0.377 | 0.130–1.097 | |
| N | 1.203 | 0.289–4.999 | |
| O | 0.367 | 0.11–1.145 | |
| P | 0.562 | 0.134–2.354 | 0.019 |
| Q | 198.400 | 0–∞ | 0.001 |
| R | 0.449 | 0.146–1.383 | |
| S | 0.206 | 0.053–0.804 | |
| T | 0.366 | 0.078–1.720 | |
| U | 0.546 | 0.207–1.443 | |
| Age | 0.994/y | 0.982–1.006 | 0.314 |
| Travel outside UK | | | |
| Y | 5.650 | 2.861–11.160 | <0.001 |
| N | 1.000 | | |
| Contact with another person with diarrhea | | | |
| Y | 4.614 | 2.449–8.691 | <0.001 |
| N | 1.000 | | |
| Touch any cattle | | | |
| Y | 3.876 | 1.419–10.04 | 0.003 |
| N | 1.000 | | |
| Usually wash before eating raw fruit and vegetables | | | |
| Always | 1.000 | | 0.108 |
| Usually | 0.966 | 0.605–1.543 | |
| Sometimes | 0.746 | 0.436–1.274 | |
| Never | 2.478 | 0.965–6.362 | |
| Toileting contact with child 5 y | | | |
| Y | 1.851 | 1.079–3.175 | 0.025 |
| N | 1.000 | | |
| Number of glasses of unboiled water drunk at home | 1.135 per glass | 1.019–2.625 | 0.019 |
| Eat ice cream | | | |
| Y | 0.472 | 0.299–0.746 | 0.001 |
| N | 1.000 | | |
| Eat raw vegetables | | | |
| Y | 0.532 | 0.346–0.820 | 0.004 |
| N | 1.000 | | |
| Eat tomatoes | | | |
| Y | 0.616 | 0.392–0.969 | 0.035 |
| N | 1.000 | | |

*Estimated from 552 observations (261 case-patients and 291 controls).
**OR, odds ratio; CI, confidence intervals; n.e., not estimable.
of or contact with domestic pets and sporadic infection. Although some researchers have suggested pets may present a risk (18), other studies indicate that pets are not a major risk factor for acquiring Cryptosporidium (19). Indeed, previous research has found various types of domestic animal contact to be protective (6).

One variable, number of glasses of unboiled water drunk at home, was significant in the model with all patients. This water consumption variable was the only one to be included in one of the multivariable models. The Australian study also found no association with drinking publicly supplied water (6). However, one of the two water catchment areas in this study was highly protected, with no livestock farming. The nature of the water catchment areas in Australia might preclude generalizing its results to other parts of the world. Interpreting this finding is difficult. Few of the drinking water variables associated with risk from water consumption were significant in the single variable analysis (online Appendix). Neither drinking unboiled tap water nor use of a water filter was significant, which suggests that drinking water from public supplies was not an important risk factor (20). In the single variable analysis, number of glasses of bottled water drunk was also associated with risk for infection, although whether or not persons drank bottled water was not associated with risk. We suggest that the significant association with amount of

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
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<td>Health authority</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>B</td>
<td>0.030</td>
<td>0.003–0.335</td>
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</tr>
<tr>
<td>C</td>
<td>n.e.</td>
<td>n.e.</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.781</td>
<td>0.206–2.960</td>
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</tr>
<tr>
<td>E</td>
<td>0.002</td>
<td>0–∞</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.169</td>
<td>0.034–0.836</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>0.277</td>
<td>0.022–3.516</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>0.229</td>
<td>0.019–2.734</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>n.e.</td>
<td>n.e.</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>0.072</td>
<td>0.011–0.456</td>
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<tr>
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</tr>
<tr>
<td>L</td>
<td>0.398</td>
<td>0.025–6.396</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>2.116</td>
<td>0.573–7.809</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5.321</td>
<td>1.098–25.78</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>0.169</td>
<td>0.017–1.685</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>0–∞</td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>n.e.</td>
<td>n.e.</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>0.126</td>
<td>0.020–0.809</td>
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<tr>
<td>S</td>
<td>0.408</td>
<td>0.065–2.539</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>1.488</td>
<td>0.273–8.104</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>1.015</td>
<td>0.288–3.579</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.997/y</td>
<td>0.982–1.012</td>
<td>0.713</td>
</tr>
<tr>
<td>Travel outside UK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>6.841</td>
<td>2.622–17.850</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>N</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spend time sleeping or sitting outside on the ground</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>0.241</td>
<td>0.060–0.968</td>
<td>0.027</td>
</tr>
<tr>
<td>N</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaper changing contact with a child &lt;5 y of age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>3.991</td>
<td>1.848–8.618</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>N</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Usually wash before eating raw fruit and vegetables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>1.000</td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>Usually</td>
<td>0.347</td>
<td>0.159–0.757</td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td>0.967</td>
<td>0.437–2.139</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.337</td>
<td>0.387–4.629</td>
<td></td>
</tr>
<tr>
<td>No. of times swum in a toddler pool</td>
<td>1.258 per time</td>
<td>0.960–1.649</td>
<td>0.077</td>
</tr>
<tr>
<td>No. of persons 5–15 y of age living with you</td>
<td>0.639 per person</td>
<td>0.413–0.991</td>
<td>0.037</td>
</tr>
<tr>
<td>Eat fresh fruit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>0.222</td>
<td>0.058–0.852</td>
<td>0.027</td>
</tr>
<tr>
<td>N</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Final multivariable model for Cryptosporidium hominis infection

*a Estimated from 433 observations (82 case-patients and 351 controls).

*b OR, odds ratio; CI, confidence interval; NE, not estimable.
unboiled water drunk may be an artifact attributable to recall bias either because the patient believes that his or her illness was waterborne (21) or because the person has been drinking more water as a result of illness. Our findings suggest that drinking tap water does not appear to be of major importance for sporadic disease.

The remaining risk factor included in the major hypotheses we tested, use of swimming pools, did not achieve significance, although number of times one swam in a toddler pool almost reached significance in the model for *C. hominis*. Use of a toddler pool and number of times swum in a swimming pool, but not use of a swimming pool, were significant in the single variable analyses (online Appendix available at http://www.cdc.gov/nci-dod/eid/vol10no7/03-0582.htm#app). Swimming pool use has previously been associated with many outbreaks of *Cryptosporidium* in the United Kingdom and elsewhere, and use of a toddler pool has been associated with sporadic cases (6). The importance of swimming pool exposure as a risk factor for sporadic cryptosporidiosis was suggested by Hunter and Quigley (22). They demonstrated a protective effect of swimming pool use in an outbreak associated with drinking water and suggested that this finding was due to immunity from an increased risk for sporadic disease in persons who go swimming.

In addition to the main hypotheses, a number of other associations were detected. These included a negative association with eating raw vegetables and tomatoes in the model with all patients and *C. parvum* only, a negative association with eating fresh fruit for *C. hominis*, a negative association with eating ice cream in the model with all patients, and an association with toileting children <5 years of age in the all-case model and diaper-changing contact in the *C. hominis* model. Also in the *C. hominis* model, spending time sleeping or sitting outside on the ground was associated with infection, the number of persons 5–15 years of age living in the same home was negatively associated with infection, and usually washing raw fruit and vegetables before eating had a protective effect.

The negative association with eating raw vegetables is also consistent with previous studies, which have suggested a protective effect from eating raw vegetables (6,18).

### Table 4. Final multivariable model for *Cryptosporidium parvum* infection

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p value</th>
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<td></td>
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</tr>
<tr>
<td>A</td>
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<td></td>
<td>&lt; 0.001</td>
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<td>B</td>
<td>0.296</td>
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<tr>
<td>C</td>
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<tr>
<td>D</td>
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<tr>
<td>E</td>
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<td>F</td>
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<td>G</td>
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<td>K</td>
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<tr>
<td>L</td>
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<tr>
<td>T</td>
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<td>U</td>
<td>2.260</td>
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<td>Age</td>
<td>0.993</td>
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<td>Touch or handle farm animals</td>
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<tr>
<td>Eat tomatoes</td>
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<td>Eat raw vegetables</td>
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<td>0.222</td>
<td>0.086–0.572</td>
<td>0.001</td>
</tr>
<tr>
<td>N</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Estimated from 392 observations (55 case-patients and 337 controls).

**a** OR, odds ratio; CI, confidence interval; n.e., not estimable.
Whether this represents the effect of immunity through repeat exposure by this route or through another mechanism is unclear (22,23). If the immunity hypothesis is correct, the fact that eating raw vegetables was strongly negatively associated with C. parvum, but not C. hominis, infection would suggest contamination of raw vegetables with animal-derived fecal material.

The negative association with ice cream was unexpected. In the single variable analysis, consuming other dairy products such as uncooked soft cheese, uncooked hard cheese, and cream were also negatively associated with illness. Unpasteurized milk products have previously been associated with Cryptosporidium infections, and consuming such products was identified as a risk factor for sporadic cases of infection in Adelaide (6). However, in the United Kingdom, unpasteurized milk is not used in ice-cream production, so this association is difficult to explain. We investigated the possibility that this finding was due to the different times of the year that patients and controls were recruited. However, in all but 1 month, controls were more likely to report ice cream consumption than patients were. A recently published case-control study on risk factors for giardiasis in the South West of England also reported a negative association with ice cream consumption (24).

Associations of toileting contact with children <5 years (all patients) and diaper-changing contact (C. hominis) were independent of whether the children were being helped to use the toilet or having their diapers changed had diarrhea. This observation would suggest that asymptomatic carriage of C. hominis may be common in very young children even in the absence of symptoms. Asymptomatic carriage of C. hominis may be the main reservoir of infection.

In conclusion, we showed that the main risk factors for C. parvum infection (contact with cattle) and C. hominis (travel abroad and changing diapers) differ. We also showed that when the case group includes both C. parvum and C. hominis as well as cases in which the species is not known, the risk factors vary again (travel abroad and contact with a case-patient). Although restricting analysis to cases where species is known reduces the power of the study by having fewer cases, analyses conducted on populations of patients that contain two pathogens with different epidemiologic features may mask species specific risk factors. Future studies of the epidemiology of and risk factors for cryptosporidiosis should ensure that strains are speciated adequately.

Acknowledgments

We thank Kristin Elwin and Anne Thomas for maintaining the national collection of Cryptosporidium oocysts and genotyping isolates; David Gomez for technical support; and David Drury, Roland Salmon, Charmian Kerr, and Mark Reacher for helpful comments.

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Dr. Hunter qualified in medicine before specializing in medical microbiology. Before his appointment to the chair of Health Protection at the University of East Anglia, he was director of the Chester Public Health Laboratory for 12 years. His main interests are in the epidemiology of waterborne disease and the management of outbreaks of infectious disease.

References


Address for correspondence: Paul R. Hunter, Professor of Health Protection, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich NR4 7TJ, United Kingdom; fax: +44-1603-593752; email: paul.hunter@uea.ac.uk

Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.
We determined the susceptibilities of 265 invasive isolates of pneumococci obtained during 1995 to 2001 in Hong Kong to 11 antimicrobial agents and their serotypes. Overall, 62.6% isolates were susceptible to penicillin, 20% were intermediately resistant, and 17.4% were resistant. The overall prevalence of levofloxacin resistance (MIC $\geq 8 \mu g/mL$) was 3.8% but increased to 15.2% among the penicillin-resistant isolates. All levofloxacin-resistant isolates were clonally related; had reduced susceptibility to penicillin, cefotaxime, and clarithromycin; and were derived from adults $>$50 years of age. Of the penicillin-nonsusceptible pneumococci, 90% from children $\leq$5 years of age and 54.8% from persons of all ages were of serotypes that are included in the 7-valent pneumococcal conjugate vaccine; 93.5% from children $\leq$5 years of age and 93% from persons of all ages were of serotypes that are included in the 23-valent polysaccharide vaccine.

The emergence of antimicrobial resistance in Streptococcus pneumoniae worldwide is an important public health issue because this organism is the leading cause of many infections, particularly community-acquired pneumonia. In many countries, rates of resistance for penicillin, macrolides, and tetracyclines have reached levels of 30% to 40% or higher and are increasing. In recent years, the emergence of fluoroquinolone resistance is being increasingly recognized among multidrug-resistant strains of S. pneumoniae. Hong Kong, Ireland, Canada, and Spain have reported increasing rates of fluoroquinolone resistance among S. pneumoniae (1–3). So far, reports on fluoroquinolone resistance in S. pneumoniae have predominantly involved respiratory tract isolates, and whether this type of resistance is emerging among the invasive isolates is unknown. In this study, we evaluated the comparative activities of five fluoroquinolones against invasive isolates of S. pneumoniae from Hong Kong that were collected during a period when fluoroquinolone resistance had increased rapidly among noninvasive respiratory isolates.

**Materials and Methods**

**Bacterial Isolates**

Stored isolates of S. pneumoniae were obtained from the blood and cerebrospinal fluid (CSF) of patients admitted to five hospitals in Hong Kong during 1995 to 2001. These five hospitals were chosen because they represent the same sentinel network that participated in an earlier study conducted by the same group (4). One hospital did not store the isolates and thus was not included in the present study. The following numbers of isolates were obtained from each of the hospitals: A (140 isolates from 1995 to 2001), B (22 isolates from 1996 to 2001), C (34 isolates from 1997 to 2001), D (64 isolates from 1998 to 2001), and E (5 isolates from 2001). All are public hospitals that provide acute patient care, including all the major specialties. Hospital A is a university teaching hospital with a bone marrow transplant unit, and the others are regional hospitals. Hospitals A and B are located in the same region, and they together serve a population of approximately 1.4 million. Hospitals C, D, and E serve populations of 0.6, 1, and 0.4 million, respectively. Thus, this network together serves approximately 53% of the 6.5 million population in Hong Kong.
Isolates included in this study represented all the invasive pneumococcal case-patients with a positive blood or CSF culture in the stated periods. All invasive isolates were tested for antimicrobial susceptibility. Only one isolate from the same patient episode of infection was included. Three patients had two episodes of infections separated by intervals of 3 months to 2 years. All isolates were subcultured and reidentified by considering the following characteristics: colony morphologic features, Gram stain results, optochin susceptibility, and bile solubility. Isolates were stored at –20°C until they were tested in batches.

**Antimicrobial Agents and Susceptibility Testing**

E-test strips of penicillin, amoxyccillin (as amoxyccillin-clavulanate 2:1), cefotaxime, cefepime, clarithromycin, vancomycin, ciprofloxacin, levofloxacin, sparfloxacin, gatifloxacin, and moxifloxacin were purchased from AB Biodisk, Solna, Sweden. E-test MICs were determined following the manufacturer’s instructions. All susceptibility testing was conducted in a single laboratory at the University of Hong Kong. Test inocula were prepared from pneumococcal colonies grown on sheep blood agar that had been incubated for 20 to 24 h in 5% CO2. Colonies were suspended in 0.9% saline to obtain a suspension equivalent to a 0.5 McFarland standard of turbidity. From this suspension, E-tests were performed on Mueller-Hinton agar with 5% sheep blood (BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD). The plates were incubated at 35°C in 5% CO2 for 20 h to 24 h. MICs falling between two marks on the E-test strip were rounded up to the next higher twofold dilution, as recommended in the instructions. For all MIC determinations, the bacterial inocula were validated by back titration in 10% of the tests to ensure the desired inoculum density. Quality control strains (S. pneumoniae ATCC 49619, Staphylococcus aureus ATCC 29213, and Escherichia coli ATCC 25922) were included with each run. Results were interpreted according to published breakpoints of the National Committee for Clinical Laboratory Standards (5). The term nonsusceptible was used to denote both intermediate and resistant isolates. For ciprofloxacin, the criteria were susceptible, ≤2 µg/mL; resistant, ≥4 µg/mL.

**Typing of Isolates**

All isolates were serotyped by the quellung reaction (6) with sera of various levels of reactivity from the Statens Seruminstitut (Copenhagen, Denmark). The subset of 11 isolates with resistance to ciprofloxacin was examined further by multilocus sequence typing (MLST) and by Hinfl restriction analysis of their php 2b and 2x genes (7). The well-defined Spanish clones of serotypes 23F and 6B (SP264 ATCC 700669 and GM17 ATCC 700670, respectively) and a strain representative of the fluoroquinolone-resistant variant Hong Kong23F-1 clone were used as controls (4).

**Polymerase Chain Reaction (PCR) and DNA Sequencing**

The quinolone resistance–determining regions of gyrA, gyrB, parC, and parE were amplified by using primers described previously (8). Nucleotide sequencing was performed by the ByeDye dideoxynucleotide chain termination method (Applied Biosystems, Hong Kong). The sequences of both strands of the amplicons were determined.

**Statistical Analysis**

Chi-square or Fisher exact test was used for statistical analysis. A p value of <0.05 was considered significant.

**Results**

**Emergence of Fluoroquinolone Resistance among Multidrug-resistant Isolates**

The number of isolates obtained from different age groups was as follows: <2 years (n = 48); 2–5 years (n = 40); 6–17 years (n = 14); 18–49 years (n = 30); 50–64 (n = 27), and ≥65 years (n = 106). Of the isolates, 256 (96.6%) were from blood, 6 from CSF, and 3 from brain abscess. The susceptibilities of the 265 pneumococcal isolates to 11 antimicrobial agents are summarized in Table 1. The annual susceptibility rates for penicillin, clarithromycin, and levofloxacin are shown in Figure 1. Overall, 166 (62.6%) were penicillin-susceptible, 53 (20%) were penicillin-intermediate, and 46 (17.3%) were penicillin-resistant. Rates of penicillin nonsusceptibility (MIC >0.06 µg/mL) do not differ significantly in the five hospitals (p = 0.1): 35.7% (50 of 140) for laboratory A, 50% (11 of 22) for laboratory B, 32.4% (11 of 34) for laboratory C, 37.5% (24 of 64) for laboratory D, and 60% (3 of 5) for laboratory E.

In children (ages ≤12 years), the rate of penicillin nonsusceptibility was significantly higher than that in adults (≥13 years of age) (48% vs. 30.9%, respectively; p = 0.005). In general, MICs of penicillin were identical or within one dilution difference of that of amoxicillin. For these two penicillins, the MIC50, MIC90, and mode MIC values were identical. On the other hand, MICs of cefotaxime were generally one dilution lower than that of cefepime. High MICs of penicillin (4 µg/mL) and cefotaxime (4 µg/mL) were found in 2 (0.8%) and 1 (0.4%) of 265 isolates, respectively.

Of the 265 isolates, 97 (36.6%) were clarithromycin-susceptible, 1 (0.4%) was clarithromycin-intermediate, and 167 (63%) were clarithromycin-resistant. In most isolates, the resistance was of a high level type. Among 168
Table 1. MICs of 11 antimicrobial agents for *Streptococcus pneumoniae* isolates based on susceptibility to penicillin

<table>
<thead>
<tr>
<th>Antimicrobial agent and penicillin susceptibility status</th>
<th>Range (µg/mL)</th>
<th>MIC (µg/mL)</th>
<th>% of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.008–4</td>
<td>0.032</td>
<td>2</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.008–0.064</td>
<td>0.016</td>
<td>0.032</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.125–1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pen-R</td>
<td>2–4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.016–4</td>
<td>0.032</td>
<td>2</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.016–0.125</td>
<td>0.016</td>
<td>0.032</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.032–2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pen-R</td>
<td>0.5–4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.016–4</td>
<td>0.032</td>
<td>1</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.016–0.125</td>
<td>0.016</td>
<td>0.032</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.032–2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pen-R</td>
<td>0.5–4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cefepime</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.016–4</td>
<td>0.064</td>
<td>2</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.016–0.25</td>
<td>0.064</td>
<td>0.125</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.032–1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pen-R</td>
<td>1–4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.032–256</td>
<td>4</td>
<td>256</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.032–256</td>
<td>0.125</td>
<td>256</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.064–256</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td>Pen-R</td>
<td>2–256</td>
<td>4</td>
<td>256</td>
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<tr>
<td>Vancomycin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.25–1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.25–1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.25–1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Pen-R</td>
<td>0.25–1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.25–32</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.25–4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.25–32</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pen-R</td>
<td>0.5–32</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td></td>
<td></td>
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<tr>
<td>All</td>
<td>0.125–32</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.125–2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.125–32</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pen-R</td>
<td>0.5–32</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.125–32</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.125–1</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.125–32</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Pen-R</td>
<td>0.25–32</td>
<td>0.5</td>
<td>32</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.064–16</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.064–1</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.125–8</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Pen-R</td>
<td>0.125–16</td>
<td>0.25</td>
<td>8</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.064–8</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>Pen-S</td>
<td>0.064–1</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>Pen-I</td>
<td>0.064–4</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>Pen-R</td>
<td>0.125–8</td>
<td>0.125</td>
<td>4</td>
</tr>
</tbody>
</table>

* N = 265; a total of 165 isolates were penicillin -susceptible (Pen-S), 53 were penicillin -intermediate (Pen-I), and 46 were penicillin -resistant (Pen-R).

** 50% and 90% MIC 50 and MIC 90, respectively.
clarithromycin-nonsusceptible isolates, 102 (60.7%) had MIC \( \geq 32 \) \( \mu \)g/mL, and 78 (46.4%) had MIC \( \geq 256 \) \( \mu \)g/mL. Again, clarithromycin resistance rate was higher among children than adults (83 [83%] of 100 vs. 85 [51.5%] of 165, respectively; \( p < 0.001 \)). The clarithromycin-nonsusceptibility rates in penicillin-susceptible and nonsusceptible isolates were 73 (44%) of 166 and 95 (95.9%) of 99, respectively (\( p < 0.001 \)).

Overall, 10 (3.8%) of 265 isolates were resistant to levofloxacin. The levofloxacin-resistance rate increased to 15.2% among the penicillin-resistant pneumococcal isolates and was 7.5% among isolates derived from persons \( \geq 50 \) years of age. One penicillin-susceptible isolate (S1D3) had a ciprofloxacin MIC of 4 \( \mu \)g/mL. This isolate remained susceptible to levofloxacin. The 10 resistant isolates had a levofloxacin MIC from 16 \( \mu \)g/mL to 32 \( \mu \)g/mL; 8 of these were resistant to gatifloxacin (MIC range, 4–16 \( \mu \)g/mL), and 6 were resistant to moxifloxacin (MIC range, 4–8 \( \mu \)g/mL). All levofloxacin-resistant isolates were also either immediately resistant or resistant to penicillin (MIC range, 1–4 \( \mu \)g/mL) and clarithromycin (MIC range, 2–256 \( \mu \)g/mL). All levofloxacin-resistant isolates were from adults (one from the 50- to 64-year age group and nine from \( \geq 65 \)-year group). Seven were nosocomial infections (having onset >2 days after admission), and three were community-acquired infections. For the levofloxacin-susceptible isolates, the rank order of potency (MIC\(_{250}/\)MIC\(_{50}\)) was as follows: moxifloxacin (0.125/0.25) > gatifloxacin (0.25/0.25) > sparfloxacin (0.25/0.5) > levofloxacin (1/1) = ciprofloxacin (1/1).

Serotype Distribution of Isolates and Resistance Patterns

Of 265 isolates, 8 isolates could not be typed; 34 different serotypes were identified among the remaining isolates. The serotype distribution of the isolates according to age group of patients and penicillin resistance is shown in Table 2. Serotype 14 was the most common serotype (24.5%). Four serotypes (6B, 14, 19F, and 23F) accounted for 92.9% of all penicillin-nonsusceptible isolates and 84.5% of all clarithromycin-nonsusceptible isolates. The capsular serotypes of the levofloxacin-resistant isolates were 14 (\( n = 4 \)), 19F (\( n = 2 \)), and 23F (\( n = 4 \)). Serotypes included in 7-valent pneumococcal conjugate vaccine formulations (4, 6B, 9V, 14, 18C, 19F, and 23F) comprised 90.4% and 90.6% of penicillin- and clarithromycin-nonsusceptible strains isolated from persons with age \( \leq 5 \) years, respectively. Coverage of the 7-valent conjugate vaccine for all isolates from young children (\( \leq 5 \) years of age) was 89.7% (79/88). Serotypes included in the 23-valent pneumococcal polysaccharide vaccine accounted for 92.9% of penicillin-nonsusceptible and 91.1% of clarithromycin-nonsusceptible isolates for all ages, respectively.

Molecular Analysis of Fluoroquinolone-resistant Isolates

Molecular analysis of the 11 fluoroquinolone-resistant isolates is summarized in Table 3. Analysis by MLST showed that a single allelic profile (4-4-2-4-4-1-1) or sequence type (ST 81) was shared by all 10 levofloxacin-resistant isolates. Fingerprint patterns after \( HinfI \) digestion of the amplified \( pbp 2x \) and 2b genes are shown in the Figure 2. One fingerprint pattern for \( pbp 2b \) was shared by nine levofloxacin-resistant isolates. The remaining levofloxacin-resistant isolate has a pattern that differed from the major pattern by one band. A single fingerprint pattern for \( pbp 2x \) was shared by all 10 levofloxacin-resistant isolates. Both \( pbp 2b \) and 2x fingerprint patterns among the levofloxacin-resistant isolates were indistinguishable from that displayed by the Spain23F-1 clone. The remaining ciprofloxacin-resistant, levofloxacin-susceptible strain had distinct \( pbp 2b \) and 2x fingerprint patterns. Furthermore, the 10 isolates all had similar pattern of mutations in \( gyrA \), \( parC \), and \( parE \) genes. In \( gyrA \), all 10 isolates had a S81F or Y substitution. In \( parC \), the 10 isolates had at least one amino acid substitution, and 6 isolates had two substitutions, an S79F plus K137N pair. In \( parE \), one isolate had no substitution. Five isolates had one substitution (I460V), and four had two substitutions (I460V plus D435 or E474K pair). The remaining ciprofloxacin-resistant, levofloxacin-susceptible isolate (S1D3) had a distinct MLST pattern and \( pbp 2b \) and \( pbp 2x \) gene profiles. This isolate had one substitution in each of \( ParC \) and \( ParE \). No strains had substitutions in \( GyrB \).

Discussion

This study showed that fluoroquinolone resistance among pneumococci that cause invasive infections is emerging. Our finding of a 3.8% resistance rate for the Spain23F-1 clone. The remaining ciprofloxacin-resistant, levofloxacin-susceptible isolate (S1D3) had a distinct MLST pattern and \( pbp 2b \) and \( pbp 2x \) gene profiles. This isolate had one substitution in each of \( ParC \) and \( ParE \). No strains had substitutions in \( GyrB \).

Discussion

This study showed that fluoroquinolone resistance among pneumococci that cause invasive infections is emerging. Our finding of a 3.8% resistance rate for...
levofloxacin is among the highest ever reported in the world and could be attributed in part to suboptimal use of the fluoroquinolones (9). In a case-control study, we have previously shown that chronic obstructive airway disease, nosocomial infection, nursing home residence, and exposure to lesser potent fluoroquinolones were independently associated with fluoroquinolone-resistant Streptococcus pneumoniae (9). Elsewhere, levofloxacin resistance among the invasive pneumococcal isolates was still rare at 1% (10–12). In the United States, Jorgensen et al. recently reported that fluoroquinolone-resistant pneumococci could be emerging in some of the Active Bacterial Core Surveillance Areas (ABCs). Of 538 invasive pneumococci collected from 1998 to 2000 from California, 3.2% had ciprofloxacin MIC of ≥4 µg/mL (13). In general, antimicrobial resistance among the pneumococci occurred more frequently among respiratory tract isolates than blood isolates (2). In our previous studies, the fluoroquinolone resistance rate among the respiratory isolates was 5.5% and 13% in 1998 and 2000, respectively (3,4).

Our findings show that invasive pneumococci with fluoroquinolone resistance in this locality were related to the multidrug-resistant Spanish 23F clone. Three different serotypes were identified among the 10 clonally related levofloxacin-resistant isolates, indicating that this clone is evolving by horizontal transfer of the capsular genes. Elsewhere, early evidence suggested that epidemic clones could be playing a role in dissemination of fluoroquinolone resistance. In an analysis of 29 fluoroquinolone-resistant pneumococci, McGee et al. reported that 6 isolates from Ireland and 1 from France were indistinguishable from the Spain9V-3 clone (14). In Birmingham, George et al. recently reported that two fluoroquinolone-resistant variants closely related to the widely distributed penicillin-resistant Spanish9V-3 clone were emerging (15). Furthermore, Alou et al. report that 30% of 82 pneumococci with reduced susceptibility to ciprofloxacin from 20 hospitals in Spain belonged to two internationally spread clones: France9V-3 and Spain23F-1 (16). The emergence of fluoroquinolone resistance among the internationally distributed S. pneumoniae clones is of concern. The Hong Kong experience is an example of how resistance to the fluoroquinolones could evolve rapidly in pneumococci as a result of clonal expansion.

This study found a high rate of macrolide resistance among the invasive pneumococcal isolates, as was reported for noninvasive isolates (4). This circumstance is likely related to the high local use of macrolides and the dissemination of several drug-resistant clones (17). Among invasive isolates, our figure was similar to that reported in

### Table 2. Distribution of pneumococcal capsular types according to age group of patient and penicillin resistance, 1995–2001

<table>
<thead>
<tr>
<th>Serotype</th>
<th>All ages</th>
<th>Age ≤5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates</td>
<td>Penicillin-resistant (%)</td>
</tr>
<tr>
<td>14</td>
<td>65</td>
<td>32.3</td>
</tr>
<tr>
<td>23F</td>
<td>46</td>
<td>82.6</td>
</tr>
<tr>
<td>6B</td>
<td>26</td>
<td>69.2</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>0.0</td>
</tr>
<tr>
<td>19F</td>
<td>19</td>
<td>78.9</td>
</tr>
<tr>
<td>18C</td>
<td>12</td>
<td>0.0</td>
</tr>
<tr>
<td>9V</td>
<td>8</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>0.0</td>
</tr>
<tr>
<td>All others</td>
<td>58</td>
<td>12.1</td>
</tr>
<tr>
<td>Total</td>
<td>265</td>
<td>37.4</td>
</tr>
</tbody>
</table>

*MIC >0.12 µg/mL. Serotypes included in 7-valent pneumococcal conjugate vaccine formulations included 4, 6B, 9V, 14, 18C, 19F, and 23F (in italics).

### Table 3. Characteristics of 11 strains of Streptococcus pneumoniae with reduced susceptibility to ciprofloxacin

<table>
<thead>
<tr>
<th>Strain</th>
<th>Y</th>
<th>Source</th>
<th>Serotype</th>
<th>MLST profile</th>
<th>MIC (µg/mL)</th>
<th>Mutation in QRDR of a,b,c,d</th>
<th>a,b,c,d</th>
</tr>
</thead>
<tbody>
<tr>
<td>S3F7</td>
<td>1996</td>
<td>A</td>
<td>23F</td>
<td>4-4-2-4-4-1-1</td>
<td>32 32 2 1</td>
<td>S81F K137N E474K, I460V</td>
<td></td>
</tr>
<tr>
<td>S2H9</td>
<td>1997</td>
<td>A</td>
<td>23F</td>
<td>4-4-2-4-4-1-1</td>
<td>32 32 4 2</td>
<td>S81Y K137N D435N, I460V</td>
<td></td>
</tr>
<tr>
<td>S1B7</td>
<td>1999</td>
<td>B</td>
<td>23F</td>
<td>4-4-2-4-4-1-1</td>
<td>32 32 8 4</td>
<td>S81F S79F, K137N I460V</td>
<td></td>
</tr>
<tr>
<td>S1B9</td>
<td>1999</td>
<td>B</td>
<td>23F</td>
<td>4-4-2-4-4-1-1</td>
<td>32 32 16 4</td>
<td>S81F S79F, K137N I460V</td>
<td></td>
</tr>
<tr>
<td>S1D5</td>
<td>1999</td>
<td>C</td>
<td>19F</td>
<td>4-4-2-4-4-1-1</td>
<td>32 32 8 4</td>
<td>S81F S79F, K137N I460V</td>
<td></td>
</tr>
<tr>
<td>S2D6</td>
<td>1999</td>
<td>A</td>
<td>14</td>
<td>4-4-2-4-4-1-1</td>
<td>32 32 8 8</td>
<td>S81F S79F, K137N I460V</td>
<td></td>
</tr>
<tr>
<td>S1D2</td>
<td>2000</td>
<td>C</td>
<td>19F</td>
<td>4-4-2-4-4-1-1</td>
<td>32 32 8 8</td>
<td>S81F S79F, K137N –</td>
<td></td>
</tr>
<tr>
<td>S1D3</td>
<td>2000</td>
<td>C</td>
<td>4</td>
<td>8-8-8-1-17-1-18</td>
<td>2 0.5 0.25</td>
<td>– K137N I460V</td>
<td></td>
</tr>
<tr>
<td>S2F3</td>
<td>2000</td>
<td>A</td>
<td>14</td>
<td>4-4-2-4-4-1-1</td>
<td>32 32 8 2</td>
<td>S81F K137N D435N, I460V</td>
<td></td>
</tr>
<tr>
<td>186G1</td>
<td>2001</td>
<td>A</td>
<td>23F</td>
<td>4-4-2-4-4-1-1</td>
<td>32 16 2 0.5</td>
<td>S81F K137N E474K, I460V</td>
<td></td>
</tr>
<tr>
<td>216D2</td>
<td>2001</td>
<td>A</td>
<td>14</td>
<td>4-4-2-4-4-1-1</td>
<td>32 32 4 4</td>
<td>S81F S79F, K137N I460V</td>
<td></td>
</tr>
</tbody>
</table>

aMLST, multilocus sequence typing. Number refers to allele of _aroE, gdh, gki, recP, spi, xpt_, and _ddiA_ genes, respectively.
bCIP, ciprofloxacin; LVX, levofloxacin; GAT, gatifloxacin; MO, moxifloxacin.
cNo strains had mutations in _GyrB_ sequence. QRDR, quinolone resistance–determining region.
d_S. pneumoniae_ numbering.
Taiwan (72%) but was higher than those reported from the United States (20.4%), Canada (14.8%), or Germany (15.3%) (18–21). Despite early skepticism, increasing evidence shows that in vitro macrolide resistance does result in clinical and microbiologic failures in systemic pneumococcal infection (22,23). Hence, in the empirical treatment of community-acquired pneumonia, our findings imply that monotherapy with a macrolide is not appropriate in this region.

Our data show that 90% or more of the resistant pneumococci that cause invasive infections in persons of all ages belonged to serotypes that are included in the 23-valent pneumococcal polysaccharide vaccine as well as the 7-valent conjugate vaccine. The 7-valent conjugate vaccine is indicated in young children and is highly effective in preventing vaccine serotype-related invasive diseases (24). In the United States, the 7-valent conjugate vaccine was added to the routine schedule in 2000. According to Whitney et al. (25), after the conjugate vaccine was introduced, the rate of invasive disease caused by vaccine and vaccine-related serotypes has markedly declined. The rate of disease caused by strains that were not susceptible to penicillin was 35% lower in 2001 than in 1999. The rate of disease in adults also declined (25). From the results of trials reported so far, the vaccine will likely also reduce carriage of vaccine types of pneumococci (26,27). Hence, resistant pneumococci might diminish as the vaccine becomes more widely available (25,28). The effectiveness of the 23-valent polysaccharide vaccine has not been as dramatic. In older adults, vaccination with the polysaccharide vaccine effectively reduced the rate of bacteremia but not that of nonbacteremic pneumonia (29). The use of the 23-valent pneumococcal vaccine in Hong Kong is low (with estimated coverage of <10% for those ≥65 years). While data on the efficacy of the 23-valent pneumococcal vaccine are considered insufficient in patients with chronic obstructive pulmonary disease (30), the benefits of vaccinating elderly people with the 23-valent pneumococcal vaccine are clear (31). In view of the findings from this and our previous study that the elderly and persons with chronic obstructive pulmonary disease are at high risk for fluoroquinolone-resistant S. pneumoniae, we believe both patient groups in this locality should receive pneumococcal vaccine.

In conclusion, this study reported high rates of fluoroquinolone resistance among multidrug-resistant strains of pneumococci that cause invasive infections among older adults in Hong Kong. Our experience leads us to call for a more prudent use of fluoroquinolones in all clinical settings. Since carriage of pneumococci is common, collateral exposure could occur anytime a person is treated with fluoroquinolones for any infection, including skin, soft tissue, or urinary tract infections. In patients with chronic obstructive pulmonary disease, high-density colonization of the airway is common, which could explain why these patients are at high risk for fluoroquinolone-resistant pneumococci (9). In young children, high numbers of pneumococci are frequently found in the nasopharynx. If fluoroquinolone use is extended from adults to children, who are frequently colonized by the antimicrobial-

Figure 2. HinfI fingerprints of the pbp genes. A) pbp2b profiles. Lanes 1, marker; 2, Spain3F-1 clone (SP264, ATCC 700669); 3, a ciprofloxacin-resistant, levofloxacin-susceptible strain S1D3; 4, Spain3F clone (GM17, ATCC 700670); lane 5–14, 10 isolates of levofloxacin resistant pneumococci (S3F7, S2H9, S1B7, S1B9, S1D5, S2D6, S1D2, S2F3, 186G1, and 216D2, respectively); B) pbp2x profiles. The lanes were arranged in the same sequence as in (A).
resistant serotypes, transmission and spread of fluoroquinolone-resistant strains would occur rapidly in the community (32). For pneumonia, all evidence so far indicates that infection caused by pneumococci (MIC <1 µg/L or 2 µg/mL) should respond well to a penicillin given in appropriate doses. In view of this and the emergence of fluoroquinolone resistance in both noninvasive and invasive isolates in this locality, we believe that fluoroquinolones should not be used as first-line treatment in community-acquired pneumonia. In local guidelines, amoxicillin-clavulanate or the combination of amoxicillin and a new macrolide are the recommended first-line drugs for empirical treatment of community-acquired pneumonia in the outpatient setting. As pneumococcal infections become increasing difficult to treat, public health authorities should give priority to pneumococcal vaccination of persons at high risk of acquiring infections by the resistant pneumococci. Since substantial changes can occur in a short period, fluoroquinolone-resistant isolates must be monitored and tracked as part of ongoing and routine pneumococcal surveillance.

Acknowledgments

We thank Frankie Chow for excellent technical support, Frances Wong for dedicated secretarial assistance, and K.P. Klugmans for kindly providing Spanish clones of serotypes 23F and 6B.

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Dr. Ho is associate professor in the Department of Microbiology, University of Hong Kong. His research interests include emerging infectious diseases and antimicrobial resistance.

References


Address for correspondence: Pak-Leung Ho, Division of Infectious Diseases, Department of Microbiology, Queen Mary Hospital, The University of Hong Kong, Pokfulam Road, Pokfulam, Hong Kong Special Administrative Region, China; fax: 852–2855–1241; email: plho@hkucc.hku.hk
Control of the 2002–2003 severe acute respiratory syndrome (SARS) outbreak was based on rapid diagnosis coupled with effective patient isolation. We used uncertainty and sensitivity analysis of the basic reproductive number $R_0$ to assess the role that model parameters play in outbreak control. The transmission rate and isolation effectiveness have the largest fractional effect on $R_0$. We estimated the distribution of the reproductive number $R_0$ under perfect isolation conditions. The distribution lies in the interquartile range 0.19–1.08, with a median of 0.49. Even though the median of $R_0$ is <1, we found that 25% of our $R_0$ distribution lies at $R_0 > 1$, even with perfect isolation. This implies the need to simultaneously apply more than one method of control.

Severe acute respiratory syndrome (SARS), a viral respiratory disease, has been reported in 32 countries as of July 11, 2003. SARS is believed to have originated in Guangdong Province, China, in November 2002 (1). Researchers at the Erasmus Medical Center in Rotterdam, the Netherlands, identified a coronavirus as the agent responsible for infecting 8,437 persons worldwide, with 813 deaths as of July 11, 2003 (2). According to recent epidemiologic data from Hong Kong (3), a person exposed to SARS enters an incubation period with a mean length of 6.4 days. Symptomatic persons in that study were hospitalized at a mean rate of 1/4.85 days$^{-1}$. Those who recovered were discharged a mean of 23.5 days after diagnosis, while the mean period to death was 35.9 days after diagnosis. Because no specific treatment for SARS exists, control of the epidemic relied on rapid diagnosis and isolation of patients (1), an approach that is reported to be effective (4). However, most early SARS cases in Toronto occurred in hospitals, with movement of SARS patients between hospitals contributing to the disease’s initial spread (5). In Taiwan, 94% of SARS cases occurred through transmission in hospital wards (6), and similar effects occurred in Hong Kong and Singapore (7). Although the SARS epidemic was eventually controlled, the measures used to achieve that control varied greatly in scope from one place to another. Control of an outbreak relies partly on identifying what disease parameters are likely to lead to a reduction in the reproduction number $R_0$. Here we calculate the dependence of $R_0$ on model parameters.

Methods

Two models of the SARS epidemic that incorporate the effects of quarantine and early detection of new cases but assume perfect isolation were recently introduced (8,9). A slightly different model was used to quantify the role that fast diagnosis and efficient isolation of patients played in Toronto’s outbreak (10). This model predicted control in Toronto and showed that lack of immediate action would have been catastrophic (11). The model incorporates differences in the population’s susceptibility (3) by dividing the population into classes $S_1$ (high risk) and $S_2$ (low risk). A low-risk group in the age range $<19$ years can be observed from the age-specific incidence in Hong Kong (3). The low-risk class ($S_2$) has a reduced susceptibility to SARS, measured by the parameter $p$ (0 < $p$ < 1). While $p = 0$ denotes no susceptibility to SARS, $p = 1$ indicates that both susceptible classes are equally susceptible to SARS. Initially, $S_1 = \rho N$ and $S_2 = (1-\rho)N$, where $N$ is the total population size and $\rho$ is the initial proportion of fully susceptible ($S_1$) persons. Susceptible persons exposed to SARS enter the exposed class (assumed to be asymptomatic) with a rate proportional to $\beta$ and remain there for a mean incubation period of 1/k. The possibility of reduced transmission from the exposed class is included through the parameter $q$ (0 < $q$ < 1), a relative measure of infec-
tiousness. Once symptomatic, exposed persons progress to the infectious class (illness not yet diagnosed), where they may recover at the rate $\gamma_1$, die at rate $\delta$, or enter the diagnosed class at rate $\alpha$. Isolation mechanisms may be put in place in the diagnosed class to reduce their impact on transmission. The relative infectiousness after isolation has begun is measured by the parameter $l$ ($0 < l < 1$) so that $l = 0$ denotes perfect isolation and $l = 1$ denotes ineffective isolation.

**Basic Reproductive Number ($R_0$)**

The basic reproductive number ($R_0$) is the average number of secondary cases generated by a primary case. If $R_0 < 1$, an epidemic cannot be sustained. On the other hand, if $R_0 > 1$, an epidemic typically occurs.

The basic reproductive number derived from our model (10) is given by the formula

$$R_0 = \frac{\beta [p + p(1 - p)]}{\gamma_1 + \delta} \frac{1}{\alpha + \gamma_1 + \delta} \frac{c d}{(\alpha + \gamma_1 + \delta)(\gamma_2 + \delta)}.$$

This equation includes 10 parameters of which 2, the diagnostic rate ($\alpha$) and the relative infectiousness during isolation ($l$), are widely recognized as being amenable to modification by medical intervention. The transmission rate ($\beta$) is defined as the number of persons infected per infectious person per day. This differs from $R_{0,p}$, which is the average number of secondary cases that an infectious person generates when introduced into a susceptible population. Definitions for the remaining parameters are provided in Table 1.

**Parameter Estimation**

Baseline values for $k$, $\gamma_2$, $\delta$, and $\alpha$ are taken from the mean values estimated in reference 3. Because whether asymptomatic persons (exposed class) can transmit the disease is not known, we have fixed $q = 0.1$ (the relative infectiousness of exposed, asymptomatic persons) as in reference 10.

The model parameters $\Theta = (\beta, l)$ are fitted to Hong Kong data (2) by least squares fit to the cumulative number of cases $C(t, \Theta)$ (equation 1 in reference 10). All other parameters are fixed to their baseline values (Table 1). We used a computer program (Berkeley Madonna, R.I. Macey and G.F. Foster, Berkeley, CA) and appropriate initial conditions for the parameters for the optimization process, which was repeated 10 times (each time the program is fed with two different initial conditions for each parameter) before the “best fit” was chosen. The best fit gives $\beta = 0.25$ and $l = 0.43$. We also estimated the relative infectiousness after isolation ($l$) for the case of Singapore ($l = 0.49$) by following the least squares procedure described above. However, for the case of Toronto, not enough data were available on the initial growth of the outbreak. Hence, we only estimated $l$ from Toronto data after control measures were put in place on March 26 (10,11), where $l = 0.1$. We used the transmission rate ($\beta$) obtained from Hong Kong data as the baseline value (Table 1).

We revised earlier estimates for $\rho$ and $p$ (10) (both affect $R_0$) using data from the age distribution of residents and the age-specific incidence of SARS in Hong Kong, as reported (3). The revised estimates are $\rho = 0.77$ (the initial proportion of the population at higher risk) and $p = 1/3$ (the measure of reduced susceptibility in $S_2$). The lower-risk subpopulation lies in the age range $\leq 19$. It constitutes approximately 23% of Hong Kong’s population (3). The fact that most of the SARS cases included in the epidemiologic studies of the Toronto outbreak (5) were transmitted in hospitals limits the use of general demographic data from Toronto in the estimation of $\rho$ and $p$. Hence, we used the parameters estimated from the situation in Hong Kong. Baseline values for all the parameters are given in Table 1.

**Uncertainty Analysis for $R_0$**

We carried out an uncertainty analysis on the basic reproductive number ($R_0$) to assess the variability in $R_0$ that results from the uncertainty in the model parameters. We

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Baseline value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho^*$</td>
<td>Reduction in risk of infection for class $S_2$</td>
<td>0.33</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Initial proportion of the population at higher risk for SARS</td>
<td>0.77</td>
</tr>
<tr>
<td>$\beta^*$</td>
<td>Transmission rate per day</td>
<td>0.25</td>
</tr>
<tr>
<td>$1/k^*$</td>
<td>Mean incubation period (days)</td>
<td>6.37</td>
</tr>
<tr>
<td>$1/\gamma_1$</td>
<td>Mean infectious period (days)</td>
<td>28.4</td>
</tr>
<tr>
<td>$1/\gamma_2^*$</td>
<td>Mean infectious period for persons with diagnosed SARS (days)</td>
<td>23.5</td>
</tr>
<tr>
<td>$1/\alpha$</td>
<td>Mean period before diagnosis (days)</td>
<td>4.85</td>
</tr>
<tr>
<td>$\delta^*$</td>
<td>Induced death rate per day</td>
<td>0.0279</td>
</tr>
<tr>
<td>$q$</td>
<td>Relative measure of infectiousness for the exposed class</td>
<td>0.1</td>
</tr>
<tr>
<td>$l$</td>
<td>Relative infectiousness after isolation has begun</td>
<td>[0,1]</td>
</tr>
</tbody>
</table>

*Baseline values for $k$, $\gamma_2$, $\alpha$, $\rho$, and $\delta$ have been taken from reference 3.

$\beta = 0.25$ is our estimated transmission rate in Hong Kong.

$l = 0$ means perfect isolation, while $l = 1$ means no isolation.
used a Monte Carlo procedure (simple random sampling) to quantify the uncertainty of $R_0$ to model parameters when these parameters are distributed. Similar methods have been used before (12–14). Parameters ($k$, $\gamma_2$, $\delta$, $\alpha$) were assigned a different probability density function (PDF) (Figure 1), which is taken from reference 3. The relative measure of infectiousness of persons after isolation procedures are put in place ($l$) was assumed to be uniformly distributed in the interval ($0 < l < 1$). The observed heterogeneity in transmission rates during the SARS epidemic is modeled here by assuming that $\beta$ is distributed exponentially with mean $0.25$ person$^{-1}$ day$^{-1}$ (our estimate of the transmission rate in Hong Kong). Parameters $q$, $p$, and $\rho$ are fixed to their baseline values (Table 1). We sampled the set of six parameters ($\beta$, $k$, $\gamma_2$, $\delta$, $\alpha$, $l$) $10^5$ times, holding $q$, $p$, and $\rho$ fixed. We then computed $R_0$ from each set. A probability density function for $R_0$ is obtained and can be statistically characterized. Here, we characterize $R_0$ by its median and interquartile range.

**Sensitivity Analysis for $R_0$**

We performed a sensitivity analysis on $R_0$ to quantify the effect of changes in the model parameters on $R_0$. Hence, we rank model parameters according to the size of their effect on $R_0$. Partial rank correlation coefficients (12–15) were computed between each of the parameters (with the exception of $p$, $q$, and $\rho$, which were held fixed) and $R_0$ as samples were drawn from the distributions, thus quantifying the strength of the parameter’s linear association with $R_0$. The larger the partial rank correlation coefficient, the larger the influence of the input parameter on the magnitude of $R_0$. Because the distribution of the parameter $l$ (relative infectiousness after isolation) is not known, we also studied the sensitivity of $R_0$ to various distributions of $l$. Distributions of $l$ used for the Monte Carlo calculation of the partial rank correlation coefficients are: a) $l - \beta$ ($a = 2$, $b = 2$) where $\beta$ is used to denote a beta distribution. Here, the likelihood of $l$ is bell-shaped with mean 0.5 and variance 0.05; b) $l - \beta$ ($a = 1$, $b = 2$), the likelihood of $l$ decreases linearly in the [0,1] interval; and c) $l - \beta$ ($a = 2$, $b = 1$), the likelihood of $l$ increases linearly in the [0,1] interval.

**Results**

**Uncertainty Analysis for $R_0$**

The resulting $R_0$ distribution lies in the interquartile range $0.43–2.41$, with a median of 1.10. Moreover, the probability that $R_0 > 1$ is 0.53. The same Monte Carlo procedure, but with fixed values of $l = 0.1$ and $\alpha = 1/3$ day$^{-1}$ for Toronto (i.e., after implementing control measures on March 26), give a median and interquartile range for the distribution of $R_0 = 0.58$ (0.24–1.18) (Table 2). Similarly, a lower rate of diagnosis $\alpha = 1/4.85$ day$^{-1}$ and the relative infectiousness after isolation in Hong Kong ($l = 0$) and Singapore ($l = 0.49$) gives $R_0 = 1.10$ (0.44–2.29) and 1.17 (0.47–2.47), respectively (Figure 2). Perfect isolation ($l = 0$) gives $R_0 = 0.49$ (0.19–1.08). Especially noteworthy is that even in cases when eventual control of an outbreak is achieved (Toronto and a hypothetical case of perfect isolation), 25% of the weight of the distribution of $R_0$ lies at $R_0 > 1$. Furthermore, the median and interquartile range of $R_0$ are larger when $p = 1$, as has been assumed (8). In Figure 3 we show the ($\beta$, $l$) parameter space when $R_0 < 1$ obtained from our uncertainty analysis (14).
Sensitivity Analysis for $R_0$

The transmission rate $\beta$ and the relative infectivity during isolation ($l$) are the most influential parameters in determining $R_0$. The systematic decline in $R_0$ with increasing $l$ in the range $[0,1]$ is illustrated in Figure 4. Furthermore, our results do not change if we assume the three distributions mentioned in the Methods section (sensitivity analysis) for the parameter $l$. Table 3 shows the partial rank correlation coefficients for the other three possible distributions of $l$. The transmission rate is ranked first independent of the distribution of $l$. The relative infectiousness after isolation is ranked second when $l$ comes from distributions (a) and (b) and ranked third when it comes from distribution (c) (see Methods). Our sensitivity analysis is corroborated by computing local derivatives on $R_0$ (see online Appendix at http://www.cdc.gov/ncidod/EID/vol10no7/03-0647_app.htm). Because bounds exist on how much a given parameter can change in practice, achieving control (i.e., $R_0 < 1$) can require changing parameters other than those with the highest partial rank correlation coefficient. For example, reference 10 showed that control of the outbreak in Toronto relied on both a reduction in $l$ and $1/\alpha$, even though $\alpha$ is ranked fairly low by the partial rank correlation coefficient.

Conclusion

We have estimated $R_0$ for the cases of Toronto, Hong Kong, and Singapore (Table 2) through an uncertainty analysis shown in equation 1. Our estimates for $R_0$ agree with the empirical $R_0$ observed from the data of the first week of the SARS outbreak in Singapore (8). A stretched exponential distribution fits the resulting distributions of $R_0$ for the different locations (Figure 2). Even though the median of $R_0$ is <1 when perfect patient isolation is assumed ($l = 0$), we find that 25% of our $R_0$ distribution lies at $R_0 > 1$. That is, implementing a single method for control may not be sufficient to contain a SARS outbreak. Control may require modifying more than one parameter amenable to intervention. In our model, these parameters include the diagnostic rate ($\alpha$), the relative infectiousness after isolation has begun ($l$), and the per capita transmission rate ($\beta$). The fact that $\alpha$ and $l$ are not independent, but are tightly coupled, favors control.

Our expression for $R_0$ incorporates the effects of diagnosis-isolation strategies. Moreover, our approach includes differential susceptibility ($p$) and effective population size ($\rho$). Most models take $p = 1$, even though data from Hong Kong show that a low-risk subpopulation lies in the age range $\leq 19$, approximately 23% of Hong Kong's population (3). The assumption $p = 1$ thus overestimates $R_0$.

Our sensitivity analysis shows that the transmission rate ($\beta$) and the relative infectiousness after isolation in

Table 2. The median and the interquartile range (IQR) of the distribution of the basic reproductive number ($R_0$) of SARS for Toronto, Hong Kong, and Singapore obtained from our uncertainty analysis

<table>
<thead>
<tr>
<th>Location</th>
<th>$R_0$ mean</th>
<th>$R_0$ median</th>
<th>$R_0$ IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toronto, Canada</td>
<td>0.86</td>
<td>0.58</td>
<td>0.24–1.18</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>1.70</td>
<td>1.10</td>
<td>0.44–2.29</td>
</tr>
<tr>
<td>Singapore</td>
<td>1.83</td>
<td>1.17</td>
<td>0.47–2.47</td>
</tr>
</tbody>
</table>

Figure 2. Empiric (dots) and stretched exponential estimated probability density function $\text{Prob}(R_0) = a \exp[-(R_0/b)^c]$ (solid line) (16) of $R_0$ for the cases of Toronto ($a = 0.186$, $b = 0.803$, $c = 0.957$, after control measures had been implemented), Hong Kong ($a = 0.281$, $b = 1.312$, $c = 0.858$), and Singapore ($a = 0.213$, $b = 1.466$, $c = 0.883$) obtained from our uncertainty analysis. The distribution for the case of perfect isolation ($l = 0$, $a = 0.369$, $b = 0.473$, $c = 0.756$) is shown as a reference.
hospitals \( (l) \) have the largest effect on \( R_0 \). With the exception of a few measures, such as closing schools, no clear policies would modify \( \beta \) directly. This means that a substantial effort must be (and has been) made by the medical community to modify other parameters, such as the diagnostic rate. Hence, the strong sensitivity of \( R_0 \) to the transmission rate \( \beta \) indicates that efforts in finding intervention strategies that manage to systematically lower the contact rate of persons of all age groups promise an effective means for lowering \( R_0 \). Such strategies may include using face masks (the probability of transmission per contact may be reduced), washing hands, and avoiding large crowds (large public events).

Associated with the role of screening, diagnosis, and the effective isolation of patients is the issue of cost. We cannot ignore or minimize the value of stringent quarantine measures and the probability of compliance combined with the economic effect of lost wages (thousands were quarantined in Taiwan, Hong Kong, and Singapore [17]), the costs associated with screening at airports and hospitals, the cost associated with closing hospitals; and the costs associated with isolating SARS patients and exposed persons (see online Appendix for a brief discussion).

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Mr. Chowell is a Ph.D. candidate in the department of Biological Statistics and Computational Biology at Cornell University. His research interests include epidemic modeling of emerging infectious diseases and social network analysis.

References


Address for correspondence: Gerardo Chowell, Biological Statistics and Computational Biology, Cornell University, 432 Warren Hall, Ithaca, NY 14853, USA; fax: 607-255-4698; email: gc82@cornell.edu
Wind in November, Q fever in December

Hervé Tissot-Dupont,* Marie-Antoinette Amadei,† Meyer Nezri,† and Didier Raoult*

Q fever, a worldwide zoonosis caused by *Coxiella burnetii*, can be transmitted from animal reservoirs to humans by the inhalation of infected aerosols. We investigated the epidemiology of Q fever in the Bouches-du-Rhone district of southern France, particularly the role of wind and rainfall in *C. burnetii* transmission. During the winter of 1998 to 1999, an unexpected number of cases were diagnosed in the area. This statistically higher incidence was associated with an increased frequency of the mistral 1 month before onset of disease, i.e., shortly after the main lambing season. These data confirm that wind plays a role in *C. burnetii* transmission, a factor that can be monitored but not prevented. Further studies are needed to identify and confirm preventable individual behavioral risk factors for Q fever.

Q fever is a worldwide zoonosis caused by *Coxiella burnetii*, an obligate intracellular bacteria which lives in the phagolysosomes of the host cell. The main characteristic of Q fever is its clinical polymorphism. Acute cases, which are symptomatic in less than 50%, generally manifest as an isolated fever or a flulike syndrome that may be accompanied by granulomatous hepatitis, pneumonia, or meningoencephalitis (1). Cases with febrile eruptions, myocarditis, and pericarditis have also been reported (2); the various clinical manifestations may depend on host factors (2). In chronic Q fever, endocarditis is the primary sign (3), although osteomyelitis, infections of vascular grafts or aneurisms (4), and pregnancy complications (5) have also been reported. Thus, a serologic confirmation is required for the diagnosis of Q fever.

Throughout the world, the most common reservoirs of *C. burnetii* are cattle, sheep, and goats (6); the bacterium is found in urine, feces, milk, and birth products of infected animals (7). Also, infected cats (8), rabbits (9), and dogs (10) can transmit *C. burnetii* to people. Human infections mainly occur after persons inhale contaminated aerosols and, rarely, after they ingest unpasteurized milk or cheese. The role of wind in aerosol transmission has been suggested since the 1950s (11). Two large outbreaks of Q fever have been studied extensively and have provided additional information about the disease’s epidemiology. In a British study (12), Q fever developed in persons who were exposed to contaminated straw, manure, and dust introduced by the vehicles that traveled along the road where these persons lived. In a Swiss study (13), Q fever also developed in 415 persons who lived on a valley road along which sheep were herded to mountain pastures. Table 1 summarizes the other main outbreaks reported over the last 20 years.

In a survey carried out from 1995 to 1997 (28), the study area (40 km northwest of Marseille) was shown to have an incidence of Q fever 5.4 times higher than that of the area of Marseille. This hyperendemicity could be due to wind blowing through an extensive sheep-rearing area before reaching the study area. The main peak of Q fever cases occurs in April and May in the disease-hyperendemic area, 1 month after the second lambing season, which takes place when the strongest winds blow. At the time of the main lambing, in October and November, the wind is infrequent, leading to a small number of Q fever cases.

<table>
<thead>
<tr>
<th>Source</th>
<th>Year</th>
<th>Country</th>
<th>No. of cases</th>
<th>Reference</th>
</tr>
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<tr>
<td>Sheep</td>
<td>1981</td>
<td>USA</td>
<td>81</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>1982</td>
<td>UK</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>1983</td>
<td>Switzerland</td>
<td>415</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>1993</td>
<td>Italy</td>
<td>58</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>Germany</td>
<td>45</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>Germany</td>
<td>18</td>
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<td>France</td>
<td>204</td>
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</tr>
<tr>
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<td>1982</td>
<td>USA</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>Poland</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>Goats</td>
<td>1992</td>
<td>France</td>
<td>40</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>1998</td>
<td>Slovakia</td>
<td>113</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>Canada</td>
<td>62</td>
<td>24</td>
</tr>
<tr>
<td>Cats</td>
<td>1984</td>
<td>Canada</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1988</td>
<td>Canada</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1989</td>
<td>USA</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>Rabbits</td>
<td>1986</td>
<td>Canada</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Pigeons</td>
<td>2000</td>
<td>France</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>Dogs</td>
<td>1996</td>
<td>Canada</td>
<td>3</td>
<td>10</td>
</tr>
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</table>

*Unité des Rickettsies, Faculté de Médecine, Marseille, France; and †Centre Hospitalier Général, Martigues, France
During the winter of 1998 to 1999, an unexpected number of cases were diagnosed in this area. Our study attempted to confirm our previous hypotheses by correlating this unusual winter peak of infections with unusual meteorologic events.

Materials and Methods

Serologic Diagnosis

The serologic diagnosis of Q fever was performed at the National Reference Center by using the immunofluorescence reference technique as previously described (29). The titers of immunoglobulin (Ig) G, IgM, and IgA against phases I and II of C. burnetii were determined. A case of evolving Q fever (acute or chronic) was diagnosed when the phase II IgG titer was \( \geq 200 \) and the phase II IgM titer was \( \geq 50 \). A diagnosis of chronic Q fever was made when the phase I IgG titer was \( \geq 800 \) (29).

Demographic, Geographic, and Meteorologic Data

Marseille is a city located in southern France with 1 million inhabitants. About 40 km northwest of Marseille is a large, natural, saltwater lake called “Etang de Berre” (Figure 1). Northwest of Etang de Berre is a 600-km², semidesert plain region called “La Crau,” which is the only steppe in Western Europe. La Crau is limited by the Alpilles Mountains (north), the Mediterranean Sea (south), the Etang de Berre (west), and the Rhône River (east). The irrigated northern area (humid Crau) is devoted to hay cultivation (120 km²), whereas the stony and dry southern part is devoted to sheep grazing.

Data on sheep breeding were obtained from the “Chambre d’Agriculture” in Aix en Provence. These data included the approximate number of sheep and the features of sheep breeding in the area.

Meteorologic data were obtained from the Meteo France weather station at Istres in the form of cumulative wind speed and direction compass cards from January 1996 to December 1999 and monthly compass cards and data sheets for November and December between 1996 and 1999. Wind speed and direction are measured every 3 hours, providing about 240 data points per month. The tables provided by Meteo France show the wind direction and speed in three meter per second (m/s) speed ranges of 2–4, 5–8, and >8. We considered only the mistral, which is the most common and strongest wind and blows for several consecutive days, in sunny and dry conditions. It mostly originates from between the west-northwest and the north and blows through La Crau before reaching the study area. Winds that occur in other local climatic conditions, such as rainfall and high humidity, come primarily from other directions, mainly the east and south, and blow for short periods only. These winds were not considered in our study. Monthly cumulative precipitation data between 1996 and 1999 in Istres, and daily precipitation for October, November, and December, 1998 and 1999, were also obtained from Meteo France (www.meteo.fr).

Using the serologic criteria described above, Coxiella burnetii–positive patients were identified from the database of the National Reference Center between 1996 and 1999. The eastern part of La Crau, along the Etang de Berre, was considered the study area. All patients had conditions diagnosed when they were inpatients or outpatients at the general hospital in Martigues or in private laboratories in the cities of Martigues, Fos-sur-Mer, Istres, Sausset-les-Pins, and Châteauneuf-les-Martigues (outpatients who were sent to the laboratory by their general practitioner). Medical practices in this area send all serum specimens for Q fever diagnosis to the National Reference Center or, if they test serum specimens by immunofluorescence with phase II antigen provided by the National Reference Center, send positive serum specimens to the center for confirmation. All serologic diagnoses of Q fever are therefore ultimately made at the National Reference Center with the reference serologic technique. Therefore, we may assume that all diagnosed cases are included in our database. For each patient with a diagnosis of Q fever, a questionnaire was filled out, which provided administrative, epidemiologic, clinical, and biologic data. Patient data were included in the study only if the patient’s place of residence (as recorded in the hospital or laboratory files) was in the study area.

Statistical Analyses

All data were managed by using EpiInfo 6 (Centers for
Disease Control and Prevention, Atlanta, GA). Pearson’s chi square or two-tailed Fisher exact test was used to compare frequencies of qualitative data. A difference was considered significant when p ≤ 0.05.

Results

Q Fever Incidence and Monthly Distribution

Between 1996 and 1999, acute Q fever was diagnosed in 73 patients in the study area. Figure 2 shows the cumulated monthly distribution of these cases (based on date of onset of symptoms) and indicates the usual main peak in incidence of infection in late spring (May–June). When this distribution was considered by year (Figure 3), an unusual peak was observed in December 1998 (5 cases) and January 1999 (2 cases). The proportion of cases occurring in December 1998, in relation to the total number of cases in 1998 (5/20), was significantly higher than the proportion of cases occurring in December in the other study years (1/53) (p < 0.006, Fisher exact test).

The seven patients whose diagnosis was made in December 1998 and January 1999 were interviewed: they were six men and one woman, with a mean age of 34 years. Two case-patients had cardiac valve disorders. Four of them reported living in rural areas. One of them lived in La Crau (Entressen), and six lived on the opposite (southeastern) shore of Etang de Berre (Châteauneuf-les-Martigues, Saint Mitre, Marignane, and Gignac) (Figure 1). None reported an occupational exposure. Two case-patients reported a usual exposure to sheep; one had been exposed to parturient sheep. Two persons owned a cat, and four owned a dog. None of these pets was a newborn or had given birth recently. No patients reported consuming unpasteurized milk or cheese.

Sheep Breeding in La Crau

Sheep have been bred in La Crau for centuries. The main lambing season (80% of births) is in October and November (Figure 2) and takes place either indoors or outdoors. In the latter case, birth products (mainly placentas) are left on the ground where they desiccate and can be a source of fomite spread of C. burnetii. Between October and February, sheep are allowed to graze hay in the northern humid part of La Crau. A second lambing season (20% of births) occurs in March. Between March and June, sheep graze in the dry southern part of La Crau. In June, the sheep are moved by trucks to their summer pastures in the Alps so sheep are generally absent from La Crau until September.

Meteorologic Factors: Wind Frequency and Rainfall

During the 4 years of the study, the mistral represented 44.9% of all winds >2 m/s, 59.4% of winds >5 m/s, and 80% of winds >8 m/s, which confirms the importance of the mistral in terms of frequency of occurrence and speed. To explain the unusual peak of Q fever cases in December 1998 and January 1999, we studied the wind frequencies 1 month before, i.e., in November and December. Table 2 shows the percentage of northwest wind for the three defined speed ranges, by year; p refers to the comparison between the year under consideration and 1998. In each speed range, the frequency of the mistral was higher in November and December 1998 than in any other year: for the speed range >2 m/s, it was 60.9% in November to December 1998, whereas it was 41.3, 40.5, and 52.4 in November to December 1996, 1997, and 1999, respectively. This unusual frequency was even more notable for the strongest winds (which are more consistent with the mistral), representing 93.5% of the wind in November to December 1998 but only 63.1%, 47.7%, and 85% in November to December 1996, 1997, and 1999, respectively. All these differences were statistically significant, except those between 1998 and 1999 in the speed range ≥5 m/s.

In terms of precipitation (Figure 4), October and November 1999 were much more rainy (190.6 mm and 54.4 mm of rainfall, respectively) than October and November 1998 (34.8 mm and 14.2 mm, respectively). The rainfall amount of December 1999 (7.2 mm) was lower than that of December 1998 (56.8 mm). Exceptionally high amounts of precipitation (156.6 mm) were noted in October 1999. The global precipitation of...
December, 1998, primarily depends on that of December 31 (43.4 mm of 56.8 mm). Figure 5 shows the mean monthly rainfall from 1995 to 1999. The main lambing in autumn occurs during the period of the most rainfall, whereas the spring secondary lambing occurs during a dry period.

Discussion

In our previous study, carried out in the same area, we showed a geographic and statistical relationship between the sheep densities, the incidence of Q fever, and the strong, local wind known as the mistral, which blows from the northwest (28). Although C. burnetii transmission is multifactorial, we speculated that the high incidence of Q fever in the study area was related to aerosol spread of organisms because the mistral blows through the local steppe where 70,000 sheep are bred (Figure 1). This study was designed to confirm this hypothesis and find an explanation for the unusual peak of Q fever cases that occurred during the winter of 1998 to 1999. We found that no changes occurred in medical practice or demographics of the region during the study period, and the Chambre of Agriculture and the veterinary services reported no unusual events in the sheep flocks in the region. Under these conditions, we have shown the following: 1) the incidence of Q fever was statistically higher in the period from December 1998 through January 1999 than in the same period in other years; 2) that the speed of the mistral, especially, was significantly higher in November to December 1998 than in other years. Our study has then shown that the increased frequency of the mistral blowing through La Crau after the main lambing season was associated with an unusual peak of Q fever cases in the study area 1 month later. However, Table 2 also shows that the mistral blew with increased frequency during the 1999 winter (although significantly less so than in 1998), without a significant increase in the incidence of Q fever cases. We suspect this might be related to variations in the conditions of C. burnetii transmission which are known to be strongly multifactorial. One of these factors seems to be rainfall, since the fall of 1998 was drier than that of other years, particularly that of 1999, when the mistral was also stronger than in the other 2 years. Moreover, we have shown that the main fall lambing takes place at a time when the mistral is unfrequent, and the environment is wet. On the contrary, the secondary spring lambing occurs at a time when a strong mistral blows, at a dry season, which enhances the aerosols.

The role of wind in the aerosol transmission of C. burnetii has been suggested since the 1950s (11). The wind probably played a role in Q fever cases which occurred in northern Kent (United Kingdom) in people living and working near a fertilizer factory which received offal from abattoirs in Kent and Sussex (11). A small outbreak occurred in a kindergarten in France caused by aerosol transmission from cattle manure infected with C. burnetii applied to nearby pastures (30). An epidemic in people exposed to packing straw has been described in eastern Kent (United Kingdom) (11), and another epidemic occurred in workers exposed to dust from maize grain used as animal food (31). The role of wind has also been

Table 2. Northwest wind (mistral) in November and December 1996 to 1999, expressed as the percentage of measures for three wind speed ranges

<table>
<thead>
<tr>
<th>Years–Dec</th>
<th>Northwest wind</th>
<th>p*</th>
<th>Northwest wind</th>
<th>p*</th>
<th>Northwest wind</th>
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<tr>
<td>1996</td>
<td>&gt;2 m/s (%)</td>
<td>41.3</td>
<td>&gt;5 m/s (%)</td>
<td>47.3</td>
<td>&gt;8 m/s (%)</td>
<td>63.1</td>
</tr>
<tr>
<td>1997</td>
<td>&lt;10^-7</td>
<td></td>
<td>&lt;10^-7</td>
<td></td>
<td>&lt;10^-7</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>&gt;2 m/s (%)</td>
<td>40.5</td>
<td>&gt;5 m/s (%)</td>
<td>50.0</td>
<td>&gt;8 m/s (%)</td>
<td>47.7</td>
</tr>
<tr>
<td></td>
<td>&lt;10^-7</td>
<td></td>
<td>&lt;10^-7</td>
<td></td>
<td>&lt;10^-7</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>&gt;2 m/s (%)</td>
<td>60.9</td>
<td>&gt;5 m/s (%)</td>
<td>76.9</td>
<td>&gt;8 m/s (%)</td>
<td>93.5</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>&gt;2 m/s (%)</td>
<td>52.4</td>
<td>&gt;5 m/s (%)</td>
<td>72.3</td>
<td>&gt;8 m/s (%)</td>
<td>85.0</td>
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<tr>
<td>Nov–Dec 1999</td>
<td></td>
<td>0.01</td>
<td></td>
<td>0.21 (NS)*</td>
<td></td>
<td>0.045</td>
</tr>
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</table>

p values refer to the comparison between the considered year and 1998.

*NS, not significant.
suspected in the infection of cotton (in fields or assembly areas) from neighboring sheep and cattle pastures (32). More recently, the role of wind was assessed in a large outbreak in Birmingham (United Kingdom) in 1989. A case-control study (26 case-patients and 52 matched controls) produced no evidence that direct contact with animals or animal products had caused the outbreak. The epidemic curve suggested a point source exposure in the week beginning April 10. The home addresses of case-patients were clustered in a rectangle-shaped area, 11 miles (18.3 km) north/south by 4 miles (6.7 km) east/west, and attack rates became lower toward the north. Directly south of this area was a region containing farms where outdoor lambing and calving took place, a potent source of *C. burnetii* spores. A retrospective computerized analysis showed that the geographic distribution of cases was associated with a source in this area (p < 0.00001). On 11 April, unusual southerly gales of up to 78 mph (130 km/h) were recorded. The probable cause of the outbreak was windborne spread of *C. burnetii* spores from farmland to more settled areas (33). The role of wind, however, was excluded in a study conducted from 1996 to 2000 in French Guiana (34). In the study of a 1987 outbreak in a Somerset (United Kingdom) secondary school, the high prevalence of unexplained infections was suspected of being related to the spread of organisms, either windborne or in straw or manure (35). In Germany, 40 outbreaks of human Q fever were documented from 1947 to 1999 (36). Sheep were implicated in the transmission in at least 24 outbreaks. Dry weather or wind blowing from areas where sheep were located to inhabited areas likely contributed to at least 14 episodes.

Evidence is now accumulating that wind is an epidemiologic factor in Q fever outbreaks occurring near sheep-rearing areas. It is a factor that can only be monitored and not prevented. Some preventive measures could be of interest in terms of public health: serologic testing and vaccinating sheep, indoor lambing, and appropriate disposal of placentas and litter. However, the feasibility of such measures in France in general and particularly in La Crau is low: Q fever is not a veterinarian-reportable disease, so testing (in cases of abortion) is carried out on request and must be paid by the farmer. An effective phase I animal vaccine is not yet available in France. Although indoor lambing is not possible in such an extensive breeding area, due to the lack of sheepfolds, recommendations have been made on proper disposal of placentas (they should not be left on the ground where they dry up, but properly collected and incinerated). This is possible only when lambing occurs in the presence of farmers.

Many other personal and behavioral factors may be involved that would be preventable. Further studies are needed to identify and confirm such preventable risk factors.

Acknowledgments

We thank Patrick Kelly for his comments on the manuscript, the technicians who performed serologic analyses, and the physicians who provided the patient data.

Dr. Tissot-Dupont is a hospital physician with a PhD in rickettsiology. His research interests include the epidemiology of rickettsial diseases. For 10 years, he managed most of the epidemiologic studies of Q fever in France.

References

**Rapid Assessment Tool for Haemophilus influenzae type b Disease in Developing Countries**

Daniel R. Feikin,* Christopher B. Nelson,† James P. Watt,*‡ Ezzeddine Mohsni,§ Jay D. Wenger,† and Orin S. Levine*‡

**Abstract**

*Haemophilus influenzae* type b (Hib) still causes a substantial number of deaths among children in developing countries, despite the availability of effective conjugate vaccines. A major obstacle in developing a Hib vaccine has been limited awareness about the impact of Hib disease. A tool was developed to estimate the national rates of Hib meningitis and pneumonia by assessing retrospective local data over 7 to 10 days. Data from 11 countries in Africa, the Middle East, and Asia were studied and showed rates of Hib meningitis from >50 cases per 100,000 children >5 years in Ghana and Uganda to <15 per 100,000 in Iran, Jordan, and Uzbekistan. Results were affected by the quality of available data. The Hib rapid assessment tool can be useful to countries that desire a timely assessment of Hib disease rates.

Among infants and young children, *Haemophilus influenzae* type b (Hib) is the leading cause of bacterial meningitis deaths and the second leading cause of bacterial pneumonia deaths worldwide and accounts for approximately 400,000 deaths of children each year (1,2). These deaths are preventable through Hib conjugate vaccines (1). In the United States, Hib conjugate vaccines were first introduced into the routine immunization program for infants in 1990. Subsequently, most industrialized, western countries have introduced these vaccines. Hib conjugate vaccines, however, are still not widely used in developing countries, where the greatest rates of Hib-related disease and deaths occur. Before 2002, only children in 2 of 51 countries with an infant death rate >70 per 1,000 live births were routinely vaccinated against Hib (Figure 1).

The major obstacles to introducing Hib conjugate vaccines into developing countries have been their cost and the population’s limited awareness about the impact of Hib disease. The Hib conjugate vaccine, even after a decade of use, is still expensive, costing approximately $U.S. 2.50 per dose, and thus, external financial resources are needed to procure the vaccine in most developing countries. Since 1999, the Global Alliance for Vaccines and Immunization (GAVI) through the Vaccine Fund has provided a mechanism of financing Hib conjugate vaccines for 5 years to the 75 poorest countries (http://www.vaccinealliance.org/home/index.php). Despite this opportunity, few countries initially requested the Hib vaccine through GAVI, partly because of limited awareness about the rate of Hib disease.

Diagnosing Hib disease is difficult; Hib primarily causes meningitis and pneumonia, two common syndromes often treated empirically. To diagnose Hib meningitis, lumbar punctures must be performed and cerebrospinal fluid rapidly processed in a microbiologic laboratory with the technical capability and supplies to culture Hib. Hib pneumonia is even more difficult to diagnose. Although blood cultures are highly specific for Hib, they have a sensitivity of 20%, thus allowing the role of Hib as a cause of pneumonia to be underestimated (3,4).

Several methods have been used to define the rate of Hib disease in developing countries. Randomized, controlled trials of the Hib conjugate vaccine in The Gambia, which looked at radiographic evidence of pneumonia as a study endpoint, showed that approximately 20% of consolidated pneumonia (diagnosed by chest x-ray) is prevented.
by vaccination and therefore presumably caused by Hib (5). In Chile, a retrospective analysis of pneumonia after a randomized, controlled trial of Hib vaccine showed a 22% reduction in consolidation or pleural effusion in the group that received vaccine (6). Despite the quality of the data produced, such trials are too expensive, time-consuming, and complex to be widely used to measure Hib disease. A more widely used method to measure Hib disease has been population-based laboratory surveillance for Hib meningitis (7). Such surveillance, although dependent on the rate of lumbar punctures and laboratory quality, can produce reliable data on the incidence rate of Hib meningitis, which can be compared across countries. Population-based surveillance for meningitis, however, requires at least a year to produce results and does not give an estimate of Hib pneumonia.

Alternatives to these longitudinal, time-consuming, resource-intense methods of measuring Hib disease are being sought, particularly for use in regions of the world, such as Africa, where studies have consistently shown high rates of Hib disease (8–12). In countries in these regions, more limited data on the local rate of Hib disease may be adequate to allow evidence-based decisions about introducing Hib vaccine and to provide a basis for national advocacy for, and commitment to, such decisions.

On the basis of these considerations, we created a rapid assessment tool to measure the national rate of severe Hib disease. We discuss the methods used in the rapid assessment tool and its results in the first 11 countries where it was used.

Methods

Logistics

Requests for Hib rapid assessments were made by national ministries of health. The Hib rapid assessment tool was usually completed in 7 to 10 days. The assessment teams consisted of local representatives from the ministry of health and one or two consultants familiar with the tool. National data relevant to the tool was collected by reviewing national health statistics and medical literature, focusing on national or regional journals. The assessment often included several days in the capital city reviewing available data and working with the ministry of health. Most of the assessment involved collecting, evaluating, and synthesizing existing data from hospitals. Data were entered into standardized spreadsheets that assist in the calculation of the national disease impact. The assessment often ended with a meeting to discuss the findings with key persons in the ministry of health and leading pediatricians.

The tool uses two different, complementary methods to estimate Hib rates. The first one works from the “bottom up,” beginning with a local estimate of Hib meningitis incidence rates; the second works from the “top down,” starting with the country’s under-5 mortality rate, which is the number of children per 1,000 live births who die before their fifth birthday.

Meningitis Incidence Rate Method

This method uses retrospective data to calculate an incidence rate of Hib meningitis, from which the annual numbers of cases of and deaths attributable to Hib meningitis and pneumonia are estimated. The first step for this method is selecting an appropriate site where the incidence of Hib meningitis can be accurately calculated. The ideal site is a region of the country where the catchment population of a hospital or a few hospitals can be well defined, where most children with meningitis go to these hospitals, and where the private sector rarely uses the Hib vaccine. In addition, physicians in these hospitals should routinely perform lumbar punctures on children with suspected meningitis, and the hospitals should have laboratories that culture *H. influenzae* and conduct cytologic tests on cerebrospinal fluid. Although few laboratories in our assessments performed serotyping, we assumed that, in the absence of vaccination, all *H. influenzae* in cerebrospinal
fluid was caused by type b (Hib), which is consistent with available studies (13,14).

Calculating Hib meningitis incidence rate begins by counting the number of cases of culture-confirmed meningitis in children <5 years of age during a defined period in a defined catchment population (Figure 2). The percentage of culture-confirmed meningitis caused by Hib is then calculated. Cerebrospinal fluid samples from neonates are excluded because Hib is very rare in the first month of life (15). Next, the number of purulent cerebrospinal fluid samples during the same period is counted. For the purposes of this tool, purulence is defined in a way that increases the specificity for bacterial meningitis: visible turbidity, ≥100 leukocytes/mm³, or 10–99 leukocytes/mm³ with glucose <40 mg/dL and protein >100 mg/dL. The culture-confirmed cases are excluded, which leaves a count of culture-negative, purulent cerebrospinal fluid samples. The tool assumes that these are bacterial in origin, and the number ascribed to Hib is calculated by applying the same percentage of culture-confirmed meningitis caused by Hib to the culture-negative, purulent specimens. The rationale for this step is that a certain percentage of Hib meningitis cases may not be culture-confirmed because of prior antimicrobial drug use or laboratory-related factors. The number of culture-confirmed Hib cases and estimated culture-negative Hib cases is added. Then this number is inflated by a factor on the basis of information from local pediatricians to account for children with suspected meningitis who did not get a lumbar puncture. The number of children <5 years of age in the catchment region of the hospitals is used as the denominator to calculate the Hib meningitis incidence rate.

In the last step, the total number of children <5 years of age in the country is used to estimate the annual number of cases of Hib meningitis in the country. By using a ratio of five Hib pneumonia cases for each case of Hib meningitis, which was found in two randomized studies of Hib conjugate vaccine, the annual number of cases of Hib pneumonia is estimated (5,6). By using the case-fatality proportions of Hib meningitis and Hib pneumonia, obtained locally if possible, the annual number of deaths attributable Hib in the country is also estimated.

**Under-5 Mortality Rate Method**

This method starts with the birth cohort in the country (Figure 3). To calculate the number of childhood deaths in the country, the under-5 mortality rate is applied to the birth cohort. The neonatal death rate, if available, is subtracted from this number. Then the percentage of childhood deaths caused by acute respiratory illness is estimated. This percentage is extrapolated from a study that showed that the higher the under-5 mortality rate, the greater percentage of deaths that are caused by respiratory illness (16). In the next step, the percentage of all acute respiratory illness deaths due to Hib is estimated to be 13%. This figure was obtained by subtracting the number of respiratory deaths unlikely to be caused by Hib, during the neonatal period and caused by measles and pertussis, and multiplying the remaining number of respiratory deaths by 20%, the percentage of severe pneumonia thought to be caused by Hib (5,6,17). This calculation yields the annual number of pneumonia deaths caused by Hib. Then, meningitis cases and meningitis deaths are calculated by using the case-fatality proportion of Hib pneumonia, the 5:1 Hib pneumonia:meningitis ratio, the Hib meningitis case-fatality proportion, and the annual number of Hib pneumonia cases.

**Review of the Rapid Assessment Tool**

The tool was pilot-tested in several countries, and a draft version was reviewed at a meeting of technical experts convened by the World Health Organization in October 2000. Suggested revisions to the tool made at the
meeting were subsequently incorporated. The minutes of this meeting (http://www.who.int/vaccines-documents/DocsPDF01/www604.pdf), along with the completed tool including worksheets, are available on the Internet (http://www.who.int/vaccines-documents/DocsPDF01/www625.pdf).

Results

The Hib rapid assessment tool has been used in 11 developing countries in sub-Saharan Africa, the Middle East, and Asia (Tables 1 and 2). The assessment of the meningitis incidence rate was performed most often in smaller cities, where the denominator population could be more easily delineated, although several assessments were performed in larger cities, such as Alexandria, Egypt, and Shiraz, Iran. In most countries, at least one hospital had cultured *H. influenzae* from cerebrospinal fluid. The exceptions were Uzbekistan, Yemen, and Ghana. Alternative strategies were used to estimate the incidence rate of Hib meningitis in these countries. In Uzbekistan, where laboratories had been unable to culture Hib from cerebrospinal fluid and the leukocyte counts were not being performed properly, data from a prospective, population-based study in Moscow, which has a very similar health care system, were applied to the rate of lumbar punctures performed in larger cities, such as Alexandria, Egypt, and Shiraz, Iran. In most countries, at least one hospital had cultured *H. influenzae* from cerebrospinal fluid. Except for a few countries (i.e., Uganda, Oman, and Kyrgyzstan), the rates of culture-confirmed meningitis were very low, <10 per 100,000 children <5 years of age. When we used the rapid assessment tool, the estimated rates of Hib meningitis tended to be several-fold higher than those based on culture-confirmed Hib. When we used the tool, the estimated incidence rate of Hib meningitis among children <5 years of age was >50 cases per 100,000 children <5 years of age in Ghana and Uganda to <15 cases in Iran, Jordan, and Uzbekistan. The under-5 mortality rate method yielded estimates of national Hib disease rates that were higher than those obtained from the meningitis incidence rate method (mean 2.8, 95% confidence interval 1.7–3.9.)

Discussion

The Hib rapid assessment tool estimated rates of Hib meningitis in Africa and the Middle East that were similar to those obtained from population-based studies in the same regions (Figure 4, 8–12,18–22). In countries in Eastern Europe and Asia where the impact of Hib meningitis is less well-defined, the tool yielded variable results, although not too discrepant from rates found from prospective, population-based surveillance performed in other countries in the same areas (23–26). These results demonstrate that the Hib rapid assessment tool can yield estimates of Hib

<table>
<thead>
<tr>
<th>Country</th>
<th>% culture-confirmed Hib</th>
<th>Unadjusted Hib meningitis rate (cases/deaths)</th>
<th>Adjusted Hib meningitis rate (cases/deaths)</th>
<th>Meningitis cases/deaths</th>
<th>Pneumonia cases/deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghana</td>
<td>NA</td>
<td>0</td>
<td>72a</td>
<td>2,030/609</td>
<td>10,148/2,030</td>
</tr>
<tr>
<td>Uganda</td>
<td>50</td>
<td>44</td>
<td>59</td>
<td>2,533/633</td>
<td>12,663/759</td>
</tr>
<tr>
<td>Egypt</td>
<td>50</td>
<td>1</td>
<td>23</td>
<td>1,795/682</td>
<td>8,977/539</td>
</tr>
<tr>
<td>Iran</td>
<td>67</td>
<td>5</td>
<td>7</td>
<td>599/12</td>
<td>2,994/180</td>
</tr>
<tr>
<td>Jordan</td>
<td>43</td>
<td>6</td>
<td>14</td>
<td>107/5</td>
<td>534/27</td>
</tr>
<tr>
<td>Morocco</td>
<td>44</td>
<td>6</td>
<td>23</td>
<td>689/34</td>
<td>3,443/172</td>
</tr>
<tr>
<td>Oman</td>
<td>43f</td>
<td>27</td>
<td>41</td>
<td>112/11</td>
<td>560/34</td>
</tr>
<tr>
<td>Yemen</td>
<td>NA</td>
<td>0</td>
<td>23f</td>
<td>790/245</td>
<td>3,950/897</td>
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<td>Kyrgyzstan</td>
<td>33</td>
<td>15</td>
<td>20</td>
<td>101/12</td>
<td>505/51</td>
</tr>
<tr>
<td>Uzbekistan</td>
<td>NA</td>
<td>0</td>
<td>4f</td>
<td>103/12</td>
<td>515/52</td>
</tr>
<tr>
<td>Bhutan</td>
<td>42</td>
<td>6</td>
<td>15</td>
<td>16/5</td>
<td>80/8</td>
</tr>
</tbody>
</table>

*a* Meningitis caused by Hib.

Unadjusted meningitis incidence rate is the number of culture-confirmed Hib meningitis cases per 100,000 children <5 years of age in the study site, as determined by the rapid assessment tool (see Methods).

Adjusted meningitis incidence rate is the number of Hib meningitis cases per 100,000 children <5 years of age in the study site, as determined by the rapid assessment tool (see Methods).

National estimates.

Based on literature review and use of data from hospital.

Based on use of data from Moscow study and rate of lumbar puncture in Uzbekistan.
disease rates similar to expected rates in many countries. Without some of the adjustments made by the tool, the incidence rate of culture-confirmed Hib meningitis would have been low in most of these countries. One strength of the tool is that its adjustments compensate for artificial reasons for low incidence rates of culture-confirmed Hib meningitis, such as low rates of lumbar puncture and the lack of appropriate microbiologic capacity.

While not meant to replace more rigorous and accurate methods used to measure Hib disease, such as prospective, population-based surveillance, the tool addresses a unique need for countries desiring local Hib disease rate estimates to assist them in making a decision to introduce Hib vaccine. The strengths of the tool in addressing this need are that it is rapid, inexpensive, uses locally obtained data, accounts for biases in these data that might underestimate the true rate of Hib disease, and provides disease impact output as the number of cases and deaths from Hib disease, which is often more tangible to decision makers than incidence rates. Moreover, the process of performing the rapid assessment engages pediatricians and decision makers in a dialogue about the clinical, epidemiologic, and laboratory aspects of Hib disease and vaccine. Consequently, the Hib rapid assessment process helps countries develop the capacity to critically evaluate existing local data and perceptions about Hib disease rates.

The Hib rapid assessment tool has several limitations. Whereas in most countries we found hospitals that effectively cultured Hib, in several countries we were unable to find any site with demonstrated capacity to culture Hib. In these countries, we were unable to use the meningitis incidence rate method as outlined in the tool. As mentioned, alternative strategies based on local and regional data were used here, which might have introduced inaccuracies. Nonetheless, the estimates of Hib impact in these countries were similar to those observed in population-based studies from the same regions (Figure 4).

A second limitation of the tool is that even when a hospital that has cultured Hib is found, the output from the tool using the meningitis incidence rate method will always be constrained by the quality of the data. Although the tool corrects for missed cases of Hib meningitis, it does not correct completely for substantial underdiagnosis of Hib meningitis. If clinical or microbiologic factors have led to such underdiagnosis, this will necessarily be reflected in the rate estimates made by the tool. This limitation was exemplified during the rapid assessment in Kyrgyzstan, where the tool found a rate of Hib meningitis of 20.4 per 100,000 in 1999 to 2000, a period when the infectious disease hospital in Bishkek was involved in a study with Aventis-Pasteur, which supplied the laboratory with appropriate reagents and materials to isolate Hib. From 2001 through 2002, after the study ended, the rate obtained from tool in the same hospital decreased to 4.7 per 100,000.

### Table 2. Results of Haemophilus influenzae type b (Hib) rapid assessment tool (under-5 mortality rate method) in 11 developing countries

<table>
<thead>
<tr>
<th>Country</th>
<th>U5MR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Meningitis cases/deaths&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pneumonia cases/deaths&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Ratio meningitis cases U5MR:MIR&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghana</td>
<td>110</td>
<td>5,465/984</td>
<td>27,326/4,099</td>
<td>2.7</td>
</tr>
<tr>
<td>Uganda</td>
<td>147</td>
<td>2,838/709</td>
<td>14,189/2,838</td>
<td>1.1</td>
</tr>
<tr>
<td>Egypt</td>
<td>65</td>
<td>6,731/2558</td>
<td>33,657/2,019</td>
<td>3.7</td>
</tr>
<tr>
<td>Iran</td>
<td>31.5</td>
<td>1,975/40</td>
<td>9,876/593</td>
<td>3.3</td>
</tr>
<tr>
<td>Jordan</td>
<td>33</td>
<td>313/16</td>
<td>1,563/78</td>
<td>2.9</td>
</tr>
<tr>
<td>Morocco</td>
<td>Not done</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oman</td>
<td>21.5</td>
<td>48/15</td>
<td>242/14</td>
<td>0.43</td>
</tr>
<tr>
<td>Yemen</td>
<td>105</td>
<td>3,085/956</td>
<td>15,427/956</td>
<td>3.9</td>
</tr>
<tr>
<td>Kyrgyzstan</td>
<td>19.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>125/15</td>
<td>625/63</td>
<td>1.2</td>
</tr>
<tr>
<td>Uzbekistan</td>
<td>21&lt;sup&gt;f&lt;/sup&gt;</td>
<td>565/68</td>
<td>2,826/283</td>
<td>5.4</td>
</tr>
<tr>
<td>Bhutan</td>
<td>63&lt;sup&gt;g&lt;/sup&gt;</td>
<td>54/18</td>
<td>270/27</td>
<td>3.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> U5MR, under-5 mortality rate method; MIR, meningitis incidence rate method.

<sup>b</sup> National estimates.

<sup>c</sup> U5MR excludes neonatal diseases.

---

**Figure 4. Comparison of incidence rates of Haemophilus influenzae type b (Hib) meningitis per 100,000 children <5 years of age between the rapid assessment tool (gray bars) and prospective, population-based laboratory surveillance (white bars), by region (8–10,12,19–26). UAE, United Arab Emirates.**
A third limitation of the rapid assessment tool is that several assumptions are incorporated into the calculations. These assumptions, although based on the available literature, may not apply to all populations. For example, the 5:1 ratio of Hib pneumonia to meningitis, which was based on the results of two randomized controlled vaccine trials in The Gambia and Chile, may not apply to all parts of the world (5,6). In countries with lower under-5 mortality rates, the 5:1 ratio may be too high, while in countries with high under-5 mortality rates, the 5:1 ratio may be too low. Moreover, this ratio may vary in different parts of the world, as suggested from the results of a recently completed Hib conjugate vaccine efficacy trial in Lombok, Indonesia, which suggested the incidence of Hib meningitis to be high and the incidence of radiographically defined Hib pneumonia to be relatively low (27).

A fourth concern is that the under-5 mortality rate method yielded higher estimates of Hib disease rates than the meningitis incidence rate method. One reason for this difference is that the neonatal death rate was not subtracted from the under-5 mortality rate in most countries (except Kyrgyzstan, Uzbekistan, and Bhutan). This omission may have led to overestimating Hib disease since neonatal deaths account for a large proportion of childhood deaths, but Hib deaths are rare in neonates (13). Also, because the under-5 mortality rate method starts with pneumonia deaths and works backwards to meningitis cases, more assumptions are made in the last estimate. Therefore, in reconciling the estimates obtained from the two methods, the meningitis prevalence may be more accurate than the meningitis incidence rate method, while estimation of the pneumonia incidence may have an accuracy in between those obtained from the two methods.

The results of the Hib rapid assessment tool need to be interpreted in light of its limitations and the validity of its assumptions critically evaluated in each country where it is used, particularly in regions, such as Asia, where the impact of Hib disease is still uncertain. Nonetheless, the tool has been useful to some countries in deciding whether to adopt Hib vaccine into their national immunization programs. Of the countries we studied, three (Ghana, Uganda, and Oman) introduced Hib vaccine, and three others (Bhutan, Kyrgyzstan, and Yemen) requested Hib vaccine on their initial application to GAVI. Subsequent to the assessments in these countries, the tool has been used in several other developing countries that were considering the introduction of Hib vaccine (Western Pacific Islands [28], Albania, Thailand, Nepal, and Sudan). Several countries (Uganda, Albania, and Egypt) have used results of the rapid assessment tool in cost-effectiveness analyses of Hib vaccine introduction. As more developing countries consider Hib vaccination programs, estimating the local impact of Hib disease will continue to be an important part of the effort to introduce and sustain Hib vaccination programs. The experience with measuring Hib disease rates, including the rapid assessment tool, might also provide a valuable base for considering the introduction of other new vaccines, including pneumococcal and meningococcal conjugates.

Acknowledgments

We thank those who participated in these assessments from the ministries of health and hospitals in these countries; the other consultants who assisted in these assessments, Shatrughan Bastola, Selma Khamassi, Salah Al-Awaidy, Robin Biellik, Mamadon Malifa Balde, Jean Bosco Ndihokubwayo, Richard Adegbola, Alexander Platonov, Amany Gayed, Fouad Youssef, and Frank Mahoney; and the technical reviewers for evaluating the tool at the meeting in Geneva in October 2000.

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Dr. Feikin is a medical epidemiologist in the Respiratory Diseases Branch at CDC. He specializes in vaccines for the prevention of bacterial pneumonia in developing countries.

References


Address correspondence to Daniel Feikin, Centers for Disease Control and Prevention, 1600 Clifton Rd., Mailstop C23, Atlanta, GA 30333, USA; fax: 404-639-3970; email: drf0@cdc.gov

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We report three patients infected with unique strains of vancomycin-dependent enterococci. Two were first infected by genetically identical strains of vancomycin-resistant enterococci (VRE). All three patients had much greater exposure to vancomycin and third-generation cephalosporins than did two control groups (patients infected with VRE and hospitalized patients without enterococcal infections). While antimicrobial pressure promotes nosocomial colonization by VRE, prolonged exposure to vancomycin may foster the transition from vancomycin resistance to dependence.

Vancomycin-resistant enterococci (VRE) are major nosocomial pathogens worldwide (1). Recent case reports, however, describe nosocomial infections caused by enterococci that require vancomycin for growth (2–12). Since 1993, we have identified three patients in our center infected by vancomycin-dependent enterococci (VDE). We report the microbiologic features and molecular epidemiology of nosocomial infection caused by these organisms.

Methods

Enterococci showing growth on media containing 6 µg/mL of vancomycin and an MIC >8 µg/mL were considered vancomycin-resistant. Strains unable to grow in the absence of vancomycin 6 µg/mL, despite multiple subcultures, were considered vancomycin-dependent.

The genotypic basis of vancomycin resistance was determined by using polymerase chain reaction to amplify sequences coding for resistance, using oligonucleotide primers for vanA (5'CATGAATAGAATTTGCTTCGCTTACCCCTTAAACG, Department of Pharmacology, University of Wisconsin-Madison, Madison, WI) and 5'CTATCAACCCCTTAAACG, Department of Pharmacology, University of Wisconsin-Madison, Madison, WI) and vanB (5'AAATTCGATCCGACTACATC and 5'AA-CGATGCCGCCATCCTCCTG, University of Wisconsin Bio-technology Center, Madison, WI). Susceptibility was assessed by National Committee for Clinical Laboratory Standards criteria using Mueller-Hinton II agar (BBL, Becton-Dickinson, Cockeysville, MD) containing vancomycin 6 µg/mL. The capacity of D-alanyl-D-alanine to support growth of the VDE was tested by using 2.5-mg and 5-mg disks of D-alanyl-D-alanine on Mueller-Hinton II agar. Screening for revertants was performed by plating serial dilutions of an overnight culture of VDE in vancomycin-containing broth to Mueller-Hinton II agar. Screening for revertants was performed by plating serial dilutions of an overnight culture of VDE in vancomycin-containing broth to Mueller-Hinton II agar with and without vancomycin, which was incubated for 48 hours at 35°C. Molecular relation of strains was determined by using pulsed-field gel electrophoresis (PFGE) after digestion of genomic DNA with restriction endonuclease Smal (Gibco BRL, Promega, Madison, WI) (13).

Case Reports

Patient 1

A 32-year-old woman with long-standing type 1 diabetes mellitus and end-stage renal disease was admitted for a kidney-pancreas transplant. Postoperatively, she had multiple complications, including transplant renal failure and intraperitoneal infection caused by vancomycin-resistant Enterococcus faecium. She received vancomycin, teicoplanin, imipenem, amikacin, cefazolin, ceftazidime, ciprofloxacin, gentamicin, metronidazole, ticarcillin-clavulanate, trimethoprim-sulfamethoxazole, and intravenous amphotericin B. On hospital day 53, intra-abdominal fluid specimens obtained at surgery yielded vancomycin-resistant E. faecium that did not grow on media without vancomycin (Figure 1). The infection was
Blood cultures were positive on radiometric monitoring. Subculturing onto media containing vancomycin confirmed bacteremia with a strain of *E. faecium* that did not grow in the absence of vancomycin. The patient remained critically ill, despite prolonged therapy with intravenous quinupristin-dalfopristin, and died after 4 days of refractory VDE bacteremia, 132 days after transplantation.

**Patient 3**

A 47-year-old woman with chronic myelogenous leukemia received a matched-unrelated donor bone marrow transplant. Subsequently, when severe graft-versus-host disease, acute renal failure, cyclosporine neurotoxicity, prolonged respiratory failure, and bacteremia with *Corynebacterium* spp. resistant to β-lactam antimicrobial agents developed, the patient received a prolonged course of vancomycin. On hospital day 80, when vancomycin-containing media was used, she was found to have catheter-associated urinary tract infection with a strain of enterococcus that required vancomycin for growth. Efforts were not made to eradicate VDE from the urine. The patient ultimately died of refractory graft-versus-host disease with multiple organ dysfunction syndrome on posttransplant day 87.

**Case-Control Study**

Potential risk factors for nosocomial infection were compared in the 3 patients and 10 randomly selected patients with nosocomial infection caused by VRE and 10 at-risk, concurrently hospitalized patients not infected by enterococci. Controls were matched by age and admission to the same hospital service as patients in the VRE cohort.

**Results**

PFGE analysis indicated that the three strains of vancomycin-dependent enterococci were clonally distinct (Figure 2) when the criteria of Tenover et al. were used (14). In the two cases in which strains of VRE were isolated before VDE were first detected, the restriction fragment patterns of the initial VRE strain and subsequent VDE isolate were identical.

All three strains of VDE were *E. faecium*; two were genotype vanA, and one was vanB. All showed resistance to penicillin, ampicillin, amoxicillin-clavulanate, gentamicin, and erythromycin. All were susceptible to quinupristin-dalfopristin; two were intermediately susceptible, and one was susceptible to teicoplanin. The rate of spontaneous reversion to nondependence on vancomycin was $1.2 \times 10^{-6}$ for strain 1, $2.5 \times 10^{-6}$ for strain 2, and $2.6 \times 10^{-3}$ for strain 3. Growth of VDE was not supported by D-alanyl-D-alanine.

All three patients infected by VDE were female transplant recipients and experienced posttransplant acute renal failure; by contrast, the VRE group had lower exposure to...
the intensive care unit (Table). No other significant differences were noted between the two groups of patients with enterococcal infection and the group of at-risk controls in underlying conditions, severity of illness, or exposure to invasive devices. However, in the 60 days before onset of the nosocomial enterococcal infection, major differences in exposure to antimicrobial agents occurred: mean ± standard deviation (SD) total antimicrobial days 103 ± 40 for VDE, 86 ± 31 for VRE, and 28.6 ± 23.1 for noninfected controls (VDE or VRE vs. controls, p < 0.01), especially vancomycin (27 ± 14 days for VDE, 9 ± 10 for VRE, and 5.7 ± 7.6 for controls; VDE vs. VRE, p = 0.03) and third-generation cephalosporins (17.0 ± 11.4 days for VDE, 15.6 ± 11.9 for VRE, and 2.9 ± 4.8 for noninfected controls; VDE or VRE vs. controls, p < 0.01). All 3 patients with VDE infection died during hospitalization, contrasted with 3 of 10 patients infected with VRE and 2 of 10 uninfected control patients who died (p = 0.03).

Discussion

Vancomycin resistance is thought to be mediated primarily by the strain’s acquiring the capacity to synthesize the cell wall by using D-alanine-D-lactate (1). In the first clinical reports of VDE infection, Fraimow et al. (2) and Green et al. (4) independently reported that D-alanyl-D-alanine supported the growth of a vancomycin-dependent E. faecalis strain (2) and a vanB E. faecium strain (4), respectively; Sng et al. (9) quantified the amount of D-alanyl-D-alanine required to support growth of their VDE strain. The phenomenon of vancomycin dependence may derive from the loss of a D-alanyl-D-alanine ligase in a VRE strain, which is then unable to survive unless vancomycin induces the production of D-alanine-D-lactate ligase (2,4). Previous reports (2–12) and our experience (Figure 2) suggest that infecting strains of VRE make the transition in situ to a state of vancomycin dependence only after prolonged exposure to vancomycin.

Sixteen patients infected by enterococci dependent on vancomycin for growth have been reported (2–12). In every case with data reported on prior antimicrobial exposure, the patients had also received a glycopeptide, vancomycin, or teicoplanin. We sought to minimize the effect of control group bias (15) by selecting as a control group concurrently hospitalized patients at risk for nosocomial infection with VRE or VDE but not infected with enterococci. We found that intense use of third-generation cephalosporins was the most important risk factor for both VDE and VRE when compared with the uninfected control group. This finding is in line with our recent observation of the striking commonality of risk factors for nosocomial colonization and infection with VRE, which, after prolonged exposure to vancomycin, may lead to the emergence of vancomycin dependence in the colonizing strain.

Renal insufficiency was the other risk factor identified in our study. The ecologic impact of vancomycin exposure is magnified and extended in patients with renal
insufficiency, especially those with end-stage renal disease requiring hemodialysis (all 3 of our patients and 5 of the 16 previously reported cases), where a single dose persists in the patient’s body for many days. The emergence of novel strains of *Staphylococcus aureus* exhibiting resistance to vancomycin has also been reported in this clinical setting (17). The prevalence of nosocomial infection or colonization with VDE can only be determined by the use of media containing vancomycin when processing cultures from patients at risk for VDE infection, namely those who have had prolonged exposure to vancomycin or third-generation cephalosporins, especially if they are already known to be colonized or infected by VRE.

These infections are clearly not trivial, although their clinical importance remains to be fully determined. Five of the 16 previously reported VDE infections were bacteremias (4,7,9,11). VDE was considered the immediate cause of death in one of our patients and a contributory cause in another. Green et al. (4) reported the spontaneous reversion of VDE to nondependence at 1 in 106, which we confirmed in all three of our strains. Thus, vancomycin discontinuation alone may not be sufficient to treat patients with VDE infection, especially if the patient has renal failure.

The best management of infection with VDE—beyond source control and treatment with linezolid, quinupristindalfopristin, or daptomycin—remains to be determined. More effective antimicrobial stewardship policies are needed to prevent VDE, VRE, and other resistant nosocomial pathogens from emerging.

### Table. Clinical and epidemiologic features of patients with nosocomial enterococcal infections and uninfected control patients

<table>
<thead>
<tr>
<th>Features</th>
<th>VDE (n = 3)</th>
<th>VRE (n = 10)</th>
<th>Uninfected control patients (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean ± SD</td>
<td>39.0±7.5</td>
<td>41.7±20.2</td>
<td>51.1±13.0</td>
</tr>
<tr>
<td>Sex, no.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Duration of hospitalization, days, mean ± SD</td>
<td>41.7±13</td>
<td>33.6±12.1</td>
<td>37.6±44.7</td>
</tr>
<tr>
<td>ICU stay, days, mean ± SD</td>
<td>9.3±4.0</td>
<td>1.0±1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4±7.4</td>
</tr>
<tr>
<td>Primary bloodstream infection</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Surgical wound infection</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Intraabdominal infection</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Urinary tract Infection</td>
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<td>Medicine or pediatrics</td>
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<td>5</td>
</tr>
<tr>
<td>Surgery</td>
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<td>5</td>
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<tr>
<td>Associated conditions, no.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Malignancy</td>
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<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Renal failure</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Trauma</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Transplant recipient</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>APACHE II score, mean ± SD</td>
<td>18.7±2.1</td>
<td>15.1±6.7</td>
<td>19.4±10.0</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL, mean ± SD</td>
<td>2.8±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5±0.8</td>
<td>1.9±1.8</td>
</tr>
<tr>
<td>Days antimicrobial agent administered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>27.3±13.7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9.1±10.3</td>
<td>5.7±7.6</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>11.3±6.7</td>
<td>9.7±9.8</td>
<td>2.5±5.1</td>
</tr>
<tr>
<td>First- or second-generation cephalosporins</td>
<td>0.7±0.6</td>
<td>1.1±2.2</td>
<td>3.6±7.5</td>
</tr>
<tr>
<td>Third-generation cephalosporins</td>
<td>17.0±11.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.6±11.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9±4.8</td>
</tr>
<tr>
<td>Quinolones</td>
<td>10.3±5.5</td>
<td>8.0±9.2</td>
<td>3.4±4.8</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2.3±4.0</td>
<td>7.1±11.7</td>
<td>1.2±3.8</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>4.3±7.5</td>
<td>4.4±6.3</td>
<td>1.8±3.8</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>32.7±18.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.6±19.2</td>
<td>2.7±5.7</td>
</tr>
<tr>
<td>Others</td>
<td>1.0±1.7</td>
<td>5.6±9.5</td>
<td>4.8±9.6</td>
</tr>
<tr>
<td>Total</td>
<td>106.3±44.7&lt;sup&gt;g&lt;/sup&gt;</td>
<td>83.5±29.4&lt;sup&gt;g&lt;/sup&gt;</td>
<td>28.6±23.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>VDE, vancomycin-dependent enterococci; VRE, vancomycin-resistant enterococci; ICU, intensive care unit; APACHE, Acute Physiology and Chronic Health Evaluation score.

<sup>b</sup>VRE vs. controls, p = 0.03.

<sup>c</sup>VDE vs. VRE, p = 0.02.

<sup>d</sup>VDE vs. VRE, p = 0.03.

<sup>e</sup>VRE vs. controls, p < 0.01.

<sup>f</sup>VDE vs. controls, p < 0.01.
Acknowledgments

We thank Bernard Weisblum for providing the primers for the polymerase chain reactions used to characterize the strains as either vanA or vanB.

Dr. Tambyah is consultant infectious disease physician and associate professor of medicine at the National University of Singapore, Singapore. This research was conducted when he was employed at the University of Wisconsin. His research interests are in nosocomial infections, in particular, emerging nosocomial pathogens such as SARS and multidrug-resistant bacteria.

References


Address for correspondence: Paul A. Tambyah, Department of Medicine, National University of Singapore, 5 Lower Kent Ridge Road, Singapore 119074, Singapore; fax: 65-67794112; email: mdcpat@nus.edu.sg
An outbreak of Q fever occurred in South Wales, United Kingdom, from July 15 through September 30, 2002. To investigate the outbreak a cohort and nested case-control study of persons who had worked at a cardboard manufacturing plant was conducted. The cohort included 282 employees and subcontractors, of whom 253 (90%) provided blood samples and 214 (76%) completed questionnaires. Ninety-five cases of acute Q fever were identified. The epidemic curve and other data suggested an outbreak source likely occurred August 5–9, 2002. Employees in the factory’s offices were at greatest risk for infection (odds ratio 3.46; 95% confidence interval 1.38–9.06). The offices were undergoing renovation work around the time of likely exposure and contained straw board that had repeatedly been drilled. The outbreak may have been caused by aerosolization of *Coxiella burnetii* spore-like forms during drilling into contaminated straw board.

Q fever is an infection caused by the bacterium *Coxiella burnetii*. The organism is found in most parts of the world and is endemic in wild and domestic animals, rodents, and arthropods, which provide a reservoir for infection (1). Most outbreaks have been associated directly or indirectly with farms or farm animals, but urban outbreaks have been described (2,3). Infected animal birth products can cause outbreaks of Q fever, and an infected placenta can contain as many as 10⁹ organisms per gram (4). *C. burnetii* produces a spore-like form, which can survive for months or years before being inhaled and causing infection (5,6). The infective dose can be as low as one organism; therefore, large outbreaks can be caused by a small source (7). A review of the literature was undertaken by one of the authors (available from H.C. van Woerden). This investigation identified 79 outbreaks reported in 48 articles in English language journals. An additional 44 papers in other languages were identified in a literature review by Williams (7) and a further 40 German outbreaks were identified in a literature review by Hellenbrand et al. (8). The literature review suggested that most outbreaks are associated with primary or secondary aerosols that arise around infected animals or contaminated fomites (5,9).

Approximately 70 cases of Q fever are identified in the United Kingdom each year as a result of routine surveillance (R. Smith, pers. comm., Zoonosis Surveillance, Communicable Disease Surveillance Centre, Wales). However, seroprevalence studies indicate that approximately 27% of farmers and 10% of the general population have antibodies, which suggests previous exposure to the organism; this finding does not appear to have changed substantially during the last 45 years (10,11). We report an investigation of an outbreak of Q fever at the premises of a manufacturer of cardboard packaging materials in Newport docks, South Wales, in the summer of 2002.

**Methods**

**Description of the Outbreak**

A possible outbreak of atypical pneumonia was reported to the local public health department on September 12, 2002, by a physician who reported that other employees at the patient’s workplace had had similar symptoms. The outbreak was verified, an outbreak control team assembled, and a case definition agreed on (12). By September 15, 2002, a total 12 potential patients had been identified and the first case confirmed as Q fever. The investigation...
was begun by contacting all relevant hospital clinicians and general medical practitioners in the Gwent area and requesting that they supply blood samples from any patients who had symptoms compatible with Q fever.

**Epidemiologic Investigation**

Several hypotheses were explored. An outbreak could have occurred in the wider community, and employees could have been infected by contaminated straw, hay, or compost; wild or feral animals; or domestic animals, particularly pregnant or newborn animals. Contamination could have been through sources brought into the factory, which included the following: contaminated personal belongings; contact with a contaminated source on the docks, which were on the way to work; windborne spread from goods passing through the docks; airborne spread from goods passing through the docks; animals or animal-based feed; contaminated hay, straw, or farm vehicles; sources in the factory premises; wooden delivery pallets contaminated with chicken carcasses returned to the factory; infection passed by red mites biting infected seagulls nesting on the roof, which then may have bitten staff in the factory; airborne spread from a cat that had given birth near the factory 2 years previously; airborne spread of contaminated dust generated by the renovation work; dust previously contaminated by an infected animal, bird, rodent, or bat; or contaminated straw or straw board aerosolized during drilling or removal.

We obtained data from a variety of sources, including a questionnaire survey, laboratories, clinicians, and factory management. A list containing details of the workforce and possible, past, and confirmed cases was developed and used to construct an epidemic curve. Data on place of work provided by factory management were used to calculate attack rates. Details were also collected on persons who had been on site for a limited number of days to help pinpoint the onset of the outbreak. Employees working on the factory floor were examined to determine whether a pattern occurred in the infected patients by calculating the relative risks for employees at each machine on the factory floor.

Two clinics were held at the factory on September 23 and 30, 2002, where blood samples were obtained from and questionnaires were completed by employees and subcontractors who had worked at the factory at any time from July 15, 2002, through September 30, 2002. A confirmed case was defined as phase 2 immunoglobulin (IgM) \( \geq 320 \) or fourfold rise in complement fixation tests (CFT) titer or IgM 20–160 + phase 2 IgG \( \geq 320 \). A past exposure was defined as phase 2 IgG but no phase 2 IgM. A noncase (control) was defined as a CFT of \( \leq 8 \) negative phase 2 IgM and IgG + either no symptoms or onset of symptoms >7 days before blood sample or (in which the onset of symptoms was within 7 days of first sample) two consecutive blood samples with a CFT of \( \leq 8 \) + one negative phase 2 IgM and IgG. A possible case was defined as all remaining cases.

Data were analyzed with Epilinfo (v. 6.04, Centers for Disease Control and Prevention, Atlanta, GA), Excel 97 (Microsoft, Redmond, WA), and Stata (v. 7, Stata Corporation, College Station, TX) software. Possible cases in patients and persons with evidence of past exposure were excluded from the nested case-control study. The analysis also excluded responses of “not sure” from odds ratio (OR) calculations. We calculated Mantel-Haenszel OR with exact 95% confidence limits (CI) (13).

**Microbiologic Investigation**

Complement fixation tests for phase 1 and phase 2 antibodies were performed at the Public Health Laboratory Service, Cardiff. IgM and IgG immunofluorescent assays were carried out on the samples by the Centre for Applied Microbiological Research (CAMR), Porton Down, UK. Laboratory staff monitored all requests for Q fever serologic testing from general practitioners and hospital clinicians to identify any additional cases that might be linked with the outbreak.

**Environmental Investigation**

Environmental information on the factory was gathered by environmental health officers and other members of the outbreak control team during site visits on September 23, 2002, and September 30, 2002. Management representatives of several other premises in or near the docks were interviewed.

On October 11, 2002, an environmental scientist collected 17 random environmental samples of straw and dust from inside and outside the factory premises. The samples were sent for polymerase chain reaction (PCR) testing at CAMR.

**Results**

**Epidemiologic Investigation**

A total of 222 employees and 60 subcontractors were working in the factory complex from July 15 through September 30, 2002. Questionnaires were completed by 214 (75.9%) of these 282 persons. Of the 253 persons who

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were tested, we identified 95 (37.5%) confirmed cases of Q fever, 42 possible cases, 8 cases of past exposure, and 108 noncases. Four persons refused blood tests but completed a questionnaire. Data for the nested case-control analysis were available on 75 (78.9%) of the 95 confirmed cases and 101 (93.5%) of the 108 noncases. The frequency and duration of symptoms are shown in Table 1 and Figure 1. Ten participants were still ill when questioned, and 5 did not provide a date of onset of symptoms. Five patients (5.3%) were admitted to the hospital with pneumonia. Some patients experienced fatigue. However, the clinical impression of one of the authors involved in follow up of patients was that very few neurologic symptoms occurred during this outbreak, compared to a previously reported U.K. outbreak (14). Further analysis of clinical symptoms is being prepared as a separate paper.

The epidemic curve for 49 confirmed cases where the date of onset of symptoms was reliably known is shown in Figure 2. A peak incidence occurs around September 1, 2002. Based on an incubation period of 5 to 40 days (1,5), these data suggest that almost all the cases can be accounted for by an exposure from August 7 to 11, 2002.

Seven confirmed patients were only present in the factory on 2 or 3 days. All these persons were present in the factory and potentially exposed to infection from August 5 through August 9, 2002.

An analysis of home postal codes of 71 participants with Q fever who completed the questionnaire showed no discernible pattern and indicates that our participants were not part of a larger Q fever outbreak with a common source in the community. Details of place of work within the factory complex were available for participants with 61 confirmed cases and 81 controls. No cases occurred among persons working exclusively outside the factory floor or office block. In addition, no cases were identified among seven participants working in a separate design office, one employee working exclusively in the dispatch building, or five sales representatives who only called into the office on an occasional basis (Table 2). The OR for having a case in office staff compared with other staff was 3.46 (95% CI 1.38–9.06). ORs for groups of staff working in all other areas were <1 (Table 2).

The relative risks of having a case of Q fever among the cohort of employees working at different machines on the factory floor are shown in Figure 3. The balcony in Figure 3 is not drawn to scale. It overhangs the adjacent machines where the relative risk to workers was zero. The relative risk for infection was greatest among people who worked in the center of the factory floor outside the shadow of the overhanging balcony; the risk for infection dropped towards the sides of the building.

Eighty-three percent of confirmed cases were in men, a similar male-to-female ratio to that of the cohort as a whole, and median age was 44 years (range 22–60 years). Questionnaire data indicated that infected employees did not own animals that had given birth or had a miscarriage nor had these employees had any contact with the birth products of animals. One subcontractor, who cleaned windows at the factory, also worked on a farm and had been in contact with animals that had given birth, but the evidence did not suggest that any of these had been infected with *C. burnetii*. Additionally, the serologic tests for this employee were negative for Q fever, and the dates on which he visited the factory suggest that his clothing or possessions could not have been the source of the outbreak.

Table 1. Frequency of symptoms in 55 symptomatic patients with confirmed cases of Q fever, Newport, Wales, August–September 2002

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes (%)</th>
<th>Not sure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>41 (75)</td>
<td>1</td>
</tr>
<tr>
<td>Sweats</td>
<td>53 (96)</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>51 (93)</td>
<td>1</td>
</tr>
<tr>
<td>Weight loss</td>
<td>26 (47)</td>
<td>2</td>
</tr>
<tr>
<td>Cough</td>
<td>24 (44)</td>
<td>0</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>25 (45)</td>
<td>2</td>
</tr>
<tr>
<td>Joint pain</td>
<td>44 (80)</td>
<td>3</td>
</tr>
<tr>
<td>Chest pain</td>
<td>20 (36)</td>
<td>5</td>
</tr>
<tr>
<td>Jaundice</td>
<td>4 (7)</td>
<td>5</td>
</tr>
</tbody>
</table>

*These responses represent a misunderstanding of the term jaundice, since none of these persons had clinical jaundice.

Case-patients were much more likely than controls (OR 5.86; 95% CI 0.55 to 291.88) to recall coming across a hay lorry entering or leaving the docks while on their way to or from work. Adjusting for cases in those whose office was refurbished reduced the OR in those who saw a hay lorry (OR 3.00; 95% CI 0.28–31.80). Employees whose offices had been refurbished were at greatest risk for infection (OR 2.60; 95% CI 0.77–9.57). Employees who described themselves as “never near an external door or window” were more likely to be infected than those who worked “near an external door or window on most days” (OR 1.98; 95% CI 0.72–5.56). Living on a farm appeared slightly protective (OR 0.35; 95% CI 0.01–4.53) as did the regular handling of compost (OR 0.14; 95% CI 0.00–1.03).
However, none of these findings, or those in Table 3, reached statistical significance value of 5%.

The work undertaken by the seven participants with the shortest incubation times was examined for unusual characteristics. A higher proportion of those with a short incubation time were women (three of seven) when compared with the general population. Four of the seven participants worked in offices that had been refurbished, and the remaining three worked on the factory floor. Their duration of illness varied from 4 to 14 days.

**Microbiologic Investigation**

Two hundred and fifty-three participants (89.7%) provided blood samples. Some participants had only one sample taken and others had up to four additional samples taken from September through December 2002 at primary care or hospital clinics. A summary of CFT and IgM results is shown in Tables 4 and 5.

As a result of informing general practitioners in the area of the outbreak, more than twice the normal numbers of general practitioner requests for Q fever serologic testing were received. Hospital samples submitted for Q fever serologic testing were also monitored. Our monitoring identified one patient with a chronic case of Q fever and one patient with an acute, neither were associated with this outbreak. No *C. burnetii* was identified by PCR testing the straw board and dust samples that were obtained from the factory.

**Environmental Investigation**

The factory consists of several buildings. The main production area consists of a large, rectangular open-plan hanger with an elevated office block at one end of the rectangle (Figure 3). The office block was undergoing extensive renovation work at the time of the outbreak. This involved drilling >100 holes in the straw board ceiling to allow the attachment of a new suspended ceiling. Some internal walls made of straw board were also removed. A temporary corridor was created from plastic sheeting which ran through the area being renovated but did not form a complete seal. No respiratory protection was used by the contractors or the workforce at any stage. The corridor was in constant use by staff in adjacent offices. Office staff and factory floor workers who visited the offices consequently had some exposure to dust generated by the renovation work.

The layout of the factory is consistent with the possibility of disseminating contaminated dust from the renovated offices to the factory floor. The office block ran along the length of one end of the factory floor. Double-swing doors led from the second floor renovated offices onto an overhanging internal balcony 30 feet above the large open-plan factory floor (Figure 3). The factory production area had no windows and no air-conditioning system. A dust extraction system existed around some of the machines on the factory floor to collect waste cardboard. The lack of win-

**Table 2. Attack rates and odds ratios (OR) for different areas of work at factory implicated in Q fever outbreak, Newport, Wales, August–September 2002**

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of persons working in area</th>
<th>No. of persons working elsewhere</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production/factory floor</td>
<td>Cases 35 Controls 52 Attack rate (%) 40.2</td>
<td>Cases 26 Controls 29 Attack rate (%) 47.3</td>
<td>0.78 (0.36–1.57)</td>
</tr>
<tr>
<td>Dispatch</td>
<td>0 1 0</td>
<td>61 80 43.3</td>
<td>0 (0–71.79)</td>
</tr>
<tr>
<td>Dispatch/factory floor</td>
<td>4 4 50.0</td>
<td>57 77 42.5</td>
<td>0.88 (0.14–2.68)</td>
</tr>
<tr>
<td>Office</td>
<td>20 10 66.7</td>
<td>41 71 36.6</td>
<td>3.46 (1.38–9.06)</td>
</tr>
<tr>
<td>Production-based but sometimes in the office</td>
<td>1 2 33.3</td>
<td>60 79 43.2</td>
<td>0.66 (0.01–12.96)</td>
</tr>
<tr>
<td>Design</td>
<td>0 7 0</td>
<td>61 74 45.2</td>
<td>0 (0–0.88)</td>
</tr>
<tr>
<td>Sales representatives</td>
<td>0 5 0</td>
<td>61 76 44.5</td>
<td>0 (0–1.42)</td>
</tr>
<tr>
<td>Dispatch but sometimes in the office</td>
<td>1 0 100</td>
<td>60 81 42.6</td>
<td>Undefined</td>
</tr>
<tr>
<td>Total</td>
<td>61 81 43.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval.
windows in the factory production area and the dust extraction system almost certainly caused a degree of negative pressure in the factory. This condition would draw air in through the double doors leading from the renovated office area and onto the factory floor.

Discussion

Environmental and epidemiologic evidence suggests that this outbreak was associated with the renovation of an office block within a cardboard manufacturing plant. One potential source identified was straw board in walls and ceilings disturbed by the renovation work. If straw board had been contaminated at some time in the past with a concentrated source of C. burnetii, drilling into this could have produced a cloud of dust containing large numbers of C. burnetii sporelike forms. Dust containing C. burnetii sporelike forms could have been sucked through the balcony doors from the renovated offices, fallen onto the workforce below, and inhaled by those infected. Workers could also have been infected when visiting the personnel or accounts offices situated adjacent to the renovation work.

No record of visits to these departments exists, which would allow this hypothesis to be further assessed. However, the hypothesis is supported by a number of factors. The pattern of relative risk for infection in groups of participants at different machines on the factory floor is consistent with this hypothesis. The highest relative risks are in the center of the factory close to the balcony, while the lowest risks are in the areas at the sides and far end of the factory floor. The overhanging balcony may have sheltered employees at some of the machines from any contaminated dust falling from above. Raised ORs for infection in employees who were decanted into neighboring offices because their offices were being renovated, and in office staff whose offices had been refurbished, also implicate the renovation work as the source of the outbreak.

The timing of the installation of the new suspended ceiling (July 17–August 9, 2002) is consistent with an outbreak source near August 5 through August 9. The raised OR in persons rarely near an open window or door compared with those often near an open window or door and the lack of cases among those who worked in the separate design office, or among sales representatives, suggest that the source of the outbreak was inside the factory.

The respirable dust fraction that is most pathogenic is generally invisible to the naked eye (15,16). We do not have a good proxy for exposure in this outbreak, and consequently the issue of a dose response has not been addressed. Exact place of work probably did not closely correlate with exposure as many staff members move around the building as part of their work.

Potential Contamination of the Straw Board

Straw board could have been contaminated either before or after manufacture. Investigating the process used to make the straw board indicated that the low pressures and temperatures involved would not kill any fungal spores present in the straw. If straw board becomes wet, these fungal spores often sprout and damage the board. The straw used to produce the board was stored in large Dutch barns and would have been accessible to rodents, cats, and other animals. Some evidence exists that a number of cases of Q fever were occurring around 1950 in the English county where the straw board was manufactured (17) and that the straw board was probably manufactured from 1950 to 1953. C. burnetii sporelike forms are

![Diagram](image-url)
resilient. They can withstand pressures of up to 20,000
lb/in², elevated temperatures, desiccation, osmotic shock,
UV light, and chemical disinfectants (18). However, experimen-
tal studies of the survival of C. burnetii sporelike forms have not demonstrated survival beyond 8 years
(Table 6) (5,6). Whether experiments for longer durations
were undertaken is not clear from the source documents.

Although not directly comparable, Bacillus anthracis and
Clostridium tetani spores are known to survive for many
years. For example, B. anthracis spores have been record-
ed as surviving for 71 years on dried silk threads (19).
Alternatively, the straw board could have been contam-
inated after manufacture by the feces, urine, birth products,
or a corpse of an infected rodent that gained access to the
inner layer of a straw board. Some holes were drilled in the
straw board ceiling in 1982 and 1983, which could have
provided a point of entry. Rodents are considered an
important potential source of C. burnetii, and in one U.K.
serosurvey, 34% of wild brown rats (Rattus norvegicus)
had antibodies suggesting previous exposure to C. burnetii
(20). The placentas of common rodents can also contain
large numbers of C. burnetii sporelike forms (21) and
could contaminate straw.

Test results of environmental samples in this outbreak
were, however, negative. This finding could have occurred for a number of potential reasons. The samples were collected by persons who did not have detailed knowledge of
the outbreak investigation, and the samples tested were
minute in comparison to the quantity of straw disrupted
during the renovation work. Concentration of potential
bacterial contaminants was attempted in the PCR tests, but
analysis was performed on small aliquots of extract, and
bacterial DNA could therefore easily have been missed.
The PCR test used was also experimental, although the
protocol followed was similar to that used in Australia,
France, and Germany. A delay of 2 months occurred be-
tween the dates when employees were probably exposed
to C. burnetii and when environmental dust samples were
collected. Consequently, contaminated dust may have been
dispersed or cleaned up in the interim. In previous out-
break investigations, test results of environmental air or
straw samples for C. burnetii have also more often been
negative (15,22–23) than positive (4,24). The environmen-
tal sampling was, therefore, like looking for a “needle in a
haystack.”

Other Hypotheses

We considered a range of alternative hypotheses but did
not find any evidence to support them. For example, wind
speeds were recorded routinely by the harbor authority but
were very low during the week of August 5 through August
9, 2002, which makes windborne spread from the nearest
farmland, 1 1/2 to 3 miles away, unlikely. No other poten-
tial wild or domestic animal sources were identified.

Animals or animal products had not been moved through
the docks in recent years. A feral cat had given birth in an
adjacent building 1–2 years previously. One of the kittens
had been adopted by an employee. However, the em-
ployee’s serologic testing for Q fever was negative. If the
feral cat had been infected with Q fever, the employee
would most likely have had evidence of past exposure to
C. burnetii. In addition, the factory strongly emphasizes
controlling vermin as some of their cardboard packaging is
used as secondary packaging in the food industry. No cats
or other animals had been identified in or around the build-

Table 4. Summary of highest phase 2 CFT results recorded for each person in the cohort in Q fever outbreak, Newport, Wales,
August–September 2002

<table>
<thead>
<tr>
<th>Highest CFT</th>
<th>AQF cases</th>
<th>Noncases</th>
<th>Past exposure</th>
<th>Possible cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8</td>
<td>4</td>
<td>104</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>32</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>64</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>128</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>256</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>512</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1,024</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals (253)</td>
<td>95</td>
<td>108</td>
<td>8</td>
<td>42</td>
</tr>
</tbody>
</table>

*CFT, complement fixation test; AQF, acute Q fever.

Table 5. Summary of highest phase 2 IgM results recorded for
107 persons in the cohort

<table>
<thead>
<tr>
<th>IgM P2 values</th>
<th>AQF cases</th>
<th>Past exposure</th>
<th>Uncertain status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Low levels</td>
<td>0</td>
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<td>2</td>
</tr>
<tr>
<td>&lt;60</td>
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<td>0</td>
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<td>160</td>
<td>5</td>
<td>0</td>
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</tr>
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<td>640</td>
<td>16</td>
<td>0</td>
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</tr>
<tr>
<td>1,280</td>
<td>4</td>
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</tr>
<tr>
<td>&gt;1,280</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

*Ig, immunoglobulin; AQF, acute Q fever.
Contaminated fomites can produce secondary aerosols of C. burnetii sporelike forms (4), and several outbreaks have demonstrated the possibility of spread on fomites such as clothing, straw, hay, contaminated shoes, and building materials (22,25–36). However, unless a mechanism exists to repeatedly reaerosolize the source, fomites are likely to pose a risk even when they are not heavily contaminated, and this view is supported by the general principles that govern the dispersion and settling out of dust particles or sporelike forms (17,37).

Neither straw nor building material is a common source of outbreaks of Q fever. However, straw has been suggested as a possible source in several outbreaks (15,27,38,39). Two case reports implicate straw: a physician who contracted Q fever after clearing out straw and rubble from his new moorland home (26) and a businessman who was attracted Q fever after cleaning out a barn that had been used for keeping livestock 10 years previously but had not been properly cleaned since (24). Moldy hay from this barn, cultured using cell growth medium, grew C. burnetii. The renovation of buildings has also been suggested as a source of Q fever in two previous outbreaks (26,39). The widespread dispersal of spores in a building has been demonstrated both by Q fever (disseminated through a large medical building school building) (23) and by anthrax (dispersed through a post office with an area of 281,387 ft² and a volume of approximately 7 million ft³) (40).

One other alternate hypothesis is that the source of the outbreak was outside the factory building. Five persons mentioned having seen a hay lorry in the docks. This hypothesis was pursued because straw from farm vehicles had been implicated as a potential cause in a previous local outbreak of Q fever (2). However, the route taken by the lorries was never closer than half a mile to the factory. The lorries passed much closer to several other factories and to residential areas where several thousand persons would have had much greater exposure than the workforce at the factory. Although two Q fever cases were identified in the neighboring factory, no evidence existed of a wider outbreak involving other premises in the docks or nearby residential areas. The hypothesis that hay lorries passing through the docks could have caused the outbreak was known to a number of employees before they completed the questionnaire, and this finding may therefore be a result of diagnostic suspicion bias (41).

Control Measures

Risk assessment and risk management was undertaken by identifying groups of persons at different levels of risk and providing relevant advice, temporarily stopping work in the area of the building considered at greatest risk, and following identified patients with Q fever. The cardboard manufactured by the factory was produced at temperatures that made survival of C. burnetii sporelike forms impossible so customers were not considered to be at increased risk. Unlike the straw board, which was produced at very low temperatures, the cardboard is produced at temperatures that would make survival of C. burnetii sporelike forms impossible. In addition, the cardboard was only used for secondary packaging and was therefore not in direct contact with any food products.

Implications of the Study

Inhaled organic particles are an important source of a number of occupational diseases (17,42), and risks from exposure to occupational dust have been addressed by the U.K. Health and Safety Executive (43,44). Q fever is also a recognized occupational disease in the United Kingdom (45) and governed by existing legislation (46), although it is not a notifiable disease (47).

Straw is an increasingly popular ecologically friendly material, and >350,000 houses have been built in the United Kingdom with this particular type for straw board as internal partitions. The product has also been exported around the world. However, this outbreak is the first where straw board was suggested as a possible source of Q fever. Further research is needed to fully investigate straw board in various venues as a potential vehicle in Q fever outbreaks. Contaminated straw board represents a potential source of Q fever and should be considered in future outbreak investigations.

Acknowledgments

We thank the other members of the National Public Health Service and Newport City Council Environmental Health Department, workers and management at the factory, and other laboratory and clinical staff who contributed to the management of the outbreak.

Dr. van Woerden works at the National Public Health Service in Wales. His research interests include health protection and environmental epidemiology.

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Table 6. Survival of Coxiella burnetii

<table>
<thead>
<tr>
<th>Environment</th>
<th>Temperature (°C)</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wool</td>
<td>15–20</td>
<td>7–9 mo</td>
</tr>
<tr>
<td>Wool</td>
<td>4–6</td>
<td>Approx. 12 mo</td>
</tr>
<tr>
<td>Sand</td>
<td>15–20</td>
<td>4 mo</td>
</tr>
<tr>
<td>Fresh meat</td>
<td>Cold storage</td>
<td>&gt;1 mo</td>
</tr>
<tr>
<td>Salt meat</td>
<td>Not recorded</td>
<td>5 mo</td>
</tr>
<tr>
<td>Skimmed milk</td>
<td>Not recorded</td>
<td>40 mo</td>
</tr>
<tr>
<td>Tap water</td>
<td>Not recorded</td>
<td>30 mo</td>
</tr>
<tr>
<td>Tick feces</td>
<td>Room</td>
<td>Conclusive evidence: 586 d Some evidence: 6 and 8 y</td>
</tr>
<tr>
<td>Not recorded</td>
<td>−20</td>
<td>2 y</td>
</tr>
<tr>
<td>Not recorded</td>
<td>−65</td>
<td>8 y</td>
</tr>
</tbody>
</table>

*References 5 and 6.*
Q Fever Outbreak in Industrial Setting

References

19. Material safety data sheet—infestious substances, section i—infec-
42. Health and Safety Executive Statistics, Table RIDDOR01: Cases of occupational disease reported under RIDDOR (a). Sheffield, United Kingdom: Health and Safety Executive; 2002.

Address for correspondence: H.C. van Woerden, Department of Epidemiology, Statistics and Public Health, University of Wales College of Medicine, Heath Park, Cardiff, CF14 4XN, United Kingdom; fax: +44-29-2040-2504; email: vanwoerdenh1@cf.ac.uk
Collecting Data To Assess SARS Interventions

R. Douglas Scott II,* Edward Gregg,* and Martin I. Meltzer*

With cases of severe acute respiratory syndrome (SARS) occurring across geographic regions, data collection on the effectiveness of intervention strategies should be standardized to facilitate analysis. We propose a minimum dataset to capture data needed to examine the basic reproduction rate, case status and criteria, symptoms, and outcomes of SARS.

First detected in China, confirmed and probable cases of severe acute respiratory syndrome (SARS) have now appeared in at least 30 countries in five continents. SARS is the first new severe infectious disease to occur in the 21st century, and little is known about its epidemiologic features (1). To assess the effect of SARS on public health and outcomes, data are needed about who becomes ill, how they contracted their illness, and the sequelae.

A minimum set of data on intervention effectiveness should be collected in a uniform manner from each identified SARS case-patient at each location. Without such standardization, datasets from different locales may not be sufficiently comparable, thereby limiting the ability to scientifically evaluate both the effect of SARS and the interventions to control and prevent its spread.

We propose a minimum set of epidemiologic and clinical variables that should be among the top priorities when designing data collection protocols related to SARS interventions. We set priorities for the variables in the minimum dataset as a guide for agencies unable to collect all the recommended data. Additionally, we summarize the health measures constructed from each of the variables, along with the possible policy implications, to provide further guidance to health agencies regarding the importance of each variable. A case study is available in an online appendix.

Previous tools have been used to understand the spread of SARS and associated illnesses (2). These tools have not provided all necessary data to facilitate modeling usefulness and cost-effectiveness of interventions. Researchers have published results from relevant epidemiologic data, but no forms of itemized data are readily available (3). Our minimum dataset differs from minimum reporting requirements recently published by the World Health Organization (WHO) (2). WHO data templates include a daily summary of SARS cases to be reported at the national level and a case-reporting form that contains detailed clinical information (based on current WHO case definitions), including patient demographics, exposure, contact follow-up, daily reporting of symptoms, hospital admission, final case classification, and final case status. The dataset we propose captures information on length of exposure, incubation period from exposure to symptom onset, and use of health care resources (e.g., length of hospitalization, length of isolation, and admission to intensive care) not currently collected by WHO’s template.

Proposed Minimum Dataset and Data Prioritization

Figures 1 and 2 (a downloadable document is available online at http://www.cdc.gov/ncidod/eid/vol10no7/03-0749-G1.htm and http://www.cdc.gov/ncidod/eid/vol10no7/03-0749-G2.htm) illustrate the minimum epidemiologic variables needed to evaluate the public health effect of SARS and the cost of interventions. These data would provide the evidence to determine key epidemiologic relationships, including the incubation period (time from exposure to onset of symptoms), the onset of symptoms leading to hospitalization, and the outcomes resulting from treatment (either discharge of patient or death). Descriptions of the variables listed in Figures 1 and 2, along with suggestions for coding, are provided in the online Appendix 1 (http://www.cdc.gov/ncidod/eid/vol10no7/03-0749_app1.htm). For all tables, the column heading corresponds with the variable name (e.g., A represents the case identification [ID] number, B represents sex, C represents age).

Figure 1 captures case-patient demographics, exposures, and symptoms. Suggested coding for demographic variables (online Appendix 1) include patient ID and age as continuous variables and sex and coexisting conditions (e.g., cardiovascular disease, diabetes) or syndromes (HIV/AIDS) as categorical variables. Other categories for coexisting conditions can be added as appropriate (e.g., smoking). An important distinction should be made between patients who have no known diagnosed coexisting conditions (coded as none known) as opposed to patients for whom information about coexisting conditions is not available or missing (coded as unknown).

In Figure 1, exposure variables and their suggested coding include date (DD/MM/YY), source (whether the source is already identified and included in the data table as an observed patient with an assigned ID or whether the source is unknown), duration of exposure (<30 minutes, 30–59 minutes, or ≥60 minutes), and locale (whether exposure occurred at home, in a hospital, or some other location). The same variables are measured for each exposure, and the table can be expanded to collect information on all known exposures.
Symptoms are categorized as either respiratory or non-respiratory. For each symptom, onset date and type (a categorical variable that can be expanded for patients with multiple symptoms) are collected. Suggested categories for symptoms include fever, myalgia, dyspnea, headache, chills, diarrhea, nausea, sore throat, arthralgia, chest pain, productive cough, nonheadache neurologic symptoms (e.g., dizziness), rhinorrhea or runny nose, vomiting, and abdominal pain. The list of symptom categories can be revised or extended as needed.

Figure 2 contains information on case criteria, along with health outcomes associated with the case. Categorical variables making up case status include the clinical case criteria (either asymptomatic or mild respiratory illness, moderate illness, severe respiratory illness, or none), epidemiologic criteria (travel within 10 days to infected area, close contact, both, or none), laboratory confirmation (yes, no, or undetermined), and case classification (probable, suspected, or noncase).

Outcome variables include hospitalization (along with admission date if hospitalized), treatment status (antiviral agent, antibacterial agent, or other treatment), isolation start date, number of days isolated (a continuous variable), number of days on ventilation or in intensive care (continuous variables), discharge date (0 if still hospitalized), death (yes or no), and date of death. The online Appendix 2 (available at http://www.cdc.gov/ncidod/eid/vol10no7/03-0749-G1.htm) provides an example of Figures 1 and 2 filled out with data from four “typical” case-patients. The variable categories from the tables in online Appendix 1 can be readily extended or revised as new information about SARS becomes available. The footnotes offer the definitions that served as the basis for the suggested categories.

Priority Classification Groups

Online Appendix 1 also provides proposed priority classification groups for each variable listed in Figures 1 and 2. Variables that are labeled “priority group 1” represent the most important set of variables, and those labeled as “priority group 3,” the least important. The table in online Appendix 1 provides a summary of how each variable contributes to important health policy questions related to the SARS outbreak. Taken together, these tables can provide guidance to health organizations regarding which data should be collected so that the needed policy analysis can be conducted (Table).

Priority group 1 variables (sex, age, date and source of exposure, date of symptom onset, and case status and criteria variables) contain the information on the transmission rate of the disease and incubation periods. These variables provide crucial information in determining the basic reproduction number of an infection (defined as the expected number of secondary infectious cases resulting from one
primary case in a susceptible population) (4,5). This measure is vital for estimating the impact of control measures to reduce the transmission of SARS (4,5). Priority group 2 variables (duration and locale of exposure; hospitalization, including start date; isolation, including start date; and death, including date of death) provide information that can be used to evaluate the risk for hospitalization or death associated with exposure, length of incubation, and impact of isolation. Priority group 3 variables (coexisting conditions; categories of symptoms; treatment status; ventilation or intensive care, including start date; and date of discharge) are not essential information for containing SARS outbreaks but provide additional information about healthcare resources (treatment and intensive care) used to treat SARS patients. Priority group 3 variables can also be used by hospital administrators and public health officials to plan and prepare for a sudden change in resource use during a catastrophic infectious disease outbreak (e.g., pandemic influenza) (6).

Conclusions

The emergence of a novel disease like SARS, which requires a global public health response to contain its spread, has illustrated the need for collecting effectiveness data in a uniform manner. Given the potential for a large variation in location-specific circumstances, producing a single questionnaire that would be entirely suitable for all locales would be difficult. Figures 1 and 2 illustrate some of the most important data needed to understand and control the disease. The tables present a standardized protocol and approach for ensuring that all the proposed data have been collected. As an illustration of the use of the tables, a case study is presented in online Appendix 2. Identifying effective interventions during an outbreak becomes important in managing public health resources. The minimum dataset proposed here provides a basis for standardizing the collection of data from various geographic locations, thereby facilitating the analysis of SARS interventions.

Dr. Scott is an economist with the Division of Healthcare Quality Promotion, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia. His areas of research include the economics of hospital infection control, infectious diseases, and patient safety.

References


Address for correspondence: R. Douglas Scott II, Division of Healthcare Quality Promotion, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop E55, 1600 Clifton Rd., Atlanta, GA 30333, USA; fax: 404-498-1101; email: Dscott1@cdc.gov
Mice Susceptible to SARS Coronavirus

David E. Wentworth,*† Laura Gillim-Ross,* Noel Espina,* and Kristen A. Bernard†

Murine models of severe acute respiratory syndrome–associated coronavirus (SARS-CoV) will greatly advance research on this emerging virus. When BALB/c mice were simultaneously inoculated intranasally and orally, replication of SARS-CoV was found in both lung and intestinal tissue.

The outbreak of severe acute respiratory syndrome (SARS) that emerged in China in November 2002 was caused by a novel coronavirus (SARS-CoV) that was detected in lungs, nasopharyngeal aspirates, and feces of infected patients (1–4). This outbreak in humans is striking because of the high rate of illness and death associated with it. The SARS-CoV outbreak likely resulted from zoonotic transmission, and natural animal reservoirs of viruses nearly identical to SARS-CoV increase the likelihood of its reemergence in humans (5).

Coronaviruses are enveloped, plus-stranded RNA viruses that cause important respiratory and enteric diseases of humans and many animal species. Large peplomers or spike glycoproteins (S) are the viral attachment proteins that protrude from the virion and give it the appearance of a corona. Coronaviruses are members of the Nidovirales, which produce 3′ co-terminal nested subgenomic mRNAs upon entry into susceptible host cells. The genome is the largest of all RNA viruses (27.6–31.2 kb), and the genomic RNA is infectious when transfected into cells from a wide variety of species and tissue types. Yet most strains of coronavirus have very restricted species and tissue tropism, illustrating the major role S-receptor interactions play in the species specificity and pathogenesis of coronaviruses. SARS-CoV sequence analysis shows that it has many of the unique characteristics of coronaviruses and that it shares the most predicted amino acid similarity and other molecular signatures with serogroup 2 coronaviruses (6).

Animal models of SARS-CoV are important for the study of virus-host interactions. Cats, ferrets, and nonhuman primates have been experimentally infected with SARS-CoV (7,8). In addition, SARS-CoV–like viruses were isolated from palm civet cats and closely related raccoon dogs, which are sold in markets in China (5). All of these animal species are important for the in vivo study of SARS-CoV. However, a murine model is also necessary to evaluate antiviral agents, vaccines, and immune response. Previous studies in suckling mice inoculated intracranially or intraperitoneally suggest that mice are not permissive to SARS-CoV (1,8). On the other hand, the infection of divergent species suggests that many animal species may be susceptible (5,8).

Human and animal coronaviruses are transmitted by the respiratory or enteric routes and initially infect epithelial cells of these tissues (9). Thus, a combined intranasal and oral injection of mice was explored as a potential animal model for SARS-CoV. Four-week-old, female BALB/c mice were inoculated intranasally and orally with 2 x 10⁵ 50% tissue culture infectious dose of SARS-CoV Urbani or were mock-inoculated with carrier alone. Mice were weighed and observed for clinical signs daily throughout the study. Three SARS-CoV–inoculated and one mock-inoculated mouse were euthanized 3, 5, 7, 10, and 28 days postinoculation (p.i.). Tissues harvested on euthanasia included blood, lungs, and small intestine (ileum). Total RNA was isolated from the lungs and intestines. All work with mice was conducted at the Wadsworth Center, New York Department of Health, Albany, under a protocol approved by the Institutional Animal Care and Use Committee. All experiments with infectious SARS-CoV were performed in a biosafety level 3 laboratory and were conducted under appropriate conditions, with precautions that adhered to, or exceeded, the requirements set forth in “Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS” (available from www.cdc.gov/ncidod/sars/sarslabguide.htm).

To specifically identify virus replication, a multiplex reverse transcriptase–polymerase chain reaction (RT-PCR) was used to simultaneously amplify glyceraldehyde 3-phosphate dehydrogenase (G3PDH), SARS-CoV genomic RNA (gRNA), and subgenomic RNA (sgRNA) (10). Upon entry into host cells the coronavirus gRNA (27–31.5 kb) serves as an mRNA to translate two large polyproteins (1a and 1ab). The polyproteins are autocatalytically processed into replicative enzymes, including the RNA-dependent RNA polymerase, which synthesizes both negative-sense and positive-sense sgRNAs, and the positive-sense sgRNAs serve as mRNAs for all of the open reading frames (ORFs) downstream of ORF1b (6,11). SARS-CoV infects Vero, Vero E6, and primary rhesus monkey kidney cells in culture, and infected cells have a nested set of eight 3′ co-terminal of mRNAs, each of which has at its 5′ end a leader sequence derived from the 5′ terminus of the genome (6,12). We took advantage of the unique features of CoV replication, sgRNA transcription in particular, to develop multiplex primers to differentiate input gRNA.
from sgRNA that is produced upon entry into the host cell. A 180-bp amplicon is produced from a sense primer (SARS-1 [5'-ATATTAGGTTTTACCTACCCAGG-3']) that is specific for the leader sequence and an antisense primer specific for the SARS-CoV spike glycoprotein gene (SARS-21,593R [5'-AGTATGGTGTGTAATTAGGAG-3']). This amplicon is produced only when sgRNA is present and indicates virus entry and replication. To identify gRNA (input and newly synthesized), a sense primer that binds the 3' terminus of the 1b gene (SARS-21,263 [5'-TGCTAATCTATTTCTGGGAG-3']) was paired with SARS-21,593R to produce a 276-bp amplicon. The amplification was performed as a multiplex reaction for SARS-CoV gRNA, sgRNA, and G3PDH by using a OneStep RT-PCR procedure (Qiagen, Inc., Valencia, CA); thus, both positive- and negative-sense SARS-CoV RNAs served as templates for reverse transcription.

Production and persistence of gRNA and sgRNA were examined in permissive and nonpermissive cells. Analysis of permissive Vero E6 cells showed that the sgRNA encoding S was not present in the input virus before RNA replication (Figure 1A, 1 h). However, S sgRNAs produced after entry were detected at 16 h and 5 d after SARS-CoV inoculation. Additionally, gRNA qualitatively increased after entry of the virus. In contrast, sgRNA was not detected in nonpermissive murine (L2) or human (MRC5) cell lines, and gRNA fell below detectable limits by 5 d. The reaction conditions were optimized to favor amplification of SARS-CoV gRNA and sgRNA; thus, when they were present at high levels, amplification of G3PDH was reduced. Amplification of G3PDH was used to demonstrate RNA integrity, and it was always detected in the absence of viral RNAs.

Lungs from the experimentally inoculated mice were analyzed by the multiplex assay. One of three mice from each group sacrificed on days 3, 5, and 7 p.i. showed evidence of lung infection (Figure 1B). The presence of gRNA on days 5 and 7 is strong evidence for viral replication since the inoculum is most likely degraded, as is seen with nonpermissive cells in vitro (Figure 1A). In addition, sgRNA, which is indicative of virus replication, was amplified from the lung RNA of a mouse on day 7 p.i. (Figure 1B, sgRNA). Heminested PCR of the first-round multiplex RT-PCR amplicons showed that SARS-CoV gRNA and sgRNA were present in the lungs of each of these mice. Another animal euthanized 5 days p.i. also showed evidence of infection by the heminested PCR (Figure 1B, HN). SARS-CoV gRNA or sgRNA was not detected in the lungs of mice 10 days p.i. or in any mock-inoculated animals (Figure 1). Infection of lung tissue in mice is consistent with the tropism of SARS-CoV in humans and experimentally inoculated nonhuman pri-mates, ferrets, and cats (4,7,8).

Coronaviruses of many animal species, including porcine, feline, canine, murine, and bovine, infect intestinal tissue (9). In humans, SARS-CoV causes interstitial pneumonia with fever and sometimes diarrhea (3,4). In our study, SARS-CoV gRNA was present in the intestines of all virus-inoculated mice at 3 and 5 days p.i. (Figure 1B). Heminested PCR of these amplicons showed that sgRNA was also present in all virus-inoculated animals on days 3 and 5. One mouse had sgRNA in the ileum 7 days p.i.; this same mouse that had qualitatively high levels of gRNA and sgRNA from RT-PCR reactions. Positive and negative controls for PCR reactions are indicated by + and −, respectively. D indicates DNA marker ladder. *The doublet observed in HN-PCR reactions results from residual primers used in the primary amplification reaction.

![Figure 1. Replication-specific multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) assay shows severe acute respiratory syndrome–associated coronavirus (SARS-CoV) replicated in the lungs and intestines of mice. A) Vero E6, murine fibroblast (L2), and human lung fibroblasts (MRC5) were inoculated with SARS-CoV at an MOI of 0.001 or were mock-inoculated (M). G3PDH, SARS-CoV gRNA, and sgRNA were amplified by multiplex RT-PCR from total RNA extracted at 1 h, 16 h, or 5 days after inoculation. Amplicons were visualized by ethidium bromide staining after electrophoresis; negative images are shown. B) Mice were inoculated with 2x10^6 50% tissue culture infective dose of SARS-CoV (lanes labeled 6–17) or were mock-inoculated (M) and euthanized after 3, 5, 7, or 10 days. G3PDH, SARS-CoV gRNA, and sgRNA were amplified by multiplex RT-PCR from total RNA extracted from the lungs (L) and intestines (I) harvested at various time points. Heminested PCR (HN) was used to amplify gRNA and sgRNA from RT-PCR reactions. Positive and negative controls for PCR reactions are indicated by + and −, respectively. D indicates DNA marker ladder. *The doublet observed in HN-PCR reactions results from residual primers used in the primary amplification reaction.](https://www.cdc.gov/eid/vol10no7/images/1294.jpg)
mice; four mice had ruffled fur for ≥3 days, including mouse 12, which had qualitatively high levels of gRNA and sgRNA in its lung (Figure 1B). No respiratory distress or diarrhea was observed throughout the study. The virus-inoculated mice tended to gain less weight than the mock-inoculated mice (Figure 2A). In addition, 3 of 15 mice lost 5%–6% body weight 3 days p.i., and 1 of 9 mice lost 6% body weight on day 7. Overall, six mice exhibited either mild clinical signs or weight loss throughout the study, while mock-inoculated mice remained unaffected. This finding suggests that SARS-CoV caused a subclinical infection or a very mild disease in mice.

Serum antibody to SARS-CoV was analyzed by a virus neutralization assay (Figure 2B). Mock-inoculated mice showed no virus neutralization, nor was neutralizing antibody detected in mice euthanized 3 or 5 days p.i. Neutralizing antibodies were detected in all mice sacrificed 7, 10, and 28 days p.i., and the titers were 8- to 16-fold higher on day 28. These neutralizing titers are similar to those reported for naturally and experimentally infected animals (5, 8). In addition, seroconversion to SARS-CoV is the accepted standard for the determination of human infections by SARS-CoV and continues to be more reliable than RT-PCR methods (4).

The presence of gRNA and, more importantly, sgRNA in the lungs and intestines shows that SARS-CoV replicates in these tissues. Furthermore, the high neutralizing antibody titers on day 28 p.i. are supportive of an active viral infection. The presence of SARS-CoV RNAs or neutralizing antibodies demonstrates that all 15 inoculated mice were infected. The results of this study suggest that SARS-CoV peaks early (days 3–5), and the immune response clears the virus from the lung and intestine by 10 days, which suggests that viral clearance in mice is more rapid than in human patients, who begin to recover 7–12 days after the onset of clinical illness (approximately 9–14 days after infection) (3, 4). In 10% to 15% of patients, the initial phase of disease is followed by more severe pulmonary disease characterized by respiratory distress, pulmonary infiltration of mononuclear inflammatory cells, multinucleated syncytia, and fibrosis (3, 4). The pathophysiology of the late complications of SARS is not understood, but immunopathology could play a critical role in the disease. This study opens many potential avenues of research using wild-type, transgenic, or knockout mice to answer questions of how age, sex, prior exposure, and immune response influence the pathogenesis of SARS-CoV.

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Dr. Wentworth is director of the influenza laboratory at the Wadsworth Center, New York State Department of Health. He is
also an assistant professor in the Department of Biomedical Sciences, School of Public Health at Albany, State University of New York. His research focuses on the interspecies transmission and pathogenesis of influenza A viruses and coronaviruses.

References


Address for correspondence: David E. Wentworth, Wadsworth Center, NYSDOH, 120 New Scotland Ave, P.O. Box 22002, Albany, NY 12201-2002; fax: 518-473-1326; email: dwentwor@wadsworth.org
Q Fever Outbreak in Homeless Shelter

Philippe Brouqui,*† Sékéné Badiaga,† and Didier Raoult*

Urban outbreaks of Q fever have occurred after exposure to slaughterhouses or parturient cats. We detected an outbreak of Q fever in a homeless shelter in Marseilles. Investigations showed that the main factors exposing persons to *Coxiella burnetii* were an abandoned slaughterhouse, used for an annual Muslim sheep feast, and wind. Homelessness, a problem that has been increasing since the mid-1980s, raises substantial public health concerns (1). We have worked with the homeless population of Marseilles since 1993 in ongoing studies on louse-transmitted diseases (2,3).

Q fever is a zoonosis caused by *Coxiella burnetii*, an intracellular bacterium transmitted by aerosols from contaminated soil or animal waste or by drinking contaminated milk (4). Domestic ungulates (cows, sheep, and goats) are the main reservoir for *C. burnetii*, but other mammals, including dogs, cats, and wild rabbits, have been implicated (5). Q fever usually occurs in rural areas when people are incidentally exposed to aerosols or infected milk or milk products (6), although urban outbreaks have been reported after occupational exposure to slaughterhouses (7–9).

While investigating louse-borne diseases in two homeless shelters in Marseilles, we systematically tested persons for antibodies to *C. burnetii* and found a significantly higher seroprevalence in the homeless population from the northern shelter (Figure 1) than in control blood donors. This shelter (A) is located 2 kilometers south of an abandoned slaughterhouse that is used 1 day each year by the Muslim population of Marseilles for the traditional sheep feast, “Aid El Khebir,” during which sheep are ritually killed. We hypothesized that the wind (the mistral) that blows over the slaughterhouse and the shelter was involved in spreading *C. burnetii*, as has been reported previously in another outbreak near Marseilles (10). We consequently investigated and followed-up the homeless population from the two shelters for 4 consecutive years and report here the first outbreak of Q fever in this population. We propose that the wind played a critical role in the outbreak.

**The Study**

The protocol was reviewed and approved by an institutional review board (Comités Consultatifs de Protection des Personnes dans la Recherche Biomédicale 99/76), and all participants gave informed consent. A medical team of 27 persons, comprising 9 nurses, 6 infectious diseases residents or fellows, and 12 infectious diseases specialists, visited the two shelters once yearly for 4 consecutive years. Each shelter can accommodate 300 persons each night, and each offers showers, food, washing machines, and clean clothes. Shelter B is located downtown, while shelter A is located in the northern part of the town (Figure 1). Homeless persons completed a standardized questionnaire, and a physical examination was performed. Nurses collected blood samples for laboratory investigation. Control subjects were sex- and age-matched blood donors enrolled during the same period and living in Marseilles. Serologic analysis was carried out at the French National Reference Center for Rickettsial Diseases. The antigen used was a phase II and phase I *C. burnetii* Nine Mile strain (ATCC VR 615) grown in our laboratory in L929 mouse fibroblasts. Phase I was obtained by injection in mice. Samples were assessed by microimmunofluorescence (MIF) as described elsewhere (11). Immunoglobulin (Ig) G phase II antibody titer >1:50 indicated *C. burnetii* exposure in the past 6 months to 5 years.

Meteorologic data were obtained from Meteo-France departmental weather stations (http://www.meteo.fr/meteonet/meteo/pcv/cdm/dept13/cdm2.htm#3). Maximum wind speeds and directions were measured three times each hour, which led to >1,400 data entries for the month observed. We asked for wind information during the month which followed the Aid El Khebir in each year: March 27–April 27, 1999; March 16–April 16, 2000; March 6–

*Université de la Méditerranée, Marseille, France; and †Centre Hospitalier Universitaire Nord Marseille, Marseille, France*
IgA antibody titers of 1:800, 1:50, and 1:200 to phase II was detected. The serologic tests showed IgG, IgM, and noted interstitial bilateral pneumonitis. No cardiac murmur showed symptoms of high-grade fever, arthralgia, myalgia; and a positive test result for C. burnetii IgG phase II antibodies were found in 17 (10.8%) of 157 persons in shelter A in 2000, compared to 14 (3%) of 460 controls (p < 0.001). In this shelter, the number of patients with a positive test result was significantly higher in 2000 than in 2001 (0/96, 0%) and 2002 (1/182, 0.54%) (p < 0.001). This difference was not significant when compared with results in 2003 (7/129, 5.4%). Among 467 controls, 217 completed the questionnaire.

C. burnetii IgG phase II antibodies were found in 17 (10.8%) of 157 persons in shelter A in 2000, compared to 14 (3%) of 460 controls (p < 0.001). In this shelter, the number of patients with a positive test result was significantly higher in 2000 than in 2001 (0/96, 0%) and 2002 (1/182, 0.54%) (p < 0.001). This difference was not significant when compared with results in 2003 (7/129, 5.4%). Among 467 controls, 217 completed the questionnaire.

C. burnetii IgG phase II antibodies were found in 17 (10.8%) of 157 persons in shelter A in 2000, compared to 14 (3%) of 460 controls (p < 0.001). In this shelter, the number of patients with a positive test result was significantly higher in 2000 than in 2001 (0/96, 0%) and 2002 (1/182, 0.54%) (p < 0.001). This difference was not significant when compared with results in 2003 (7/129, 5.4%). Among 467 controls, 217 completed the questionnaire.

C. burnetii IgG phase II antibodies were found in 17 (10.8%) of 157 persons in shelter A in 2000, compared to 14 (3%) of 460 controls (p < 0.001). In this shelter, the number of patients with a positive test result was significantly higher in 2000 than in 2001 (0/96, 0%) and 2002 (1/182, 0.54%) (p < 0.001). This difference was not significant when compared with results in 2003 (7/129, 5.4%). Among 467 controls, 217 completed the questionnaire.

When exposure to cats, kittens, or dogs; Muslim religion; and living in shelter A were considered, only contact with a kitten (p = 0.031) was associated with C. burnetii positivity in the univariate analysis. However, multivariate analysis using a stepwise linear regression model with all variables present in the univariate analysis showed that living in shelter A was the only factor independently associated with a positive test result for C. burnetii. Moreover, one person in 2002 and seven in 2003 were found positive in shelter A, compared to none in shelter B in those years. Acute Q fever was diagnosed in three homeless persons with IgM anti–phase II antibodies > 1:50, one person in 2000 and two in 2003. Two were asymptomatic, and one showed symptoms of high-grade fever, arthralgia, myalgia, and dyspnea. He was hospitalized, and a chest x-ray noted interstitial bilateral pneumonitis. No cardiac murmur was detected. The serologic tests showed IgG, IgM, and IgA antibody titers of 1:800, 1:50, and 1:200 to phase I antigen and 1:400, 1:25, and 1:200 to phase II antigen respectively. He was treated with 200 mg oral doxycycline each day for 15 days, and he recovered.

Weather records showed that the cumulative number of windy days with the wind blowing from the north (N), north-northwest (NNW), and northwest (NW) was significantly higher in the month that followed the Aid El Khebir in 1999 compared to 2000 (12/32, p = 0.002) and 2001 (2/32, p = 0.0006) (Figure 1) but not to 2002. The strength of the mistral measured as a mean of the daily recorded maximum speed was not significantly different among the investigated years (Figure 1).

Conclusions

Q fever is a disease caused by C. burnetii, a strict intracellular bacterium that can survive in the environment for up to 10 months at 15°–20°C, for >1 month on meat in cold storage, and for >40 months in skim milk at room temperature (5). Two distinct sets of symptoms of Q fever are prevalent. In the acute phase, patients may have fever, granulomatous hepatitis, or interstitial pneumonitis; the chronic phase is primarily characterized by culture-negative endocarditis. The acute phase is asymptomatic in >50% of cases, which explains why an outbreak might be unnoticed (9,12).

Q fever is primarily transmitted to humans when aerosolized fluids are inhaled during or after parturition of an infected animal. The organism can stick on wool and dust and be spread by wind. The wind has been shown to spread C. burnetii in other circumstances. In a small town in southern France, wind blew through a steppe where sheep were gathered after lambing, and persons whose homes were exposed to the wind were more often infected with Q fever than their neighbors (10). In cities, the role of slaughterhouses in the spread of Q fever is well-known (7,8). The last reported slaughterhouse-related outbreak of Q fever in France was related to contaminated waste from sheep sacrificed for a Christian Easter feast. The waste had been left uncovered outside the slaughterhouse, which was near a heliport. Helicopters might have facilitated airborne transmission of the infectious agent (9). C. burnetii has also been shown to be transmitted by dogs (13), wild rabbits (14), and parturient cats (15), and transmission has been associated with religious practices (16). In this study, contact with kittens and correlation with wind from the

Table. Homeless persons positive for Coxiella burnetii phase II antibodies > 1:50 compared to controls

<table>
<thead>
<tr>
<th>Group</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelter A residents</td>
<td>17/157 (10.8)</td>
<td>0/96 (0)</td>
<td>1/182 (0.54)</td>
<td>7/129 (5.4)</td>
</tr>
<tr>
<td>Shelter B residents</td>
<td>2/104 (1.9)</td>
<td>0/75 (0)</td>
<td>0/114 (0)</td>
<td>0/73 (0)</td>
</tr>
<tr>
<td>Controls</td>
<td>14/460 (3)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NA, not applicable.

p < 0.001.
slaughterhouse were the only identified risk factors. We showed here that homeless persons were likely exposed to *C. burnetii* in shelter A during the month that followed the Aid El Khebir in 1999, with the wind playing a critical role in this outbreak. Some controversy surrounds this feast in France because of the way sheep are ritually sacrificed. Several hundred sheep are maintained for a few days inside and outside the slaughterhouse before having their throats slit and being bled outside. They are then displayed to buyers, as shown in Figure 2. That sheep are maintained under conditions of poor hygiene, without veterinary counsel, and that the bleeding and sale takes place outside may explain how *C. burnetii*–infected particles could have contaminated soil, wool, or loose straw, and particles could have blown downwind. Veterinary control of sheep flocks would help avoid such contamination.

Shelter B is further south than shelter A. Since no significant differences in incidence of Q fever were found in shelter B, homelessness itself is not associated with Q fever. Access to health care is problematic for this population, so an outbreak of Q fever could go unnoticed unless Q fever testing was a part of disease surveillance in homeless persons. The risk of an unnoticed outbreak emphasizes the need to systematically survey this population and nearby residents. Persons in other areas surrounding the slaughterhouse were also likely exposed to *C. burnetii* in 1999.

**Acknowledgment**

We thank Patricia Crocquet-Valdes for suggestions and English review of the manuscript.

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Dr. Brouqui manages the infectious disease ward in the university hospital in Marseilles, France, and conducts research at the World Health Organization collaborative national reference center for rickettsioses. His main research interests are rickettsial diseases in the homeless population.

**References**


Address for correspondence: Pr. D. Raoult, Unité des rickettsies, UMR CNRS 6020, Faculté de médecine, 27 av J Moulin, Marseille 13385, Cedex 5, France; fax: 33-491-38-77-72; email: didier.raoult@medecine.univ-mrs.fr

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SARS Coronavirus Detection

Andreas Nitsche,* Brunhilde Schweiger,* Heinz Ellerbrok,* Matthias Niedrig,* and Georg Pauli*

We developed a set of three real-time reverse transcription–polymerase chain reaction (PCR) assays that amplify three different regions of the SARS-associated coronavirus (SARS-CoV), can be run in parallel or in a single tube, and can detect <10 genome equivalents of SARS-CoV. The assays consider all currently available SARS-CoV sequences and are optimized for two prominent real-time PCR platforms.

The Study

Recently, a new coronavirus was identified as the suspected causative agent of an increased number of atypical pneumonia cases reported from Hong Kong, Singapore, Vietnam, and Canada (1–4). Subsequent publications demonstrated that this new coronavirus was detectable in patients with severe acute respiratory syndrome (SARS) (5,6), classified according to the World Health Organization’s case definition (7). During the first 6 months of 2003, a total of 8,422 patients were affected. This fact, together with the reappearance of the SARS-associated coronavirus (SARS-CoV) in China in late 2003, makes it clear that rapid and reliable diagnostic tools are essential for accurate disease reporting and subsequent disease management.

Because a defined treatment program and vaccination strategy are lacking, the main strategy to counteract the spread of this emerging virus is timely identification and isolation of infected persons. SARS patients’ typical initial symptoms include fever, cough, and headache, similar to many acute viral respiratory infections. Therefore, molecular-based diagnostic methods are applied to rapidly identify SARS-CoV–infected persons. Recently, nested and real-time reverse transcription–polymerase chain reaction (RT-PCR) assays to detect SARS-CoV have been published (5,8). These assays—the first tools to detect SARS-CoV in patients with SARS—were based on the short stretches of viral sequence identified as the RNA-directed RNA polymerase of a new microbe.

Subsequently, sequences from several SARS-CoV isolates were determined, and all of these sequences were closely related, as would be expected during the clustered outbreaks in 2003. However, the genomes of RNA viruses, including those of coronaviruses, tend to vary over time and with location (9–12). Recently, the sequence variations of SARS-CoV during the first epidemic phases in China in 2003 were reported. The neutral mutation rate for SARS-CoV was almost constant and similar to that of known RNA viruses; the S protein, responsible for virus-host receptor recognition, displayed the most extensive amino acid changes (13). In addition, the sequence analysis of isolates from recent SARS patients in China in 2004 has shown that 98.8%–99.4% of the 3,768 bases of S gene, 99% of 658 bases of M gene, and 99% of 1,068 bases of N gene are isogenous with those submitted to public databases, which date back to the first epidemic in spring 2003 (14). However, even these minimal changes could render existing PCR assays ineffective should SARS-CoV reemerge (15).

To improve the ability to detect SARS-CoV safely and reduce the risk of eliciting false-negative results caused by genome sequence variations, we established three individual real-time RT-PCR assays. Target sequences were chosen by using the following criteria: 1) the regions are distributed over the whole genome, including the nonstructural polyprotein 1a and 1ab genes and the spike glycoprotein gene (Table 1); 2) the regions are highly conserved among the 89, 90, and 100 respective sequences available in public sequence databases; 3) the regions are suitable for the design of a real-time RT-PCR assay; and 4) the designed primers, 5′-nuclease probes, and amplicons displayed no considerable homology to other viruses, including human CoV OC43 and 229E in BLAST searches (available from http://www.ncbi.nlm.nih.gov/BLAST/).

These assays were based on the fluorogenic oligoprobes chemistry, which uses the 5′-exonuclease activity of the DNA polymerase to generate a more specific signal than that produced by the use of SYBR Green I (8). The real-time RT-PCR assays were successfully run on the Applied Biosystems real-time PCR systems (SDS7700 and SDS7000; Applied Biosystems, Foster City, CA) as well as on the Roche LightCycler (Roche Diagnostics GmbH, Mannheim, Germany). All assays were designed as one-step RT-PCR reactions to be run under identical conditions on the respective PCR platform. This system allowed the simultaneous detection of different SARS-CoV regions in a single PCR run. Moreover, we could combine the three assays in a single tube, which might be important when clinical material is limited. Finally, the assays were compared to the 5′-nuclease assay published recently (5) and to a commercially available real-time PCR kit (Real-Art HPA-Coronavirus LC RT PCR Reagents, Artus GmbH, Hamburg, Germany).

After optimization of primer and 5′-nuclease probe concentration and annealing temperature, reaction conditions for our 5′-nuclease assay were as follows. For the RT-
PCR performed on the Applied Biosystems platforms, each 25-µL reaction contained 12.5 µL of 2xQuantitect Probe RT-PCR Master Mix (Qiagen, Hilden, Germany), 10 pmol of each primer, 3 pmol of 5'-nuclease probe, and 0.25 µL of QuantiTect Probe RT Mix. RNase-free water was added up to 23 µL, and 2 µL of RNA was used. Cycling conditions were 30 min at 50°C for RT reaction, 15 min at 95°C for inactivation of RT, activation of the Taq DNA polymerase, and cDNA denaturation, followed by 45 cycles of 15 s at 95°C and 30 s at 60°C. Total running time was 140 min.

For LightCycler RT-PCR reactions, each 20-µL reaction included 7.5 µL of 2.7xLightCycler RNA Master Hybridization Probes mix (Roche Diagnostics GmbH), 10 pmol of each primer, 3 pmol of the 5'-nuclease probe, and 1.3 µL Mn (OAc)₂ (50 mmol/L). RNase-free water was added up to 18 µL, and 2 µL of RNA was used. Cycling conditions were 20 min at 55°C for the RT reaction, 30 s at 95°C for initial denaturation, followed by 45 cycles of 1 s at 95°C, 10 s at 55°C, and 10 s at 72°C. Total running time was 55 min. The combined assays were set up by adding all primers and probes in the same concentration; the amount of water was reduced accordingly. Protocols are also available from the Robert Koch-Institut homepage (www.rki.de/INFEKT/SARS/PCRPROTOCOL.PDF).

The human L13 gene and the human cyclophilin gene (16) were regarded as unconfirmed SARS patients. In addition, 35 more with suspected disease were negative by RT-PCR. Moreover, these patients remained seronegative and are regarded as unconfirmed SARS patients. In addition, 35
### Conclusions

None of the assays displayed cross-reactivity to clinical samples containing human cDNA from blood; human CoV 229E; influenza viruses A and B; parainfluenzaviruses 1, 2, and 3; respiratory syncytial virus; rhinoviruses; enteroviruses; adenoviruses 1–10; human metapneumovirus; *Mycoplasma pneumoniae*; or *Chlamydia pneumoniae*. For these pathogens, we obtained neither a fluorescent signal nor an amplification product in subsequent agarose gel analysis (selection shown in online Figure). Although we focused on a one-step RT-PCR to decrease handling and total assay time, the three real-time RT-PCR assays can also be performed as two-step RT-PCR, including a separate cDNA synthesis step followed by PCR, and then finally using appropriate ready-to-use master mixes and the same cycling condition, omitting the RT step.

The single assays and the combined assay were also used in an external quality assessment to detect SARS-CoV, organized by the ENIVD. All assays could detect SARS-CoV in 7 of 11 samples with virus loads ranging from $5 \times 10^6$ to $2 \times 10^3$ GE of two isolates of SARS-CoV per milliliter sample without false-positive or false-negative results. While the application of three single assays to detect SARS-CoV leads to a higher reliability of negative results, reflecting the negative outcome of three independent amplification reactions, it is a more expensive approach than combining the assays.

In conclusion, the real-time RT-PCR assays we describe provide a fast and reliable tool that can complement and improve recently introduced techniques for SARS diagnostics. Parallel amplification of two human reference genes, L13 and cyclophilin, confirmed negative results in clinical samples by demonstrating amplifiable RNA. The separation of the control reaction was chosen to guarantee the high sensitivity of the SARS-CoV detection of $<10$ GE of SARS-CoV per reaction. An RT-PCR run is completed in $<1$ h, depending on the real-time PCR platform. In cases of small amounts of material or in an emergency situation with a high throughput of samples, the three SARS-CoV–specific assays can be combined into one RT-PCR reaction without loss of sensitivity. Furthermore, as the ambiguous diagnostic results in a hospital in Canada have recently shown (17), targeting three different regions distributed over the whole genome considerably reduces the risk for false-negative results caused by virus sequence modifications.

### Acknowledgments

We thank Sabrina Wendt for excellent technical assistance, Ian M. Mackay for critically reading the manuscript, and Artus (Artus GmbH, Hamburg, Germany) for kindly providing the Real-Art HPA-Coronavirus LC RT PCR Reagents Kit.

Dr. Nitsche is a research fellow at the Centre for Biological Safety of the Robert Koch-Institut, Berlin. His primary interest is the molecular detection of human pathogens with special focus on emerging viral infections.

### References


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#### Table 2. Ct values and standard deviation of serial dilutions of SARS-CoV RNA subjected to different real-time RT-PCR assays

<table>
<thead>
<tr>
<th>GE</th>
<th>Artus kit n = 4</th>
<th>Drosten et al. n = 6</th>
<th>NS pp1a (5) n = 7</th>
<th>NS pp1ab (5) n = 7</th>
<th>SS GP (5) n = 7</th>
<th>NS pp1ab/SS GP (5) n = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>28.69 ± 0.46</td>
<td>28.16 ± 0.04</td>
<td>29.57 ± 0.87</td>
<td>29.56 ± 0.18</td>
<td>29.47 ± 0.53</td>
<td>28.53 ± 0.26</td>
</tr>
<tr>
<td>50</td>
<td>32.68 ± 0.26</td>
<td>45.00</td>
<td>33.85 ± 0.60</td>
<td>32.83 ± 0.41</td>
<td>32.83 ± 0.41</td>
<td>32.48 ± 0.69</td>
</tr>
<tr>
<td>10</td>
<td>34.42 ± 0.20</td>
<td>45.00</td>
<td>36.72 ± 0.42</td>
<td>35.99 ± 0.55</td>
<td>35.33 ± 0.48</td>
<td>34.50 ± 0.25</td>
</tr>
<tr>
<td>5</td>
<td>35.66 ± 0.40</td>
<td>45.00</td>
<td>35.00</td>
<td>36.94 ± 0.10</td>
<td>32.93 ± 0.81</td>
<td>32.01 ± 0.44</td>
</tr>
</tbody>
</table>

Note: Ct, threshold cycle; SARS-CoV, severe acute respiratory syndrome–associated coronavirus; RT-PCR, reverse transcription–polymerase chain reaction; GE, genome equivalents/assay; pp1ab, nonstructural polyprotein 1ab gene; n.d., not determined.

*See Table 1.*

Address for correspondence: Andreas Nitsche, Robert Koch-Institut, ZBS 1, Nordufer 20, 13353 Berlin, Germany; fax: +49-1888-7542605; email: nitschea@rki.de

EMERGING INFECTION DISEASES

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Family Cluster of Mayaro Fever, Venezuela
Jaime R. Torres,* Kevin L. Russell,† Clovis Vasquez,‡ Roberto Barrera,‡ Robert B. Tesh,§ Rosalba Salas,¶ and Douglas M. Watts§

A cluster of protracted migratory polyarthritis involving four adult family members occurred in January 2000 after a brief overnight outing in a rural area of Venezuela. Laboratory testing demonstrated Mayaro virus as the cause of the cluster. These results documented the first human cases of Mayaro virus in Venezuela.

Mayaro virus (MAYV), the cause of Mayaro fever, is a member of the genus Alphavirus, family Togaviridae, and is closely related to Chikungunya, O’nyong-nyong, Ross River, Sindbis, and Sindbis viruses (1–3). Infection by these viruses produce similar clinical illnesses in humans (4–8). Mayaro fever is typically a dengue-like acute febrile illness 3–5 days in duration, characterized by headache, retroorbital pain, arthralgias, arthritis, myalgias, vomiting, diarrhea, and rash (8). However, joint involvement in Mayaro fever may persist for several months and in some cases precede the fever. Moderate-to-severe polyarthritis, occasionally incapacitating, is a prominent feature of the disease (8).

MAYV is enzootic in South America, where the suspected vectors are forest-dwelling Haemagogus mosquitoes, and the vertebrate hosts are marmosets and other nonhuman primates (8). Most human cases occur sporadically and involve persons who work or reside in humid tropical forests (8,9). Nevertheless, several small outbreaks of Mayaro fever have been described in residents of rural communities of the Amazon region of Brazil, Bolivia, and Peru (8–10). Airborne transmission has been reported, with the rainy season normally lasting from May to December.

Four adult members of the same family (age range 26–58 years) spent a single night together in early January 2000 near the Padrón Agriculture Station. While sharing an outdoor dinner, they were frequently bitten by mosquitoes. Three days later, all four had a sudden onset of malaise, fever (up to 45°C), retroocular pain, generalized headache, conjunctival suffusion, flushing of the face and neck, myalgias, and severe incapacitating polyarthralgias and polyarthritis which mainly involved the small joints of the hands, wrists, ankles, and toes. Joints became swollen and tender, but effusion was not evident. Pain was intense and worsened with motion. Limbs felt weak and very sensitive to touch. Joint stiffness in the morning and after inactivity was a prominent complaint. On day 5 of illness, a rapidly spreading maculopapular rash developed, which involved neck, trunk, and limbs. The rash persisted for 2 days, followed by desquamation. In three of the patients, painful cervical, preauricular, and retroauricular lymphadenopathies occurred and lasted approximately 2 weeks. Beyond week 2 of illness, only severe joint symptoms and lower limb hyperesthesias persisted, but they steadily resolved during a 6-month period. Clinical laboratory results were unremarkable except for a transient and mild increase in erythrocyte sedimentation rate and serum levels of alanine aminotransferase, and a moderate lymphocytosis.

Serum samples were obtained 3 months after onset of symptoms, when the patients were first seen at consultation by one of the authors. Samples were also collected an additional 3 months after the initial samples were collected. The patients’ initial signs and symptoms resembled a classical febrile syndrome, and the patients had a history of suspected risk for arboviral infection. Therefore, all samples were tested initially at a 1:100 dilution for immunoglobulin (Ig) M antibodies to MAYV; VEEV; dengue viruses (DENV) 1, 2, 3, and 4; yellow fever virus (YFV); and Oropouche virus (OROV) by using an IgM

*Universidad Central de Venezuela, Caracas, Venezuela; †U.S. Naval Health Research Center, San Diego, California, USA; ‡Ministry of Health and Social Welfare, Caracas, Venezuela; §University of Texas Medical Branch at Galveston, Galveston, Texas, USA; and ¶Caribbean Epidemiology Centre, Republic of Trinidad and Tobago

The Study
Clinical cases resembling dengue fever were studied in the vicinity of Padrón Agriculture Station, in Miranda State, north-central Venezuela (10°13′22″ N; 66°17′56″ W; 50 m elevation), a location where entomologic and epidemiologic studies on Venezuelan equine encephalitis virus (VEEV) and other arboviruses were conducted from 1997 to 1998 (12). This area, originally covered by lowland tropical rain forest, was converted into cacao (Theobroma cacao) plantations. Indigenous tall trees (Erythrina poeppigiana, Ceiba pentandra, Ficus sp., Hura crepitans, Bauhinia sp.), were preserved so that the area resembled a natural forest habitat. The mean temperature and annual rainfall were 27.2°C and 2,324 mm, respectively, with the rainy season normally lasting from May to December.
antibody-capture enzyme-linked immunosorbent assay (MACEIA) (9,12). Reactive samples were subsequently retested for IgM antibody at serial dilutions ranging from 1:200 through 1:102,400 to determine endpoint titers. Serum samples were also tested by an indirect ELISA for IgG antibodies to the above-mentioned viruses (9,13). A patient with MAYV disease was defined as a person with IgG antibodies to the above-mentioned viruses (9,13). Serum samples were also tested by an indirect ELISA for IgM antibody at serial dilutions ranging from 1:200 through 1:102,400 to determine endpoint titers. Reactive samples were subsequently retested for IgM antibody at serial dilutions ranging from 100 to 800. Assay results for DENV and OROV IgM and IgG were negative. Similarly, the patients were negative for YFV IgM antibody but had IgG antibody to this virus.

### Results

Serologic results indicated that three of the four family members had a MAYV viral infection. Assay of serum samples obtained 3 months after onset of symptoms from the three members showed high specific Mayaro viral IgM antibody antibody ranging from 3,200 to 6,400 and IgG antibody titers ranging from 6,400 to 12,800 (Table). Testing of samples from the fourth patient were positive for MAYV IgG antibody only. Subsequent samples taken approximately 3 months later were IgM negative but remained positive for MAYV IgG antibody. All patients were negative for VEEV IgM antibody but had VEEV IgG antibody ranging from 100 to 800. Assay results for DENV and OROV IgM and IgG were negative. Similarly, the patients were negative for YFV IgM antibody but had IgG antibody to this virus.

### Conclusions

MAYV has not been isolated in Venezuela, but isolates have been obtained from humans, wild vertebrates, and mosquitoes in Colombia, Brazil, Suriname, Guyana, French Guiana, Peru, United States, and Bolivia (2,8–10,14–17). In addition, serologic survey data suggest that MAYV infection is relatively common among humans in rural populations of northern South America and the Amazon River basin (2,8,9,14–16). This virus is believed to be maintained in a sylvan cycle involving wild vertebrates, such as nonhuman primates and possibly birds, and Haemagogus mosquitoes (2,8,18). Three species of that genus, H. celeste, H. equinus, and H. lucifer, have recently been identified in the area where the Venezuela patients acquired MAYV infection (12). Also, the red howler monkey (Alouatta seniculus), a suspected host of MAYV in nature, is common in the area. Thus, the results of this study suggest that these first documented cases of Mayaro fever in Venezuela were acquired during an overnight outing in a rural area where MAYV may have been circulating in a cycle involving Haemagogus mosquitoes and red howler monkeys.

Convalescent-phase serum samples from an additional unrelated patient (a 40-year-old woman who lived in a nearby rural location), obtained approximately 4 months after she had recovered from a self-limited febrile illness with polyarthritus similar to that described in the patients involved in this report, showed high (25,600) MAYV IgG antibody titers. These samples were negative for IgM antibody, however, which provides further evidence that MAYV was enzootic in the area.

As observed in this study, Mayaro fever cases are usually sporadic and occur in persons with a history of recent activities in humid tropical forests (4,8,9,19). Typically, Mayaro fever ensues approximately 1 week after infection (4,8). However, shorter incubation periods, similar to those observed in these Venezuelan cases, are occasionally observed. Members of the family described in this outbreak had symptoms and clinical courses consistent with previously documented MAYV patients. Abrupt onset of fever, frontal headaches, myalgias, and incapacitating arthralgias were predominant complaints. A maculopapular rash, also a common manifestation in up to 90% of children and 50% of adults (4,8,9,18), was prominent in these patients, lasting 2 days and followed by desquamation. Up to one third of patients initially have nausea, vomiting, and diarrhea (4,8,9,18,19), but these symptoms were not experienced in this family.

Little information is available on the kinetics of MAYV IgM antibodies for Mayaro fever patients during long-term follow-up examinations. While obtaining acute-phase blood samples from the patients in this study was not possible, existing data indicate that detectable IgM antibody develops after viremia subsides, which is usually 4–5 days after the onset of symptoms (9,19). Our data indicated that

---

**Table. Mayaro and Venezuelan equine encephalitis viral IgM and IgG antibodies demonstrated by an antibody-capture ELISA in serum samples obtained from three Venezuelan family members**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Convalescent-phase</th>
<th>Late convalescent-phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAYV</td>
<td>VEEV</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>1</td>
<td>3,200</td>
<td>12,800</td>
</tr>
<tr>
<td>2</td>
<td>6,400</td>
<td>12,800</td>
</tr>
<tr>
<td>3</td>
<td>6,400</td>
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|MAYV, Mayaro virus; VEEV, Venezuelan equine encephalitis virus; Ig, immunoglobulin; ELISA, enzyme-linked immunosorbent assay.

1Reciprocal of highest serum dilution at which a positive result occurred.

2Three months after onset of illness.

3Six months after onset of illness.
IgM antibody persisted for >3 but <6 months for our patients. These are the first documented data on the persistence of IgM antibody following a Mayaro viral infection and will be useful for interpreting diagnostic test results. To our knowledge, this is the first report of human cases of MAYF in Venezuela and, therefore, further documents the public health importance of this disease.

Acknowledgments

We thank the Virology Laboratory at the U.S. Naval Medical Research Center, Lima, Peru, under the guidance of Carolina Guevara, for processing human specimens, and Eduardo Gotuzzo for his support of this study.

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Dr. Torres is a tropical medicine professor at the Universidad Central de Venezuela in Caracas. He is head of the Infectious Diseases Section of the Tropical Medicine Institute. Internal medicine, infectious diseases, and tropical medicine are his specialties. Primary research interests include epidemiology and pathogenesis of tropical endemic infections.

References


Address for correspondence: Jaime R. Torres, Instituto de Medicina Tropical, UCV, Apartado 47019, Caracas 1041-A, Venezuela, fax: +58-212-987-6590; email: torresj@mailandnews.com
Emergence of Multidrug-resistant Salmonella Paratyphi B dT⁺, Canada

Michael R. Mulvey,* David Boyd,* Axel Cloeckaert,† Rafiq Ahmed,* Lai-King Ng,* and the Provincial Public Health Laboratories1

We document an increase in the number of multidrug-resistant Salmonella enterica serovar Paratyphi B dT⁺ identified in Canada. Most of these strains harbor Salmonella genomic island 1 (SGI1). Further studies are needed to determine factors contributing to the observed emergence of this multidrug-resistant strain.

Salmonella genomic island 1 (SGI1) was first characterized in Salmonella enterica serovar Typhimurium definitive phage type 104 (DT104). It consists of a 43-kb DNA segment harboring genes responsible for the pentarresistance phenotype ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline (ACSSuT) and is inserted into the chromosome at the end of the thdF gene (1). Complete nucleotide sequence of this region revealed 44 open reading frames, of which some displayed homology to genes associated with plasmid transfer, which suggests SGI1 may be at least partially plasmidic in origin (2). The SGI1 is associated with the multidrug-resistant DT104 clone that has disseminated worldwide (3). Recently, a clone that has disseminated worldwide (3). Recently, a

The Study

This report is a result of a collaborative effort between the National Microbiology Laboratory (NML), Health Canada, and the Provincial Public Health Laboratories (PPHLs) in Canada. The PPHLs represent every province in Canada and also include the Yukon and North West Territories. All S. Paratyphi B dT⁺ identified at the PPHLs were forwarded to the NML for additional characterization. Between 1998 and 2002, 252 S. Paratyphi B dT⁺ strains were submitted to the NML, of which 246 were from a human source. Distribution of the strains over time is shown in Figure 1. Incidence of S. Paratyphi B dT⁺ has generally increased since 1998; the spike in S. Paratyphi B dT⁺ in the third and fourth quarters of 1999 can be attributed to an outbreak in British Columbia, Alberta, and Saskatchewan caused by contaminated alfalfa sprouts (7). In addition to this large outbreak, additional outbreaks were reported between 1998 and 2002; however, each outbreak was small, and most involved fewer than six persons.

Antimicrobial susceptibility testing was performed on all strains by using the disk-diffusion method as described by the National Committee for Clinical Laboratory Standards (8). Susceptibilities were determined for ampicillin (A), chloramphenicol (C), ciprofloxacin (Cp), streptomycin (S), sulfamethoxazole (Su), tetracycline (T), and trimethoprim (Tm). Of the 237 strains examined for susceptibility, 123 (52%) were susceptible to all antimicrobial agents tested. Sixty-seven strains (28%) displayed the pentaresistance phenotype (ACSSuT), and the second most prevalent resistance profile was Su (n = 41, 17%). Single strains displayed the phenotypes A, T, CSSu, ASuTm, ASSu, and ACSuTTm. A significant increase was observed in ACSSuT strains over the time period from 1998 to 2002 (p ≤ 0.001). Rates for the years are as follows: 1998, n = 2 (2%); 1999, n = 4 (18%); 2000, n = 2 (10.5%); 2001, n = 23 (39%); and 2002, n = 36 (58%). No large outbreaks were reported during this time period. To determine if the pentaresistance phenotype was caused by SGI1, polymerase chain reaction (PCR) was used to detect integrons and the left (thdF-S001) and right (S044-yidT) junctions of SGI1 as previously described (2). Of the 67 strains identified with the ACSSuT phenotype, 63 contained 1.0-kb and 1.2-kb integrons and left and right junctions of SGI1, which suggests that these strains contained SGI1 inserted into the same location on the chromosome as was described for DT104 (1). One strain with the ACSSuT phenotype contained 1.0-kb and 1.2-kb integrons and gave a PCR product for the left junction amplification reaction but not the right junction, which suggests that a portion of SGI1 downstream of the integrons was missing.

1Provincial Public Health Laboratory Members: Lewis Abbott (Prince Edward Island), Kevin Forward (Nova Scotia), Glenna Hardy (New Brunswick), Greg Horisman (Saskatchewan), Judy Isaac-Renton (British Columbia), Frances Jamieson (Ontario), Jean Joly (Quebec), Jutta K. Preiksaitis (Alberta), Sam Ratnam (Newfoundland), Paul Van Caeseele (Manitoba).

*Health Canada, Winnipeg, Manitoba, Canada; and †Institut National de la Recherche Agronomique, Nouzilly, France.
Three strains with the ACSSuT phenotype did not give the characteristic size products for integron (0.7 kb, n = 2; 2.0 kb, n = 1), and all were negative for the junction PCR, which suggests that these strains most likely harbored other resistance genes giving the ACSSuT phenotype. Only four strains with the ACSSuT phenotype were identified from a nonhuman source (one poultry, three environmental). Although the three environmental isolates contained SGI1, the ACSSuT strain isolated from poultry did not; instead, it contained a 2-kb integron. Of the human isolates for which source was reported for the ACSSuT strains (n = 53), all were isolated from stool, with the exception of three that were isolated from blood. To ensure the ACSSuT phenotype were grouped into three closely related clusters named clusters 1 to 3 (Figure 2). The three strains that did not cluster with the other ACSSuT strains did not harbor SGI1 as described above.

Cluster 1 contained 32 strains that represented 10 subtypes. Cluster 2 contained 17 strains that represented 7 subtypes, all of which showed <7 band differences between strains from cluster 1. Cluster 3 contained 15 strains that represented 6 subtypes, all of which showed fewer than seven band differences between subtypes in cluster 2, but had more than seven band differences between the cluster 1 fingerprints. In addition, six other strains identified before 2000 were subtyped. Five were other ACSSuT identified in 1998 (n = 1) and 1999 (n = 4), and all were identified in cluster 2. In addition, one S. Paratyphi B dT+ recently shown to harbor SGI1 that was isolated in Singapore from a fish was grouped into cluster 3 (4). The Canadian isolates in clusters 1 to 3 have been identified from multiple provinces, including British Columbia, Alberta, Manitoba, Ontario, and Québec, representing western and central regions of Canada.

Conclusions

The emergence of multidrug-resistant S. Paratyphi B dT+ was documented recently in the Netherlands and Scotland (11,12). Some isolates had the ACSSuT susceptibility pattern and did not harbor any plasmids, which suggests the resistance is of chromosomal origin (12).
However, in neither study was the presence of SGI1 or other resistance genes examined. We demonstrate the rapid emergence of multidrug-resistant *S. Paratyphi* B dT⁺ in Canada. The increase is due to three clusters, all of which contain the multidrug-resistant genomic island SGI1. That three closely related clonal groups were present suggests SGI1 may have inserted into the genome of *S. Paratyphi* B dT⁺ in Canada and make up 46% (64 of 139) of all strains identified in 2001 and 2002. Furthermore, three additional *S. Paratyphi* B dT⁺ strains with the ACSSuT phenotype were identified that do not harbor SGI1 and do not appear to be rapidly increasing in Canada. In this hypothesis, SGI1 may predict the next emerging *Salmonella* serotype. Other factors, such as processing food products and the structure of the food distribution system, could play a role in disseminating these organisms. We continue to monitor this pathogen and are designing studies to improve understanding of the epidemiology of *S. Paratyphi* B dT⁺ in Canada. We suggest all strains of *Salmonella* with the ACSSuT phenotype be examined for SGI1.

**Acknowledgments**

We thank Dave Spreitzer for PFGE analysis, Kevin Nelson for PCR analysis, and Derrick Ozunko for antimicrobial susceptibility testing.

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Dr. Mulvey is chief of Antimicrobial Resistance and Nosocomial Infections at the National Microbiology Laboratory, Health Canada. His main interests are antimicrobial resistance mechanisms and the dissemination of resistance genes.

**References**


Dispatches


Instructions for Emerging Infectious Diseases Authors

Research Studies: Articles should be 2,000 to 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author—both authors if only two.

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

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

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

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

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

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

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

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

Manuscript Submission. Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch) and verifying that the final manuscript has been seen and approved by all authors. Submit an electronic copy (by e-mail) to the Editor, eeditor@cdc.gov.
Bartonella spp. DNA Associated with Biting Flies from California

Crystal Y. Chung,* Rickie W. Kasten,* Sandra M. Paff,* Brian A. Van Horn,* Muriel Vayssier-Taussat,† Henri-Jean Boulouis,† and Bruno B. Chomel*†

Bartonella DNA was investigated in 104 horn flies (Haematobia spp.), 60 stable flies (Stomoxys spp.), 11 deer flies (Chrysops spp.), and 11 horse flies (Tabanus spp.) collected on cattle in California. Partial sequencing indicated *B. bovis* DNA in the horn fly pool and *B. henselae* type M DNA in one stable fly.

*Bartonella* spp. are vector-borne bacteria associated with numerous emerging infections in humans and animals (1). Four *Bartonella* species have been isolated from wild and domestic ruminants. *B. schoenbuchensis* and *B. capreoli* were recovered from wild roe deer (*Capreolus capreolus*) (2,3) in Europe, whereas *B. bovis* (formerly *B. weissii*) was recovered from domestic cattle in the United States and Europe (3–5). Strains similar to *B. bovis* and *B. capreoli* were also isolated from mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus*) from California (3,4). Recently, *B. chomelii* was recovered from bacteremic cows in France (6). A high prevalence of infection with various *Bartonella* species has been reported in domestic and wild ruminants in North America and Europe (2–4). Of the herds investigated in California, 95% of beef cattle and 17% of dairy cattle were bacteremic for *B. bovis* and 90% of the mule deer were bacteremic for *Bartonella* spp. (4). The main vector of these ruminant-infecting *Bartonella* spp. has not been identified.

The role of ticks as potential vectors for *Bartonella* in cattle was investigated (7,8). In Europe, >70% of 121 *Ixodes ricinus* ticks collected from roe deer had 16S rRNA gene sequences for *Bartonella* or other closely related species (7). In California, *Bartonella* DNA was detected in approximately 19% of 151 questing adult *I. pacificus* ticks (8), but the direct role of ticks in *Bartonella* transmission among ruminants has never been established. In a search for an efficient *Bartonella* vector, which could explain such high prevalence of infection in wild and domestic ruminants, we tested biting flies for *Bartonella* spp. DNA to establish the potential role of biting flies as vectors of *Bartonella* in cattle.

The Study

Flies were collected by hand, with a bug net, at various locations on the University of California campus, mainly the dairy barn, beef barn, and feedlot, from early July to mid-August 2003. Flies were identified on the basis of morphologic characteristics visually or under binocular lenses for the smaller flies by an experienced entomologist. Of the 370 biting flies collected, 104 (62%) of the horn flies (Haematobia spp.), 60 (33%) of the stable flies (Stomoxys spp.), 11 (92%) of the deer flies (Chrysops spp.), and 10 (91%) of the horse flies (Tabanus spp.) were tested for *Bartonella* DNA. The stable flies were collected from the dairy and the feedlot barns. The horn flies, deer flies, and horse flies were collected from the beef barn.

Before DNA extraction, the flies were placed in a sterile 1.5-mL microtube, washed with 70% ethanol, and rinsed with sterile water. Because of size differences among the flies, 2–3 horn flies were grouped together in a single microtube, while each stable fly was placed in an individual vial. The abdomen of deer flies and horse flies was first removed and then placed in individual vials. DNA extraction was performed by using the DNeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions, with some minor adjustments. The amount of reagents for the deer and horse flies were doubled, and the flies were incubated in a waterbath overnight at 55°C.

*Bartonella* DNA was detected by polymerase chain reaction (PCR) using primers for the citrate synthase (*gltA*) gene, as previously published (9). Undiluted DNA extracted from the flies was used as the DNA template. As a positive control, a low concentration of *B. henselae* was added to a separate set of the same DNA template. A negative control was made by using sterile water instead of the DNA template. Using gel electrophoresis, we analyzed PCR products for the appearance of an ~380-bp fragment. Any evidence of a 380-bp fragment was further analyzed by restriction fragment length polymorphism (RFLP) procedures, by using *TaqI* (Promega Corp., Madison, WI), *HhaI*, *AciI*, and *MseI* endonucleases (New England Biolabs, Beverly, MA), and DNA sequence analysis (Davis Sequencing, Davis, CA).

Four of the 60 stable flies and one pool (2 flies) of the 45 horn fly pools showed a 380-bp fragment. PCR/RFLP analysis confirmed *Bartonella* DNA in one of the four stable flies and in the horn fly pool. However, for the three other stable flies, the PCR/RFLP profiles did not match any known *Bartonella* digestion profile. The sequence obtained from the horn fly pool (Haematobia spp.) collected in the beef cattle barn was identical to that for *B. bovis*.
The sequence obtained from a stable fly (Stomoxys spp.) collected in the dairy cattle barn was identical to that for *B. henselae* type M (Marseille) (Figure 2). The highlighted area indicates the divergence between *B. henselae* type H (Houston I) and *B. henselae* type M, as previously described (10).

**Conclusions**

This identification of *Bartonella* DNA is the first associated with horn and stable flies and the first identification of *B. henselae* from a biting fly. It is also the first report of identification of *Bartonella* DNA from flies from North America. This finding demonstrates, as for ticks, that *Bartonella* DNA is present in various biting insects. We found a very low percentage of *Bartonella* DNA–positive flies, in contrast to the very high prevalence (57 [88%] of 65 observed in *Hippoboscidae* adult flies (*Lipoptena cervi* and *Hippobosca equina*) collected from domestic cattle and wild roe deer in France (H.J. Boulouis, pers. comm.).

Identification of *B. henselae* DNA in a stable fly indicates the wide range of blood-sucking arthropods that can harbor this human pathogen. The partial *gltA* sequence was identical to that for *B. henselae* type Marseille, the most common type found in cats and humans in California (11). Fleas have been shown to be an efficient vector of *B. henselae* (12–14). More recently, *B. henselae* DNA was identified in adult questing *I. pacificus* ticks from California and from *I. ricinus* ticks collected on humans in Italy (8,15). The role of ticks as potential vectors of *B. henselae* in humans has also been suggested (16–18).

Since *Bartonella* are likely to be present in biting flies, investigating the potential of biting flies as either mechanical or biologic vectors of *Bartonella* in cattle and possibly humans should be pursued.

**Acknowledgments**

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Ms. Chung is a second-year student at the School of Veterinary Medicine, University of California, Davis. This study was performed as her NIH summer fellowship through the Center for Comparative Medicine at University of California, Davis.
References


Address for correspondence: Bruno B. Chomel, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616, USA; fax: 530-752-2377; email: bbchomel@ucdavis.edu
Phylogenetic Analysis of West Nile Virus, Nuevo Leon State, Mexico

Bradley J. Blitvich,* Ildefonso Fernández-Salas,† Juan F. Contreras-Cordero,† María A. Loroño-Pino,* Nicole L. Marlenee,* Francisco J. Díaz,* José I. González-Rojas,† Nelson Obregón-Martínez,‡ Jorge A. Chiu-García,‡ William C. Black IV,* and Barry J. Beaty*

West Nile virus RNA was detected in brain tissue from a horse that died in June 2003 in Nuevo Leon State, Mexico. Nucleotide sequencing and phylogenetic analysis of the premembrane and envelope genes showed that the virus was most closely related to West Nile virus isolates collected in Texas in 2002.

The Study

Cerebellar tissue was taken from a dead 12-year-old stallion from a privately owned ranch in the municipality of Juarez in Nuevo Leon State, Mexico, approximately 240 km south of the Texas border. The horse was first observed with neurologic symptoms on June 20, 2003, and it was euthanized 7 days later. The horse had never been outside the state of Nuevo Leon and had not been vaccinated against WNV. The tissue sample was immediately placed on dry ice and transported to the biosafety-level-3 facilities at Colorado State University for processing. Although we were unable to isolate virus from the sample by passing brain homogenate in Vero cells, we successfully amplified viral RNA.

Total RNA was extracted from approximately 100 µg of cerebellar tissue with Trizol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions. The prM-E genes were amplified as two fragments by reverse transcription–polymerase chain reaction (RT-PCR) by using primers designed from the nucleotide sequence of the prototype WN-NY99 strain (GenBank accession no. AF196835). PCR amplifications were performed by using Ex Taq DNA polymerase (Takara Biomedicals, Shiga, Japan), which has 3′→5′ exonuclease activity. Amplification products were separated by agarose gel electrophoresis, visualized with crystal violet, and extracted by using the rapid gel extraction system (Invitrogen, Carlsbad, CA). The resulting DNA fragments were reamplified by PCR because of the low RNA copy number in the original material and purified by using the QIAquick PCR purification kit (Qiagen, Valencia, CA). Purified DNAs were sequenced on both strands with an ABI 377 DNA sequencer (Davis Sequencing, Davis, CA) and eight pairs of WNV-specific primers.

The nucleotide sequence of the prM-E genes of the WNV from Nuevo Leon State, Mexico (designated MexNL-03) was submitted to GenBank (GenBank accession no. AY426741). This region comprises 2004 nucleotides and corresponds to nucleotides 466 to 2469 of the genomic RNA of the WN-NY99 strain (8). Alignment of the MexNL-03 sequence with other known sequences in the GenBank database showed that it was most closely related to the homologous regions of three WNV isolates collected in Harris County, Texas in 2002 (13).

More recently, Estrada-Franco et al. (5) reported the first isolation of WNV from Mexico. The isolate (TM171-03) was from a corvid that died on May 5, 2003, in Tabasco State, southern Mexico. We identified WNV RNA in the brain of a dead horse from Nuevo Leon State, northern Mexico. Nucleotide sequencing and phylogenetic analysis of the prM-E genes showed that this WNV from Mexico was most similar to isolates collected from noncoastal areas of Texas in 2002.
Table. Nucleotide and deduced amino acid differences in the premembrane and envelope genes of the West Nile virus from Nuevo Leon State compared with various other West Nile viruses.

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<td>WN-NY99</td>
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In all cases, one change was in the prM gene at position 549, and two changes were in the E gene at positions 1179 and 1356. All substitutions were in the third codon position, and none resulted in an amino acid change.

The nucleotide sequence of MexNL-03 differed from that of the WN-NY99 strain (GenBank accession no. AF196835) in six positions (0.30% divergence; Table). Two mutations were in the prM gene (positions 549 and 660), and four mutations were in the E gene (positions 1179, 1356, 1442, and 2466). The U to C substitution at 1442 resulted in an amino acid change (Val → Ala); all other substitutions were silent. The U to C substitution at 549 and A to G substitution at 1179 have not been reported in any WNV isolates from the United States. However, an isolate collected in Illinois in 2002 (GenBank accession no. AY428521) has a U to A substitution at position 549. Similarly, an isolate from Randall County, Texas (GenBank accession no. AY428519), has an A to C substitution at position 1179. Several more divergent strains of WNV, such as a Kunjin virus isolated in Australia in 1960 (GenBank accession no. D00246), have a G at position 1179. Several more divergent strains of WNV, such as a Kunjin virus isolated in Australia in 1960 (GenBank accession no. D00246), have a G at position 1179. Similarly, an isolate from Randall County, Texas (GenBank accession no. AY428519), has an A to C substitution at position 1179. Several more divergent strains of WNV, such as a Kunjin virus isolated in Australia in 1960 (GenBank accession no. D00246), have a G at position 1179. Several more divergent strains of WNV, such as a Kunjin virus isolated in Australia in 1960 (GenBank accession no. D00246), have a G at position 1179.

In the Bayesian tree, the WNV isolates from North America formed a monophyletic group consisting of two sister clades (denoted as clade 1 and 2). MexNL-03 shared a close phylogenetic relationship with isolates from inland Texas, consistent with our nucleotide sequence alignments. These viruses, along with isolates from Colorado, Illinois, Alabama, and Tabasco State, belonged to a nested clade (denoted as 1A) within clade 1. The statistical support for clade 1A by parsimony and distance bootstrap analyses were 64% and 66%, respectively. The other WNV isolates that clustered in clade 1 were from the eastern United States. Clade 2 contained WNV isolates from the southeast coastal area of Texas and the northeastern United States. The WNV isolates from coastal Texas formed a nested clade (denoted as 2A), confirming previous results (13,14).

The parsimony-informative sites, corresponding to nucleotide positions 660, 1442, and 2466, were important in defining the topologic features of the tree (data not shown; see online figure, available from http://www.cdc.gov/ncidod/EID/vol10no7/03-0959-G2.htm). All 21 WNV strains in clade 1A contained a unique C to U substitution at 2466. All, except for the five most basal isolates, contained a unique C to T substitution at 1442. TM171-03 was basal to the other WNV strains in clade 1A, and this topologic arrangement was due to the single nucleotide difference at 1442. However, we consider it unlikely that TM171-03 was the predecessor of the other viruses in clade 1A. In addition, the parsimony and distance bootstrap analyses did not significantly support this topologic arrangement (bootstrap values = 50% and 51%, respectively). Moreover, WNV isolates from the northeastern United States occupied the basal position of clade 1. Similarly, the basal position of clade 2 was occupied by WNV isolates from the northeastern United States. Thus, our findings suggest that the
WNV isolates circulating in the United States and Mexico diverged from a common ancestor from the northeastern United States.

The trees generated by NJ, MP, and ML analyses showed the same overall topologic features to the Bayesian tree, except that all of the coastal Texas isolates were basal to the Israeli isolate (data not shown). The bootstrap support for this topologic arrangement ranged from 58% to 73%. Similar findings were also reported by Estrada-Franco et al. (5). Furthermore, the WNV isolates from Maryland and New Jersey, as well as most of the isolates from New York, occupy the basal positions of clade 1 of the NJ/MP/ML trees. As a result, we have not shown the bootstrap values for clades 1 and 2 of the Bayesian tree because their composition does not match exactly to the corresponding clades of the NJ/MP/ML trees. However, the Bayesian analysis provides a more robust and efficient phylogenetic tool compared to more conventional phylogenetic techniques (15). Additional sequencing and phylogenetic analyses will be necessary to clarify these issues.

Conclusions
The data presented here indicate that WNV was introduced into Nuevo Leon State, Mexico, from inland Texas. A likely mode of introduction was by infected birds traveling for short distances (16). Earlier studies have provided serologic evidence of WNV infection in horses or birds in the nearby Mexican states of Coahuila, Tamaulipas, and Chihuahua (4,5,17). Taken together, our sequence data and the findings from the serosurveys indicate that WNV activity is now widespread in northern Mexico, as well as in other regions in Mexico (5,6,18). The geographic distribution of WNV in the Americas will likely continue to expand; thus, enhanced WNV surveillance in Mexico is warranted.
Acknowledgments

We thank Alan Barrett and Scott Weaver for providing unpublished WNV sequences that were used in the phylogenetic analyses and Charles Calisher for critically reading the manuscript.

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Dr. Blitvich is a postdoctoral scientist in the Department of Microbiology, Immunology and Pathology at Colorado State University, Fort Collins, Colorado. His research interests include the mechanisms of vector and host interactions in arbovirus transmission cycles.

References


Address for correspondence: Barry J. Beaty, Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523, USA; fax: 970-491-8323; email: bbeaty@colostate.edu

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Human Metapneumovirus and Severity of Respiratory Syncytial Virus Disease

Isaac Lazar,* Carla Weibel,* James Dziura,* David Ferguson,* Marie L. Landry,* and Jeffrey S. Kahn*

We screened 23 children with severe respiratory syncytial virus (RSV) disease and 23 children with mild RSV disease for human metapneumovirus (HMPV). Although HMPV was circulating in Connecticut, none of the 46 RSV-infected patients tested positive for HMPV. In our study population, HMPV did not contribute to the severity of RSV disease.

In the United States, 100,000 infants and young children are hospitalized each year with respiratory syncytial virus (RSV) bronchiolitis (1). Although the risk factors for severe RSV disease, such as prematurity and bronchopulmonary dysplasia, are well defined, severe RSV disease may develop in otherwise healthy children. The pathogenesis of severe RSV disease is poorly defined.

In 2001, van den Hoogen et al. reported the isolation of a novel paramyxovirus, human metapneumovirus (HMPV) from children with respiratory tract disease (2). HMPV has been identified worldwide (3–7) and appears to have a seasonal distribution (winter and spring) (8). Since the circulation of HMPV may overlap with that of RSV, simultaneous infection with both RSV and HMPV may contribute to severe disease. Greensill et al. (9) reported that 70% of RSV-infected children who required admission to the Pediatric Intensive Care Unit (PICU) in Liverpool, U.K. were co-infected with HMPV. We sought to determine whether infection with HMPV was associated with the severity of RSV disease. We determined the frequency of HMPV infection in children with either mild or severe RSV disease. Disease severity was assessed by both the disposition (PICU vs. non-PICU) and by a clinical severity score.

The Study

As part of an ongoing epidemiologic study of viral respiratory infections in children, we collected all the RSV direct fluorescent antibody (DFA)–positive respiratory specimens from the Clinical Virology Laboratory at Yale-New Haven Hospital from November 1, 2001, to October 31, 2002. All respiratory specimens were also screened by DFA for para-influenza viruses 1–3, influenza A and B viruses, and adenoviruses (10). Any RSV-positive patient who also tested positive for one of the viruses listed above was excluded from the study. All RSV-positive children admitted to PICU during this yearlong period were identified. Because the peak time of infection with RSV and with HMPV may differ, each RSV-positive child from PICU was matched by date of diagnosis with a child with mild RSV disease. All RSV-positive children who were not admitted to PICU and diagnosed within 2 days of the diagnosis of the PICU-admitted child were identified. Of these, the child whose age most closely matched the age of the PICU-admitted child was selected. If no child diagnosed with RSV was identified within 2 days of the PICU-admitted child, the child with the closest date of diagnosis was identified and matched to the PICU-admitted patient.

RNA extraction and reverse transcription–polymerase chain reaction (RT-PCR) were performed as previously described (4). Primers used for the amplification of the RSV L gene were as follows: forward primer 5’-GGTAGAATCTACATACATTACCTAAGTG-3’ (genome location 14881–14909, [reference strain RSV A Melbourne, Australia/2/'61] GenBank accession no. M74568), reverse primer 5’-CGAGATATTAGTTTTGACAC-3’ (genome location 15190–15210, GenBank accession no. M74568). The HMPV forward primer, 5’-CGCGTCTGAAGGTAG-3’ (HMPV genome location 3163-3180, GenBank accession no. AF371367, G/C clamps are underlined) and reverse primer, 5’-CGAGATATTAGTTTTGACAC-3’ (HMPV genome location 15190–15210, GenBank accession no. M74568). The HMPV forward primer, 5’-CGCGTCTGAAGGTAG-3’ (HMPV genome location 3163–3180, GenBank accession no. AF371367, G/C clamps are underlined) and reverse primer, 5’-CGCGTCTGAAGGTAG-3’ (3425–3444, GenBank accession no. AF371367, G/C clamps are underlined) used for the amplification of the HMPV F gene were based on regions of the F gene conserved in New Haven, Netherlands, and Australian strains (4). PCR reactions were performed by using HotStar (Qiagen, Inc., Valencia, CA) and amplification cycles were as follows: 95°C for 15 min followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final 10-min cycle at 72°C. Each set of reactions contained appropriate negative (water) and positive control samples for the RT (HMPV-positive nasal wash) and the PCR (HMPV cDNA) steps.

A clinical severity score (CSS) was adapted from the severity score described by Martinello et al. (11). Two points were assigned if the patient required positive-pressure ventilatory support during the illness, and one point was assigned for each of the following: hospital admission,
hospitalization for >5 days, oxygen saturation <87% (at least one measurement), and any use of supplemental oxygen. Therefore, CSS ranged from 0 to 6. Medical records from all patients were abstracted and scored by a reviewer who did not know the patient’s HMPV status.

On the basis of the published literature (9), we expected the frequency of RSV/HMPV co-infection in the PICU patients to be approximately 70%, and in the non-PICU patients to be closer to the DFA-negative–HMPV-positive infection rate of 6.4% found in our population (DFA-negative refers to samples that tested negative for RSV, parainfluenza viruses, influenza viruses, and adenovirus) (4). Power calculations were performed by using PASS 2002 (J. Hintze, NCSS and PASS, Number Cruncher Statistical Systems, Kaysville, Utah). By using a power of 90% and α of 0.05, a sample of 12 patients in each group would be sufficient to support these findings (70% vs. 6.4%). By using the same power calculations, 23 patients in each group would provide adequate power to show as little as a 45% difference in proportions co-infection between the two groups. Comparisons were made by using the chi-square test and Wilcoxon rank sum tests, as appropriate (Table). Exact 95% confidence intervals were calculated in SAS V8.2 (SAS Institute, Cary, NC).

Twenty-three RSV DFA-positive patients were admitted to PICU during the study period, and 23 matched patients were identified. Demographic and clinical information were obtained for each patient from the medical record. Of the children admitted to PICU, 7 (30.4%) of 23 had a known predisposing risk factor for severe RSV disease. All the PICU-admitted children had CSS ≥3 and most (16 [70.0%] of 23) had a CSS ≥5. Eighteen (78.2%) of 23 PICU patients were hospitalized for >5 days. None of the RSV-positive patients admitted to the PICU tested positive for HMPV by RT-PCR.

Children with mild RSV disease were initially seen in the emergency department, and according to their severity of illness, either were discharged or were admitted to the pediatric ward. Eight patients (34.8%) of the 23 mild RSV disease group were admitted to the hospital, although only 4 (17.4%) of these children were hospitalized for ≥5 days. The CSS range for this group was 0–4. Most patients (14 [61.0%] of 23) had a CSS of <3. None of these patients were positive for HMPV by RT-PCR.

Statistically significant differences between the PICU group and the mild disease group were observed in admission age (median age 7 weeks vs. 54 weeks, p = 0.025), hospital admission rate (23/23 vs. 8/23, p = 0.004) and CSS (median CSS 5 vs. 1, p = 8 x 10^-12) (Table). Positive pressure ventilation was required by 17 (73.9%) of 23 PICU patients and 10 of these 17 patients needed it for >5 days. None of the patients screened in either group of RSV-DFA positive patients had evidence of HMPV infection (p = 1.0). To ensure that the methods for RT-PCR were adequate, we performed RT-PCR for RSV for each patient’s respiratory specimen. Overall, 44 (95.7%) of 46 of children had a positive RT-PCR test for RSV.

Conclusions
The possibility that HMPV plays a role in the pathogenesis of infections with other respiratory viruses is not known. The importance of identifying HMPV in persons with SARS remains to be explained (5,12). Greensill et al. observed a 70% co-infection rate with HMPV and RSV and a 90% co-infection rate among intubated infants with HMPV and RSV admitted to their PICU (9). Although Greensill et al. did not include an appropriate control group in their study, these findings suggest that co-infection with both HMPV and RSV is common and that together the two viruses may contribute to increase the severity of disease.

We did not observe HMPV infection in children with either mild or severe RSV disease. Our findings cannot be explained by the absence of HMPV in Connecticut. From

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*PICU, pediatric intensive care unit.
*χ^2 test.
*Wilcoxon rank sum test.
*RSV, respiratory syncytial virus.
*DFA, direct fluorescent antibody screen.
*CSS, clinical severity score.
*PPV, positive pressure ventilation.
*ND, non-comparable because patients requiring PPV are admitted to the PICU.
*HMPV, human metapneumovirus.
November 2001 to April 2002 (the period of time when all of the RSV-positive PICU-admitted children were identified), HMPV was detected in 11% of 446 patients who tested negative by DFA for RSV, parainfluenza viruses, influenza virus, and adenovirus (13). Furthermore, we matched children with mild RSV disease to children with severe RSV disease by date of diagnosis to eliminate the possibility that the temporal distribution of the viruses might influence our results.

The potential difference in the sensitivity of the screening tests used by Greensill et al. and our group likely does not account for the differences in the observed rates of co-infection. We used a similar RT-PCR–based approach. We are confident that our methods to detect HMPV are both sensitive and specific (4,13). The observed rate of HMPV in respiratory specimens in our previous study (8.1%) (13) is comparable to rates observed elsewhere (6,14). Furthermore, in our previous studies, we have used sequence analysis of RT-PCR amplicons to confirm the identification of HMPV (4,13). An increased prevalence of HMPV in Liverpool, UK, may account for the high rate of RSV/HMPV co-infection observed by Greensill et al., although no data at this point support this hypothesis.

Our relatively small sample size limited the power of our analysis. However, on the basis of the sample size calculations with 90% power, our patient numbers were sufficient to detect a difference of >45% above the rate of HMPV infection in the non-PICU group (6.4%). Nonetheless, our results demonstrate that the rate of co-infection is low (0% of 23 patients, 95% confidence interval 0%–14.8%). Other studies also support our findings that the frequency of co-infection with HMPV and RSV is rare (7,14).

The basis of the pathogenesis of severe RSV disease is multifactorial. Since severe RSV disease may develop in apparently healthy children, known host risk factors cannot completely account for instances of severe illness. Preexisting or maternally acquired immunity, innate immunity, viral factors and genotypes and environment all likely contribute to disease pathogenesis. Although we did not detect co-infection, HMPV may worsen RSV disease in a small percentage of infants. Nonetheless, HMPV most likely does not play an important role in the severity of RSV disease in the population.

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Dr. Lazar is a pediatric intensive care postdoctoral fellow in the Division of Pediatric Critical Care and Applied Physiology, Department of Pediatrics, Yale University School of Medicine. His research focuses on the pathogenesis of RSV disease.

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Address for correspondence: Jeffrey S. Kahn, Department of Pediatrics, Division of Infectious Diseases, Yale University School of Medicine, PO Box 208064, New Haven, CT 06520-8064, USA; fax: 203-785-6961; email: Jeffrey.Kahn@yale.edu
Atypical Avian Influenza (H5N1)

Anucha Apisarnthanarak,* Rungrueng Kitphati,† Kanokporn Thongphubeth,* Prisana Patoomanunt,* Pimjai Anthanont,* Wattana Auwanit,† Praneet Thawatsupha,† Malinee Chittaganpitch,† Siriphan Saeng-Aroon,† Sunthareeya Waicharoen,‡ Piyaporn Apisarnthanarak,‡ Gregory A. Storch,§ Linda M. Mundy,§ and Victoria J. Fraser§

We report the first case of avian influenza in a patient with fever and diarrhea but no respiratory symptoms. Avian influenza should be included in the differential diagnosis for patients with predominantly gastrointestinal symptoms, particularly if they have a history of exposure to poultry.

Influenza A viruses are classified into subtypes (hemagglutinin and neuraminidase subtypes) based on antigenic differences in their surface glycoproteins (1). Of 15 identified hemagglutinin (H1–H15) and 9 neuraminidase subtypes (N1–N9), only 3 hemagglutinin subtypes (H1, H2, and H3) and 2 neuraminidase subtypes (N1 and N2) have established stable lineages in humans (1). Because the natural reservoir of known influenza A subtypes is found in birds and waterfowl (2), subtypes other than those typically found in humans have the potential to cross the species barrier and infect humans (3).

Avian influenza A virus H9N2 was isolated from two children in Hong Kong in 1999, and avian influenza H7N7 infected 89 persons during a simultaneous outbreak in poultry in the Netherlands in 2003 (4–7), although these infections resulted in only mild illnesses. The first outbreak of a highly pathogenic avian influenza (H5N1) in humans occurred in Hong Kong in 1997; 6 of 18 people with confirmed infection died (5,8). Despite attempts to prevent disease, two cases of influenza A H5N1 occurred in Hong Kong in February 2003 (1), followed by outbreaks in Vietnam and Thailand in January 2004 (9,10). Data are limited on the epidemiologic characteristics, signs and symptoms, and outcomes of avian influenza H5N1 exposure in healthcare workers. We report atypical avian influenza H5N1 and follow-up surveillance of 35 exposed healthcare workers; we also review relevant literature in this area.

The Case

On March 9, 2004, a 39-year-old woman with no underlying disease was transferred to our hospital with rapidly progressive pneumonia. At the referring hospital, she reported fever for 1 week, diarrhea, nausea, and vomiting, with no early respiratory symptoms. Initial laboratory values at the referring hospital included the following: leukocyte count 3,300 cells/mm³, total lymphocyte count 640 cells/mm³, hemoglobin 13 g/dL, and platelet count 400,000 cells/mm³. Stool samples and cultures were negative for bacteria and parasites. Because the patient had no respiratory symptoms, no chest radiograph was performed on initial admission. Norfloxacin was prescribed. On hospital day 5, cough and shortness of breath developed, and chest radiograph was performed (Figure 1A). Norfloxacin was changed to ceftriaxone and amikacin, and the patient was transferred.

Upon arrival at our hospital, her temperature was 39.4°C, respiratory rate 44/min, blood pressure 110/80 mm Hg, and heart rate 140 beats/minute. She was intubated, and the examination showed bilateral crackles. Laboratory data included leukocyte count 2,200 cells/mm³ with total lymphocyte counts of 440 cells/mm³, hemoglobin 11.1 g/dL, platelet count 330,000 cells/mm³, aspartate aminotransferase 474 U/L, alanine aminotransferase 106 U/L, alkaline phosphatase 546 U/L, blood urea nitrogen 11 mg/dL, creatinine 1.6 mg/dL, partial thromboplastin time 35.4 s, prothrombin time 11.9 s, and lactate dehydrogenase 1,832 mg/dL. Imipenem, azithromycin, and doxycycline were administered as adult respiratory distress syndrome progressively developed. Infectious disease consultation was requested the next day, and additional exposure history was obtained from the family.
The patient and five family members live together in a rural area of Ayudhaya in central Thailand. Her family reported that she was exposed to several dead chickens in her neighborhood. Neighborhood chickens were noted frequently to roam around the patient’s house, and some had died in front of it. All her family members were well, with no symptoms of influenzalike illness. After the exposure history was obtained, droplet and contact precautions were implemented, and the patient was evaluated for H5N1 influenza. Nasopharyngeal aspirates underwent a rapid influenza A test by enzyme immunoassay (ELISA), HIV by enzyme-linked immunosorbent assay (ELISA), Legionella by microimmunofluorescence, mycoplasma by microparticle agglutination assay, Chlamydia by microimmunofluorescence, and Legionella by enzyme-linked immunosorbent assay (ELISA), HIV by ELISA, Burkholderia pseudomallei (meliodi) by immunohistochemical assay, dengue titer by hemagglutination inhibition using all four serotypes, Leptospira titer by microscopic agglutination test, Widal test, Weil-Felix test, and viral culture. Several dead chickens around the neighborhood near the patient’s house tested positive for H5N1 by viral cultures. The patient’s family declined to permit an autopsy.

Thirty healthcare workers at our hospital and five healthcare workers at the referring hospital were exposed to this index patient without using appropriate personal protective equipment. All of them were monitored for 2 weeks for temporally related influenzalike illness, and temperatures were measured twice weekly. No temporally related influenzalike illness or fever developed. The characteristics and types of exposure reported by influenza A H5N1–exposed healthcare workers are summarized in the Table.

Viral culture for avian influenza H5N1 was conducted on Madin-Darby canine kidney cell monolayers at the Department of Medical Sciences, National Institute of Health, Bangkok. Nasopharyngeal aspiration specimens tested positive by an RT-PCR assay specific for the hemagglutinin gene of influenza A H5N1. The specimen was tested with the primer set for the H5 gene (forward primer H5-1 GCC ATT CCA CAA CAT ACA CCC, reverse primer H5-2 TAA ATT CTC TAT CCT CCT TTC CAA), with an expected product size of 358 bp (8). The specimen was confirmed positive by different RT-PCR primers (forward primer H5-1456 ACG TAT GAC TAT CCA CAA TAC TCA, reverse primer H5-1685 AGA CCA GCT ACC ATG ATT GC), which amplify a DNA fragment of 229 bp. This specimen was further confirmed as positive by using the real-time RT-PCR method, including primer and probe, described by Spackman et al. (11). This assay amplifies a conserved region of European and Asian avian influenza virus and was modified to run on the LightCycler (Roche Molecular Systems, Indianapolis, IN). Precautions for preventing cross-contamination were observed (12).

Conclusions

The clinical signs and symptoms of avian influenza H5N1 may be more protean than originally described. During the 1997 epidemic in Hong Kong, patients exhibited fever, headache, malaise, myalgia, sore throat, cough, and rhinitis (5,8). Although uncommon, conjunctivitis and gastrointestinal symptoms were also reported (5,8). In the 2004 epidemic in Vietnam, prominent clinical signs and symptoms of avian influenza H5N1 were those of a severe influenza syndrome with fever, cough, diarrhea, and shortness of breath (9). Of note, diarrhea was present in 7 (70%) of 10 patients along with lower respiratory symptoms (9). The preliminary clinical features of avian influenza H5N1 in the 2004 epidemic in Thailand included fever, cough, sore throat, rhinorrhea, myalgia, and shortness of breath (10). Laboratory findings of patients with severe avian...
influenza H5N1 are undistinguishable from those of patients with prevailing human influenza; findings include leukopenia, lymphopenia, impaired liver function, prolonged clotting times, and renal impairment (5,8–10). To our knowledge, this patient has the first reported case of H5N1 with fever and gastrointestinal symptoms but no respiratory symptoms.

As of this submission, 22 patients in Vietnam and 12 in Thailand have confirmed cases of avian influenza H5N1. Twenty-three (67%) of 34 infected patients have died. The death rate of H5N1 was 33% (6 of 18 patients) in Hong Kong in 1997, 73% (15 of 22 patients) in Vietnam in 2004, and 67% (8 of 12 patients) in Thailand in 2004. Risk factors associated with severe disease and poor outcome of H5N1 included older age, being symptomatic for a longer period before admission, pneumonia, leukopenia, and lymphopenia (8). Patients <5 years of age had mild disease compared with hospitalized adults (8). However, all cases of H5N1 from Thailand occurred in pediatric patients, except for our patient and one previously reported patient (10). No H5N1 patients in Thailand had underlying or concomitant disease (10).

Avian influenza (H5N1) can be isolated by conventional viral culture methods (1). Several reports suggested that rapid influenza tests, H5-specific RT-PCR, and real-time RT-PCR could aid a rapid diagnosis (1,2,11,13,14). However, rapid diagnostic tests for influenza have low sensitivity, which may limit their usefulness to reliably detect H5N1, especially if illnesses are diagnosed later in their clinical course (1), as in our patient. Thus, clinical findings and a history of poultry exposure may be more helpful in identifying patients with H5N1 infection than the result on rapid diagnostic tests for influenza.

Data are limited on human-to-human transmission of avian influenza H5N1. Whether H5N1 could be efficiently transmitted from human to human is a matter of concern.

In a matched case-control study of 15 patients with H5N1, exposure to live poultry in the week before symptom onset was significantly associated with H5N1 disease, while traveling, eating or preparing poultry products, and recent exposure to persons with respiratory illness had no significant association (15). A cohort study conducted among persons infected with H5N1 to detect anti-H5 antibody among their household and social contacts suggests that human-to-human transmission might have occurred through close physical contact with H5N1-infected patients, whereas social exposure to an infected person was not associated with H5N1 infection (16).

Another cohort study, which included healthcare workers from three hospitals where H5N1 patients had been admitted, found a significantly higher rate of seropositivity for H5N1 among exposed workers (8 [3.7%] of 217 persons) than among nonexposed workers (2 [0.7%] of 309 persons), which provides evidence of H5N1 transmission from infected patients to healthcare workers (17). In our study, although 33 (94%) of 35 healthcare workers were exposed to the index patient within 1 m, they did not exhibit fever or influenzalike illness during our 2-week follow-up period (Table). However, we cannot rule out mild or subclinical infection because of the lack of data on anti-H5 serology. Together, these studies confirm that healthcare workers are at low risk of acquiring H5N1 from patients, but continued precautions and monitoring are essential in case the virus evolves to become more transmissible among humans. In conclusion, physicians working in areas where H5N1 is endemic should be aware of unusual cases. A high index of suspicion will facilitate prompt diagnosis and proper management.

Dr. Apisarnthanarak is an infectious disease specialist and hospital epidemiologist at Thammasat University Hospital, Thailand. He is a member of the Thai Infection Control Work Group. His major research focus is in preventing and controlling nosocomial infections, with a secondary focus on investigating outbreaks and emerging infectious diseases.

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Address for correspondence: Anucha Apisarnthanarak, Faculty of Medicine, Thammasart University Hospital, Klong Luang, Pratumthani, Thailand 12120; fax: 662-997-2175; email: anapisarn@yahoo.com

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Transporting Patient with Suspected SARS

To the Editor: The severe acute respiratory syndrome (SARS) outbreak in Taiwan can be traced back to a Taiwanese businessman who returned from mainland China to Taiwan in March 2003 (1). In May 2003, several outer islands belonging to Taiwan reported SARS, and on June 2, 2003, Penghu Army Hospital reported a 40-year-old man with suspected SARS. The patient complained of shortness of breath and a dry cough. He had visited a person with confirmed SARS 7 days earlier. He had a temperature of 38.4°C and leukocyte count of 7,920 cells/µL, and his chest x-ray showed infiltration in both lower lobes.

Because medical facilities are limited on these islands, the Department of Health authorized the National Aeromedical Consultation Center (NACC), a physician-based 24-hour control center that coordinates all aeromedical transport of critically ill or injured patients within Taiwan, to coordinate transporting these patients to designated SARS hospitals in Taipei. The NACC dispatched an aircraft (Fokker 50) with a specialized team of two flight physicians, one flight paramedic, and a PIU (portable isolation unit) on board. During the flight, the medical crew prepared equipment and dressed themselves in three layers of personal protective equipment. On arrival at Penghu, only essential equipment was taken into the hospital. One physician took the PIU into the isolation room. The rest of the crew and equipment remained in the pre-isolation room. The patient was briefly about the transport and given 10 mg of metoclopramide to prevent motion sickness. He was asked to get into the PIU. A pulse oximeter was attached to his finger and placed inside the PIU so that it could be read from the outside. A thermometer was also placed inside the unit. The patient was given a squeeze-bottle of water, and the unit was sealed and inflated.

When leaving the pre-isolation room, the physician and the PIU were sprayed with a sodium hypochloride solution before the first layer of personal protective equipment was removed. At the exit, the entire medical crew removed a layer of personal protective equipment after being sprayed with sodium hypochloride solution. The team returned to the airport for the flight back to Taiwan. No other personnel or family member was allowed to accompany the patient on the flight.

The patient remained stable and calm throughout the flight. His oxygen saturation remained 97%–99% with heart rate of 90 to 100 beats per minute. Humidity was maintained at 60% and temperature at 28°C. On arrival, the team proceeded to the isolation ward. The physician accompanied the patient into the isolation room; the patient was released from the PIU and transferred to the receiving medical team.

On exiting the isolation room, the empty PIU and the medical team were sprayed with sodium hypochloride. All equipment was sprayed and put into biohazard bags. The medical team then discarded the last layer of impermeable clothing. The PIU was left in biohazard bags for 24 hours before being sprayed with water and air-dried.

After the assignment, the medical crew self-documented their temperature twice daily for 10 days. All staff remained asymptomatic with normal body temperatures during this period. The patient’s temperature remained normal, and results of a polymerase chain reaction of throat swab were negative for SARS-associated coronavirus (SARS-CoV). He was discharged on June 10, 2003.

When the SARS outbreak occurred in Taiwan, many medical and ambulance personnel were exposed to SARS-CoV while transporting or caring for patients with suspected SARS. As SARS was an emerging infectious disease, the mechanism of transmission was still unclear. Although one report by Christopher and Eitzen (2) suggested the value of an aeromedical team to evacuate patients with suspected lethal, infectious diseases, limited evidence supported a safer means of transportation that would possibly reduce transmission of SARS to persons taking part in the mission.

When the SARS epidemic spread to remote islands, aircraft companies refused to transport patients with a case of suspected SARS unless certain precautions were implemented. Smaller aircraft used on domestic routes in Taiwan do not meet the standards set for transporting SARS patients (3,4), which prompted the design of the PIU, an airtight polyvinyl chloride bag with a one-way inlet valve and an exhalation valve. The valves were modified by incorporating HEPA filters on both sides of the valves and then connecting a ventilator with an oxygen source to the inlet valve. The respiratory rate and tidal volume are set, depending on weight and oxygen requirements of the patient. By regulating the exhalation valve, the minimum pressure inside the bag can be manipulated to keep it from collapsing, since the bag has no internal or external frame.

The PIU has some limitations. No physical contact with the patient is possible after the PIU is sealed and inflated. Very strict criteria on the suitability of a patient to be transported are followed. Any patient who is unconscious, uncooperative, or whose condition may deteriorate is not transportable in this unit. Because of possible discomfort, a maximum total transport time of 2 to 4 hours is suggested. This time frame works well in Taiwan; all locations in the country, including the outer islands, are within a 4-hour limit.
The use of PIU during the SARS crisis had a number of positive effects in Taiwan. It enabled the safe transport of SARS patients between hospitals by air and road and decreased the risk of cross-infecting transport personnel. The anxiety of transport personnel was decreased, as was the fear felt by the population of the outer islands. In addition, the credibility of the local health authorities was improved among the general population in Taiwan.

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Shin-Han Tsai,*†‡ Chin-Man Tsang,§ Hsueh-Ru Wu,*†¶ Li-Hua Lu,# Yung-Chia Pai,# Mark Olsen,§ and Wen-Ta Chiu†‡

*National Aeromedical Consultation Center, Taipei, Taiwan; †Taipei Medical University, Taipei, Taiwan; ‡Wan Fang Hospital, Taipei, Taiwan; §International SOS, Taipei, Taiwan; ¶Chun Shan Hospital, Taipei, Taiwan; and †Mackay Memorial Hospital, Taipei, Taiwan

References


Address for correspondence: Wen-Ta Chiu, Institute of Injury Prevention and Control at Taipei Medical University and Wan Fan Hospital, 250 Wu-Shin Street, Taipei, Taiwan, 110; fax: 886-2-27390387; email: shtsai@tmu.edu.tw

Psychosocial Impact of SARS

To the Editor: An outbreak of severe acute respiratory syndrome (SARS) occurred from February to May 2003 in Hong Kong, China, Singapore, and Canada. According to the World Health Organization, 1,755 people were infected in Hong Kong; 386 of these were healthcare workers. A total of 300 persons died from SARS, constituting a death rate of 17% (1).

Evidence suggests that persons infected with SARS recovered physically, but SARS is associated with social and psychological problems poorly understood by the scientific community. A survey in a convalescent hospital in Hong Kong showed that approximately 50% of recovered SARS patients showed anxiety (2), and approximately 20% were fearful (2). Approximately 20% of the rehabilitated patients showed some negative psychological effects (3), which included insomnia and depression. Some patients with serious cases could not rid themselves of the memories of fighting SARS, and these memories disrupted their daily activities. These psychosocial problems may be due to the complications of SARS medications, such as ribavirin and corticosteroid. Persons who took these drugs had hair loss, major memory loss, impaired concentration, and depression. A medical practitioner in Hong Kong who recovered from SARS attempted suicide because complications from drugs made him unable to earn his living (4).

In addition to SARS patients themselves, an estimated 50% of family members of SARS patients had psychological problems, including feelings of depression or stigmatization (5). They had difficulties sleeping, and some children who had lost parents cried continuously. Some children also felt embarrassed to be a member of a SARS family (6). The spouse of one healthcare worker who died from SARS attempted suicide at her workplace (7). The loss of parents who were SARS patients also impaired the growth of their children (7). A study conducted in China (8) reported that negative SARS-related information increased persons’ perception of their risk and led to irrational nervousness or fear.

Although data from systematic studies of SARS do not exist, evidence suggests that this disease has psychosocial consequences for SARS patients, their families, and society. While biomedical scientists must continue their efforts to clarify the genetic makeup of the SARS coronavirus, look for new medications, and develop vaccines (9–13), the social and psychological aspects of SARS should not be overlooked. Since nearly all resources are devoted to biomedical research and medical treatment, psychosocial problems of SARS patients and their families are largely ignored. Our review of the literature using the ISI Web of Knowledge on January 17, 2004, substantiated this observation. To date, no systematic study examining psychosocial consequences of SARS has been published in scientific journals. A systematic exploration of how SARS negatively affects patients’
mental health is needed so that appropriate interventions may be implemented at individual, family, and societal levels.

Hector W.H. Tsang,*
Rhonda J. Scudds,*
and Ellen Y.L. Chan*
*The Hong Kong Polytechnic University, Hong Kong

References


Address for correspondence: Hector W. H. Tsang, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Hung Hom, Hong Kong; fax: 852-2330-8656; email: rhshtsang@polyu.edu.hk

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**Highly Pathogenic Avian Flu, Japan**

To the Editor: More than 15,000 chickens on an egg farm in Yamaguchi Prefecture (Chugoku area) have died since the end of 2003. A highly pathogenic avian influenza virus, which had not appeared in Japan for 79 years, was detected in the dead chickens. Of the 34,600 chickens on the farm, dozens to hundreds have died daily since December 28. Moreover, the deaths have increased during 2004. The Ministry of Agriculture, Forestry, and Fisheries ascertainment that the same H5N1 avian influenza virus had caused the bird flu epidemic that started in 1997 in East Asia, including Hong Kong, Vietnam, and South Korea. The H5N1 type is a virulent pathogen that can also infect humans as demonstrated by the >20 deaths in Hong Kong, Vietnam, and Thailand. After the influenza infection was confirmed, the ministry immediately ordered the henryey to recall all eggs that had been shipped. The hen-ryey was then disinfected, and non-workers were restricted from entering. Yamaguchi Prefecture also restricted transfer of the chickens and eggs within a 30-km radius of the infected henryey. The henryey was the first facility infected in Japan. Since mid-February, an additional three outbreaks have occurred (one in Ohita Prefecture in Kyushu Island and two in Kyoto Prefecture in the Kansai Area). In a big poultry farm in Kyoto, 40,000 deaths of chickens, caused by H5N1, were confirmed. The H5N1 virus was also detected by polymerase chain reaction in crows found dead near the chickens in Kyoto. All four sites with infected chickens are in western Japan.

Modern stock raising that involves breeding a large number of domestic animals and fowl in high density has become a risk factor for large-scale outbreaks. The globalization of the marketplace and easy mobility of people and goods have facilitated the spread of many pathogens. Avirulent pathogens that mutate easily may acquire stronger infectious and toxic properties as confirmed in the influenza pandemic of 1918 (1).

Several possibilities exist for the appearance of avian influenza virus in Japan. First, migratory birds from disease-epidemic areas might be the primary vectors of the virus. Yamaguchi Prefecture is located 200 km southeast of South Cholla Province, South Korea, where avian influenza is epidemic. The two areas are close enough for wild birds to cross the Korean Strait. Ito et al. reported that avirulent viruses found in wild waterfowl and bearing the consensus avirulence type sequence R-E-T-R have the potential to become pathogenic when present in chickens (2). Thus, migratory birds that are asymptomatic carriers may cross the Korean Strait harboring the H5N1-type virulent viruses generated in Korea. Alternatively, people, cars, and feed grains instead
of migratory birds could carry the virulent viruses. To identify the source of infection, the genetic sequence of the virus will be compared with the sequences of viruses acquired in other epidemic areas.

The avian influenza virus did not originally infect other animals, including humans. The virus in Japan had different DNA sequencing from the viruses responsible for human deaths in Hong Kong and Vietnam. However, mutations of the virus in pigs as a result of hybridization are possible, since both avian and human influenza viruses can infect pigs. According to the Food and Agriculture Organization of the United Nations, the H5N1-type virus was detected in pigs raised on farms that also raise chickens infected with the virus in Vietnam. Thus, a new virus that can infect other animals may emerge. In fact, a clouded leopard died of avian influenza in Thailand.

The worst scenario would be that the new virus could be spread from person to person. An avian influenza vaccine is not available in Japan. Because a vaccine may not be developed quickly enough, this new influenza might become pandemic. Therefore, to prevent the virus from infecting humans, bird-to-bird transmission must be stopped.

Kazuo Inoue*
*University of Tokyo, Tokyo, Japan

References

Address for correspondence: Kazuo Inoue, Department of Public Health, Graduate School of Medicine, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033 Japan; fax: 81-3-3816-4751; email: inoue@mu-tokyo.ac.jp

Alexander the Great and West Nile Virus Encephalitis

To the Editor: Marr and Calisher suggest the cause of Alexander the Great’s death in Babylon in 323 B.C. was West Nile encephalitis (1). They were intrigued by the fact that as Alexander entered Babylon, ravens fell dead from the sky. The authors postulated the ravens might have had West Nile encephalitis, and because of the endemicity of mosquitoes in ancient Babylon, Alexander could have died of West Nile encephalitis. The authors are to be complimented on coming up with a novel explanation for his death, but this explanation has several problems (2,3).

Determining the exact cause of Alexander’s death is impossible. Classical scholars are hampered by difficulties with translations from ancient Greek texts as well as differences in terms used by Plutarch in his description of Alexander’s demise. We are left with a description that is incomplete, but nevertheless contains cardinal features of his terminal illness (4–6). In infectious disease practice, a syndromic diagnosis is the basis of the clinical approach. Astute infectious disease clinicians must discern between consistent and characteristic features in syndromic diagnosis. In addition to characteristic clinical features, syndromic diagnosis also depends on time relationships of clinical features. That splenomegaly is a feature of Epstein-Barr virus infectious mononucleosis is important, but equally as important is the late rather than early appearance of splenomegaly in the illness. A laundry list of features associated with various infectious diseases tells only part of the story and is diagnostically unhelpful unless placed in the proper time sequence.

In the authors’ table, the clinical symptoms associated with Alexander’s final days are listed (1). In my review of translations of ancient sources, chills are never mentioned as accompanying Alexander’s slowly rising fever. After a steady increasing fever, Alexander first became weak, then lethargic, and finally died after a 2-week febrile illness. These features and time course are inconsistent with various explanations that have been given for Alexander’s death, i.e., influenza, poliomyelitis, alcoholic liver disease, malaria, schistosomiasis, leptospirosis, and poisoning (6–8).

The death of Alexander was certainly caused by an infectious disease and not poisoning or alcoholic liver disease. Although Alexander had an appetite for alcohol, his terminal illness is inconsistent with liver failure attributable to alcoholic cirrhosis or delirium tremens. Poisoning, which has been postulated by some, is not a reasonable diagnostic possibility either, since toxins or poisons are not accompanied by fever. Therefore, we are left with an infectious disease that was endemic in ancient Babylon and was fatal after approximately 2 weeks. The infectious disease that resulted in Alexander’s demise was characterized by a slow but relentless increase in temperature during 2 weeks, unaccompanied by chills or drenching sweats. While remaining mentally alert, he drifted into an apathetic state, according to Alexander’s Royal Diaries. Details of his death do not provide additional details other than he was febrile, weak, and gradu-
ally became lethargic, lapsed into coma, and died. Are the features of his illness and temporal sequence of events characteristic of West Nile encephalitis (9)?

West Nile encephalitis is a mosquito-borne infectious disease that may have been endemic in ancient Babylon. Ravens could have had West Nile encephalitis, and if West Nile encephalitis was present at the time, certainly it was transmitted to animals as well as humans. No one would argue with the possibility of West Nile encephalitis in the ancient Middle East; however, proving that West Nile encephalitis explains Alexander’s death is more difficult. West Nile encephalitis begins acutely, with initial signs and symptoms of mental confusion and muscle weakness. Fevers are not usually the most conspicuous feature of West Nile encephalitis, and in most cases the fever does not usually increase or last more than a 2-week period. Other forms of viral encephalitis, including West Nile encephalitis, all begin with an abrupt change in mental status, e.g., encephalitis, at the outset of the illness. The patient’s mental status may change over time, but encephalitic symptoms are present initially. This symptom is a characteristic feature of viral encephalitis, whether it is due to West Nile encephalitis or western equine encephalitis, Venezuelan equine encephalitis, St. Louis encephalitis, or Japanese encephalitis. Even non–arthropod-borne causes of viral encephalitis, e.g., herpes simplex virus I encephalitis, occurs with encephalitis as an initial, not terminal feature.

Alexander’s final illness is more characteristic of typhoid fever than West Nile encephalitis. On Alexander’s return to Babylon, he was confronted by many portents and omens and correctly assumed that they were a forewarning of his death. Not only were ravens falling from the sky, but the birds that were sacrificed to foretell the future were devoid of a liver lobe, which was thought by the ancients to be an ominous sign. A docile animal in the royal menagerie, in a violent outburst, kicked the royal lion to death. A mysterious person entered the royal chamber and sat on Alexander’s throne bypassing the household guards. He claimed that he was divinely sent. West Nile encephalitis could explain these unusual phenomena.

However, the time course and characteristic clinical features of West Nile encephalitis are inconsistent with the cause of Alexander the Great’s death (10). On the basis of characteristic features and time course of the illness, typhoid fever is the most likely explanation for Alexander the Great’s death. The ravens in this case were the red herrings.

Burke A. Cunha†

†Winthrop-University Hospital, Mineola, New York, USA; and †SUNY School of Medicine, Stony Brook, New York, USA

References
describe as “history.”

When approaching the time of Caesar’s assassination, Plutarch wrote, “...many strange prodigies and apparitions are said to have been observed shortly before this event... the wild birds which perched in the forum” (5). As Cicero fled Antony’s death sentence, Plutarch wrote, “...a flight of crows rose with a great noise, and made towards Cicero’s vessel, as it rowed to land, and lighting on both sides of the yard, some croaked, others pecked the ends of the ropes” (6). On the founding of Rome, he wrote, “...concluding at last to decide the contest by a divination from a flight of birds... Remus, they say, saw six vaults, and Romulus double that number... Hence it is that the Romans, in their divinations from birds, chiefly regard the vulture” (7). (For Remus, who died shortly thereafter, this appears to have been a less propitious sighting.)

When writing on the lost grave of Theseus, Plutarch wrote, “...he, by chance, spied an eagle upon a rising ground pecking with her beak and tearing up the earth with her talons” (8).

On the defeat of the Persian armada at Salamis, he wrote, “...an owl was seen flying to the right hand of the fleet, which came and sat upon the top of the mast” (9). These examples, to which we could add others, should suffice to make our point.

Yet, we do not seek to diminish the contribution of Marr and Calisher. Plutarch, renowned for his expositions on notable men, sought in doing so to identify elements of greatness. In this vein, we note the qualities that these three fine writers share. Truly, all are erudite. All share a remarkable awareness of the importance of birds. For this, both as physicians and as birders, we applaud them. In this age of emerging infections, including WNV and avian influenza viruses, we ignore bird health at our peril. We thank the doctors for this reminder and have increased our vigilance. We recommend, however, a grain of salt with Plutarch.

David Oldach,*† R. Michael Benitez,*† and Philip A. Mackowiak*†
*VA Maryland Health Care System, Baltimore, Maryland, USA; †University of Maryland School of Medicine, Baltimore, Maryland, USA

References

Address for correspondence: David Oldach, University of Maryland, School of Medicine, Room 552, Institute of Human Virology, 725 W. Lombard Street, Baltimore, MD 21201, USA; fax: 410-706-1992; email: oldach@umbi.umd.edu

To the Editor: The article by Marr and Calisher (1) concerning the causes of the death of Alexander the Great triggered our curiosity about the possibility of supporting this hypothesis by determining the evolutionary time of West Nile virus (WNV). WNV is a member of the *Culex*-transmitted clade of flavivirus (which also includes Japanese encephalitis virus, St. Louis encephalitis virus, and Murray Valley encephalitis virus) whose reservoir is birds (1). Like most of the RNA viruses, flaviviruses are characterized by a high degree of genomic variability (2,3). Strains of WNV currently are divided into two distinct lineages on a molecular basis: one with a worldwide distribution and the other, which includes the prototypic strain isolated in Uganda in 1937 that is only found in sub-Saharan Africa and Madagascar.

To estimate the time of divergence among the different WNV strains, we conducted a phylogenetic analysis of a number of WNV sequences available in GenBank using a maximum likelihood (ML) method that makes it possible to estimate the branch lengths of a phylogeny with dated isolates under the SRDT (single rate dated tip) model (4). In particular, we retrieved sequences included in the envelope (E) gene of 38 WNV isolates: 18 lineage 1 strains representative of all of the proposed type I subtypes, including one Kunjin virus isolate (5), and 20 lineage 2 strains, including the original 1937 isolate from Uganda (6,7). The date of isolation was available for all of the viruses for which sequences where considered.

The 227-bp sequences were aligned with ClustalW (Thompson 1994), and distance-based unweighted pair group method with arithmetic mean (UPGMA) and ML methods were used to make the analysis. The distance matrix and the ML trees were obtained using the PAUP* program (version 4.0b10, Swafford 2001). The
Kimura’s two-parameters model of nucleotide substitution was used with \( \gamma \)-distributed rates. The substitution model, \( \alpha \) shape, Ti/Tv ratio and base frequencies were estimated using Modeltest version 3.06 (8). The trees were obtained by means of a DR heuristic search and were rooted by using Japanese encephalitis virus as the outgroup. The trees were used to estimate branch lengths in accordance with the single rate dated tips (SRDT) model using the Tipeate program implemented in PAML version 3.13 (9). A likelihood ratio test (LRT) was used to examine the fit of each model to the data.

The high mean divergence between the two lineages (0.891 [SE 0.294] substitutions/site) was a good reason for analyzing them separately. The mean distance between the lineage 2 strains was 8.3 times shorter than that between the lineage 1 strains (0.018 [SE 0.05] sub/site vs. 0.154 [SE 0.036] sub/site). Analysis of the goodness-of-fit of the models showed that the likelihood of the SRDT and DR models was similar for lineage 2 (\(2 \Delta \ln L \approx 0.05 \text{ LRT}\)), whereas DR was significantly better than SRDT for lineage 1 (\(2 \Delta \ln L = 47.08, \text{df} = 15-p < 0.001 \text{ LRT}\)).

The substitution rates estimated with the SRDT model were very similar in the two lineages (1.25 \( \times \) \(10^{-4}\) [\(\pm 7.07 \times 10^{-4}\)] in lineage 1, and 1.20 \( \times \) \(10^{-4}\) [\(\pm 7.03 \times 10^{-4}\)] in lineage 2). On the basis of these substitution rates, the most recent common ancestor (MRCA) for lineage 1 can be dated back 1,159 years ago (95% confidence interval [CI] 1,043–1,274, i.e., between 729 and 961 AD) and the MRCA for lineage 2 back to 208 years ago (95% CI 105–311; i.e., between 1,693 and 1,899 AD) (Figure).

Our calculated substitution rates are very close to those reported for other RNA viruses, including some flaviviruses. A phylogenetic study of the entire E gene of various flaviviruses (3) estimated a rate of \(7.5 \times 10^{-5}\) nonsynonymous nucleotide substitutions/site/year, and the divergence times estimated on this basis showed that the Flavivirus genus is relatively young (<10,000 years). As suggested by the phylogenetic trees, the divergence of the three groups of Flavivirus (mosquito-borne, tick-borne, and no known vector viruses) is the earliest event in their evolution and dates back to no more than 5,000 years ago (2), and the divergence of the Culex-transmitted group (including WNV) and Aedes-transmitted flaviviruses (including dengue and yellow fever viruses) has been placed at approximately 3,200 years ago (3).

One possible limitation of our study is the fact that the goodness-of-fit of the DR model is better than that of the SRDT model for lineage 1. However, on the basis of the results of a simulation study, the estimated substitution rates should still be reliable indicators of the average rate of evolution and can be used to infer the divergence times correctly also in this case (10).

In conclusion, our divergence time estimate suggests that WNV is a relatively young virus and reduces the probability of incidental infections of humans before 1,000 years ago. Encephalitis itself became a frequent complication of WNV fever in 1996 (11), which suggests the recent appearance of more pathogenic viral strains. Although the present spread of WNV lineage 1 may be compatible with its presence in the geographic area of ancient Babylon, the molecular dating of its origin acquires it of any responsibility for Alexander’s death.

Massimo Galli,* Flavia Bernini,* and Gianguglielmo Zhender*

*Istituto di Malattie Infettive e Tropicali, Università degli Studi di Milano, Milan Italy

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In reply: The response by Oldach et al. is wonderfully whimsical (a word that was unfortunately deleted from our manuscript by the EID reviewers). We do not think a witty response is indicated and cannot think of one anyway. We note, however, that both our paper (1) and the Oldach et al. response are examples of the serendipitous pleasures that can be brought to the literature by classical citations, and that all orthodox medical theories on historical causations should be periodically reexamined. Because we are the type who do not mind crawling around in dirty places, we consider computer-based epidemiology for the birds and are willing to eat Corvus brachyrhynchos.

We also thank Cunha for his exhaustive differential diagnosis. We felt we had to address all previously cited diagnoses as well as those not posited in the literature, even though, like Cunha, we did not think most of them were likely causes. We concur that most plant toxins do not induce fever, but some do contain anticholinergic alkaloids that may interfere with perspiration and elevate body temperature. (A most enjoyable recent book discusses a variety of poisons and their widespread use by the Greeks, Romans and Scythians [2]. The book illuminates the widespread use of poisons not only on persons but also as weapons in battle and sieges.) Since thermometers were not available at that time, it remains impossible to document this critical vital sign, but since poisoning was specifically mentioned by Plutarch, we felt we could not ignore this possibility. Who are we to ignore Plutarch?

We also agree that typhoid fever remains high on the list of probable causes, as Oldach eloquently argued 5 years ago (3). Although individual cases of this disease usually occur in a camp setting, one would expect reports of other similar cases (the same for malaria), which was apparently not the case. A singular case of West Nile encephalitis, however, is the rule, not the exception.

Cunha stresses the importance of “acute infectious diseases clinicians” arriving at a procrustean diagnosis. In our diagnosis, we chose to emphasize previously overlooked environmental and public health considerations, such as climatic conditions and the deaths of ravens. As stated earlier, we also had an ulterior motive in our writing: to continue the legacy of others in heuristic discussions of the classics (4). In that sense, we have achieved our goal. Cunha considers the diagnosis of West Nile encephalitis as a “red herring.” We point out that Clupeus harengus was quite bountiful in ancient times (5), and at least some must have been erythematous.

As for the marvelous letter from Galli, Bernini, and Zehender, which minimizes Plutarch’s assertions, we can only say that perspective is everything. That these investigators have gone to such lengths to investigate our diagnosis, we chose to emphasize Plutarch’s assertions, we can only say that perspective is everything. That these investigators have gone to such lengths to investigate our diagnoses must have been erythematous.

As the rule, not the exception. ”best guess” is reward enough for us. That these investigators have gone to such lengths to investigate our diagnoses must have been erythematous.

always prefer a “hot” topic to an important one, we have been successful in promoting intellectual debate. We would be delighted to be proven right or wrong in our thesis, but we are not convinced that Galli et al. are correct in their estimation that West Nile virus did not exist at the time of the death of Alexander the Great. Various phylogenetic studies of flaviviruses (6–8) have discussed the time period when flaviviruses have emerged or diverged, with estimates based on nucleotide substitution rates. However, most groups seem to be retreating from their former definitive positions on this subject because of various technical discrepancies originating from assumptions made regarding the sequence dating methods themselves. Some investigators believe that such dating methods are unreliable for all but the most recent divergence events. At the very least, these methods remain controversial, as does the cause of death of Alexander the Great, who is, after all these years, still causing trouble.

John S. Marr* and Charles H. Calisher†

*Virginia Health Department, Richmond, Virginia, USA; and †Colorado State University, Fort Collins, Colorado, USA

References


Syndromic Surveillance

To the Editor: As public health practitioners directly involved in constructing, maintaining, and interpreting syndromic disease surveillance systems, we offer the following comments on the Buehler et al. article, “Syndromic Surveillance and Bioterrorism-related Epidemics” (1). In general, this article was well-crafted. It reviewed the potential for syndromic surveillance to detect various diseases of bioterrorism, specifically an anthrax event based on the inhalational anthrax cases of 2001. However, the reader may conclude that hospital-based syndromic surveillance is potentially ineffective and unproven.

Buehler et al. describe how, within 18 hours, a presumptive diagnosis of anthrax would prompt a full-scale response. We think that functional syndromic surveillance can respond to the rapid onset of hospital-based disease. To isolate and positively identify Bacillus anthracis from a blood culture would take ~48 hours. Syndromic surveillance should detect a large number of cases within 24 hours. A fully functional hospital syndromic surveillance system that uses automated analysis (such as the daily emergency department–based surveillance with SaTScan in New York City) should identify a substantial increase in a relevant syndrome within 12 to 24 hours after data submission (2). A continued daily rise in any disease category would most certainly set off alarms in a syndromic surveillance network. If active statewide laboratory surveillance is included in syndromic surveillance, such as the gram-positive rod surveillance conducted in Connecticut (3), this surveillance should rapidly detect even single cases of anthrax concurrent with the presumptive diagnosis within the hospital.

The authors also state that syndromic surveillance would not detect outbreaks too small to trigger statistical alarms. The combination of active and passive surveillance in the hospital admissions–based syndromic surveillance in Connecticut allows a number of syndromes to be tracked immediately upon notification; these syndromes include pneumonia and acute respiratory disease in healthcare workers admitted to a hospital, all disease clusters, and fever with rash illness. This system is very flexible, and active surveillance of other syndromes can be quickly instituted as required. This active surveillance component has been proven useful. The first 2 of Connecticut’s 17 confirmed human cases of West Nile virus during 2002 were discovered in August when a health director, who regularly monitored the syndromic admissions data for the hospital in his municipality, requested immediate West Nile virus testing from the hospital’s infection-control department when he received two late summer reports of neuritic illness.

Buehler et al. state that specificity for distinguishing bioterrorism-related epidemics from ordinary illness may be low because the early symptoms of bioterrorism-related illness overlap with those of many common infections. Illness specificity can be modulated within a syndromic surveillance system by making changes in the definition of the information requested, the method of analysis used, or by incorporating varying amounts of active surveillance into a passive reporting system. In Connecticut, annual rates of hospital admissions for pneumonia and respiratory illness have significantly increased (>3 standard deviations) during winter months. These increases have corresponded temporally with peaks in laboratory-confirmed influenza reports and in our state-based and the national sentinel physician influenzalike illness reports. Similarly, in the military-based syndromic surveillance system, respiratory outbreaks are detected by monitoring routine outpatient visits and pharmacy prescriptions. Absolute numbers of visits, as well as percentage of visits, to primary care clinics for influenzalike illness provide up-to-date information on respiratory disease conditions at military installations in both active-duty personnel and family members.

Connecticut has added additional active surveillance categories to its syndromic surveillance for potential SARS cases by gathering extensive data on all healthcare providers hospitalized with respiratory illness. In the absence of an identified pathogen, the entire United States was conducting syndromic surveillance for SARS during the spring of 2003.

What are existing alternatives to rapid, patient-based reporting through syndromic surveillance for bioterrorism and emerging illness? Will individual physicians (i.e., the “astute clinicians”) truly recognize an increase of nonspecific symptoms among their patients in time to warn public health authorities of an impending bioterrorism event? During the past 4 years in the U.S. military population, unless


Address for correspondence: John S. Marr, Office of Epidemiology, Virginia Health Department, Room 123, 1500 East Main Street, Richmond, Virginia 23218; fax: 804-786-1076; email: john.marr@vdh.virginia.gov
In reply: The thoughtful letter of Drs. Dembek, Cochrane, and Pavlin draws attention to several key themes emerging in the ongoing dialogue about the utility and role of syndromic surveillance. First, as illustrated by their work, the growing body of experience in conducting syndromic surveillance should advance this dialogue beyond the hypothetical framework described in our manuscript to a more evidence-based assessment of epidemic detection. Second, in the absence of a bioterrorism-related illness since 2001, the utility of syndromic surveillance for detecting naturally occurring events is coming into greater focus, particularly for detecting the onset of anticipated seasonal upswings in infectious diseases, including West Nile virus disease, gastrointestinal illness, and influenza.

Syndromic surveillance coupled with follow-up investigations can assist clinicians by alerting them to communitywide problems likely to be manifest among their patients. This recognition may occur at the hospital level, as reported by Dembek et al. in the initial recognition of West Nile virus disease in Connecticut in 2002, or at the community level, as illustrated by public health alerts in New York City to notify clinicians about viral gastrointestinal illness (1). Multiple studies have documented that newer syndromic surveillance systems can recognize the onset of the annual influenza season (2), but it is not clear what these systems add to existing syndrome-based systems that track “influenzalike illness” as part of a larger array of influenza-specific surveillance methods. While Dembek et al. note that syndromic surveillance has detected multiple outbreaks that would have been otherwise unrecognized, Sichel et al. observed that syndromic surveillance did not detect outbreaks recognized through more traditional means (1). This discrepancy emphasizes the need to further assess the characteristics of epidemics and surveillance systems that favor detection by using syndromic methods.

We recommend distinguishing between the increasing practice, prompted by concerns about bioterrorism, of syndromic surveillance for epidemic detection and the longstanding and common practice of using syndrome-based case definitions in public health surveillance. Such case definitions have been used in situations in which a wide net is cast to identify potential cases of a particular disease (e.g., acute flaccid paralysis as part of global efforts to eradicate poliomyelitis [3]), liver disease associated with a new therapy for latent tuberculosis infection [4], and inhalational anthrax in New Jersey in 2001, after bioterrorism-related cases were clinically detected [5]), when resource and infrastructure constraints do not allow routine use of laboratory-based definitions (e.g., surveillance for sexually transmitted diseases in infrastructure-weak countries [6]), and when surveillance is initiated for a new disease of unknown origin (e.g., toxic shock syndrome [7], AIDS [8], and severe acute respiratory syndrome [SARS] [9]). Although SARS surveillance did not represent syndromic surveillance according to this distinction, relationships between health departments and hospitals, fostered in establishing syndromic surveillance, likely facilitated SARS surveillance.

New guidelines offer an approach for evaluating syndromic surveillance systems, including what is learned from follow-up of statistical alarms and whether syndromic surveillance or other methods lead to the earliest detection of outbreaks (10). These guidelines also provide a framework for modeling exercises to test syndromic surveillance under various bioterrorism scenarios, supplementing experience gained from real-life, but typically less severe, seasonal illness or community epidemics. Eventually, this information should be...
useful in developing guidance for health departments seeking to determine whether and how to implement syndromic surveillance.

James W. Buehler,*
Ruth L. Berkelman, *
and Clarence J. Peters‡

*Emory University, Atlanta, Georgia, USA;†University of Maryland School of Medicine, Baltimore, Maryland, USA; and‡University of Texas Medical Branch, Galveston, Texas, USA

References:


Address for correspondence: James W. Buehler, Rollins School of Public Health, Rm. 416, Emory University, 1518 Clifton Rd., NE, Atlanta, GA 30322, USA; fax: 404-712-8345; email: jnbuehle@sph.emory.edu

Staphylococcus aureus and Escherichia hermanii in Diabetes Patient

To the Editor: Polymicrobial invasive infections are infrequent, representing <10% of the invasive infections of known etiology (1). They are often correlated with a predisposing factor: immunodeficiency (e.g., diabetes mellitus, malignancies, extremes of age) or use of a central catheter. Escherichia hermanii is an extremely rare etiologic agent for invasive infections; only four cases were published from 1980 to 2002. We report the first case of double invasive infection by E. hermanii and Staphylococcus aureus and emphasize the importance of screening of all the septic foci for demonstrating a polymicrobial invasive infection.

In August 2000, a 54-year-old comatose man was admitted to our infectious diseases department with a 10-day history of fever. He had a medical history of vertebral arthropathy (lumbar laminectomy in 1989) and insulin-dependent diabetes mellitus. Six weeks before, he had received for 3 days gluteal injections with ketobu-sone (an intramuscular nonsteriodal antiinflammatory drug [NSAID]) for acute lower back pain. Twenty-eight days after the first injection, a gluteal abscess developed, which was surgically drained, without perioperative antimicrobial therapy. Three days later, he became febrile, and pyrexia persisted despite local wound management and treatment with oxacillin, 4 g/day for 3 days; cefuroxime, 3 g/day; and gentamicin, 160 mg/day for another 7 days.

The patient became comatose and was transferred to our department. At that time, the physical examination showed fever (40.2°C), neck stiffness, Brudzinski sign, thoracic dullness, and bilateral crackling rales. The level of C-reactive protein was 123 mg/L. Renal failure was noted with a creatinine blood level of 312 mmol/L and uncontrolled diabetes with fasting glucose of 24.75 mmol/L. Computed tomographic (CT) scan of the brain did not show brain abscesses or tumors. Examination of the cerebrospinal fluid (CSF) indicated a protein level of 2.67 g/L, decreased glucose concentration of 0.55 mmol/L, and a leukocyte count of 2.3 x 10^9/L with 96% neutrophils; no microbial pathogens were demonstrable under direct examination of CSF. Chest x-ray identified bronchopneumonia and bilateral pleural effusion. The pleural fluid analysis revealed a purulent exudate—protein, 4.5 g/L—containing 55% neutrophils. A urine specimen and three blood samples were obtained for cultures over the first 4 hours after admission. A bacterial invasive infection was considered and the antibiotic therapy was started with ceftriaxone, 2 g/day, and rifampin, 1,200 mg/day. Concomitantly, the patient received colloids to reestablish blood volume, intravenous dexamethasone, 6 mg four times daily, to diminish the cerebral edema; and fast-
acting insulin to control hyperglycemia.

On day 3, the urine and CSF cultures were positive for *E. hermanii*, and the pleural fluid and all three blood cultures yielded methicillin-susceptible *S. aureus*. The *E. hermanii* strain produced a yellow pigment. The drug susceptibility was assessed by AtB Expression system (BioMerieux, Marcy l’Etoile, France). The *Staphylococcus* strain was susceptible to oxacillin, cotrimoxazole, tetracycline, and ciprofloxacin and resistant only to penicillin. *E. hermanii* is naturally resistant to aminopenicillins and carbencillin; this strain was susceptible to third-generation cephalosporins, carbapenems, cotrimoxazole, and quinolones and resistant to aminoglycosides.

The patient’s clinical central nervous system status improved, and he came out of the coma, but his temperature remained >37.5°C. He started to report lumbar pain. On day 5, the antimicrobial regimen was switched to meropenem. After 24 hours, the patient became apyretic, and glucose and creatinine levels were normal on day 8. However, on day 10, fever, inflammation of the right thumb, and intensified lower back pain developed. The abdominal CT and bone scintigraphy indicated abscess of the psoas, L4-L5 spondylitis, and thumb periostitis. Intravenous ciprofloxacin was added, 400 mg twice daily, and apyrexia occurred on day 14. On day 21, open surgery was performed, consisting of drainage of the psoas abscess and curettage of the L4-L5 disc. On day 30, clinical improvement and C-reactive protein level of 4.2 mg/L, led to a change to oral antimicrobial agents: cotrimoxazole 2 g/day and ciprofloxacin 1.5 g/day. This regimen was continued for 2 months while the patient was seen as an outpatient. The patient remained afebrile and inflammation-free for the entire 24-month followup period.

Polymicrobial invasive infections represent a major therapeutic problem. However, they are infrequent: only 3.2% of infectious endocarditis (2) cases and 6.27% of 2,188 community-acquired bacteremia cases (3) were polymicrobial. Polymicrobial infections and elevated bacteremia levels are more frequently associated with diabetes; 20%–35% of the skin and soft tissue infections in persons with diabetes are polymicrobial (4), and 15.7%–20% of the community-acquired bacteremia cases were registered in persons with diabetes (3,4). The probable entry site for *S. aureus* was cutaneous. Intramuscular NSAIDs are known to cause aseptic necrosis, predisposing the patient to staphylococcal abscesses. Hyperglycemia itself is a risk factor for soft-tissue infections. The role of perioperative antimicrobial therapy in preventing the dissemination of infection from a surgically drained abscess is controversial (5). *E. hermanii* usually produces wound or gastrointestinal tract infections; in our patient, *E. hermanii* probably originated from the skin or from the gastrointestinal tract (6–9). *E. hermanii* could be involved more frequently in polymicrobial invasive infections; of *E. hermanii* invasive infections noted in four published reports, two were polymicrobial (6,7).

Initial antimicrobial drug therapy was established empirically for probable staphylococcal meningitis. The ongoing fever and persistent metabolic disturbances led to an escalation of therapy. Some authors recommend carbapenems as the initial regimen against invasive methicillin-susceptible *S. aureus* infections with meningeal or bone involvement (10). The patient’s lower back and the invasive staphylococcal infection urged us to consider septic bone involvement. The imaging studies confirmed the existence of vertebral osteomyelitis; ciprofloxacin was used based on its excellent bone diffusion and its in vitro activity on the two isolated strains. Surgical intervention eradicated one of the septic foci and decreased risk for spinal cord injury; 27 (47%) of the 58 patients with spondylodiscitis who were treated surgically had a better outcome than the other patients with medical care only (11). Control of the infection allowed changing to an oral regimen after 1 month. We selected cotrimoxazole and ciprofloxacin for their in vitro effectiveness against the two pathogens. Although this agent is not usually used to treat bone infection, we used cotrimoxazole on the basis of evidence provided by several communications that indicated a superior efficiency to referential regimens (12).

In conclusion, the identification of all organisms involved in polymicrobial invasive infections may require cultures of specimens from all accessible septic foci. For *E. hermanii*, a role of “associated” pathogen in a polymicrobial invasive infection could be considered. Medical therapy alone could be insufficient, and the combined therapy allowed for a successful outcome in invasive infection with lumbar spondylodiscitis.

Gabriel Adrian Popescu,*‡ Ioana Daha,†‡ Cristina Popescu,*‡ and Elena Mitache*

*Matei Bals Infectious Diseases Institute, Bucharest, Romania; †Colentina Hospital, Bucharest, Romania; and ‡Carol Davila University of Medicine, Bucharest, Romania

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Multiple rpoB Mutants of Mycobacterium tuberculosis and Second-order Selection

To the Editor: Rad and colleagues recently described variation in some genes involved in DNA repair (mutT2, mutT4, ogt) in Mycobacterium tuberculosis strains of different genotypes (1). This approach can also be used to investigate developing rifampin resistance in the context of emerging mutator alleles. Resistance to rifampin in M. tuberculosis strains is usually caused by the point mutations in the rpoB gene encoding the β-subunit of the DNA-dependent RNA polymerase, which is a target of the drug. Although a single point mutation is sufficient for developing rifampin resistance, a number of articles (2,3) describe multiple rpoB mutants for M. tuberculosis, i.e., rifampin-resistant strains harboring mutations in different codons of rpoB. Double, triple, and quadruple mutations in M. tuberculosis clinical isolates were reported in studies conducted throughout the world (2,3). Such emergence, albeit infrequent, of M. tuberculosis rpoB multiple mutants raises questions about their biologic importance and underlying mechanisms; answers to both remain elusive.

I propose an explanation of these observations in terms of second-order selection of hypermutable (mutator) alleles based on alterations in DNA repair genes. Unlike that of other antibacterial drugs, resistance to rifampin is acquired in most M. tuberculosis isolates by altering a single target molecule and offers the most appropriate and straightforward model to demonstrate possible hypermutability in this species. In mycobacteria, hypermutability was demonstrated in vitro for M. smegmatis, a surrogate model for M. tuberculosis, as an increase in reversion (mutant to wild-type) rate in rpoB526 or rpsL43 under counterselection by streptomycin or rifampin, respectively (4). A correlation between high mutation rate and antimicrobial resistance was reported for Pseudomonas aeruginosa isolates from lungs of cystic fibrosis patients (5). The mutator P. aeruginosa strains resulted from a defective mismatch-repair system (5). In M. tuberculosis, mismatch-repair genes (mutH, mutL, mutS, and recJ) were not found in its genome (6). However, the nucleotide pool in this species is exceptionally clean because of the presence of several copies of the mutT gene (1,6); the MutT protein removes oxidized guanines (8-Oxo-dGTP), thus counteracting replication or transcription errors. Consequently, the MutHLS mismatch-repair system simply may not be required in M. tuberculosis (6). Therefore, hypermutability in some strains of this species resulting in multiple rpoB mutants might develop under certain special (in vivo) circumstances through inactivation or down-regulation of some mutT genes. Further, the two most frequently described rpoB mutations are 531TCG→TTG and 526CAC→TAC. Both are cytosine-to-tyrme transitions, which easily occur by spontaneous cytosine deamination to uracil. Indeed, M. tuberculosis is a G+C rich organism, therefore, it is naturally at high risk for cytosine deamination. Furthermore, pathogenic mycobacteria are at increased risk for deamination because of the production of reactive oxygen and nitrogen intermediates inside host macrophages. This deamination process is normally counteracted by uracil-N-glycosylase, the product of the ung gene, and organisms defective in the removal of uracil from DNA have an increased spontaneous mutation rate and more G:C→A:T base-pair transitions (7).
Merchant et al., by using ung+ and ung– Escherichia coli strains, demonstrated that total nitric oxide exposures in the μmol/L range can lead to C→T mutations by a mechanism probably involving cytosine deamination (8). On the other hand, in M. smegmatis, the abrogation of the Ung activity leads not only to increased mutator phenotype but also to growth inhibition by reactive nitrogen intermediates (7). In summary, I speculate that mutations in ung that do not completely impair function, but do decrease synthesis of its product, might tolerably increase the spontaneous C→T mutations, including those in the respective positions in the rpoB codons 531 and 526. This assumption seems likely because both of the aforementioned particular mutations were described in spontaneous mutants of H37Rv obtained in vitro and had a Darwinian fitness slightly less than or equal to that of the rpoB wild-type-susceptible parental strain (9). In contrast, the translesion synthesis-based pathways appear less likely to contribute to emergence of such mutants, although at least one of the translesion synthesis genes (dinP) is present in the genome of M. tuberculosis. In the E. coli in vitro model, a translesion synthesis enzyme (dinB encoded DNA polymerase IV) activity clearly promoted more important frameshift mutations (single-base deletions) in two thirds of the spontaneous mutants (10).

From an evolutionary point of view, the multiple rpoB mutations in M. tuberculosis have been hypothesized to arise as a compensatory mechanism to ameliorate the fitness costs of the original resistance mutation by a secondary mutation (11). The process of adaptation to the fitness costs of chromosomally encoded resistance has been studied in E. coli and Salmonella enterica serovar Typhi for mutations that affect translation in the rpsL and fusR genes (11) and for rpoB mutations in E. coli K12 strain (11). In the last instance, the rpoB multiple mutants were selected in vitro in a stepwise fashion, and one double mutant, L511Q+D516G (also described in M. tuberculosis strain [3]), exhibited a relative fitness either greater than or equal to either single mutant or the wild type. Reynolds (11) suggested that this allele is favored not merely as a combination of two low-level resistance mutations but also because these mutations together boost resistance and preserve fitness. Whether the same is true for other multiple mutant alleles in M. tuberculosis rpoB remains to be seen. Studying the costs of resistance of multiple rpoB mutations in a more realistic environment of animal models of TB infection seems promising.

Igor Mokrousov*
*St. Petersburg Pasteur Institute, St. Petersburg, Russia

References


Address for correspondence: Igor Mokrousov, Laboratory of Molecular Microbiology, St. Petersburg Pasteur Institute, 14, Mira Street, St. Petersburg, 197101, Russia; fax: +7 812 232 92 17; email:imokrousov@mail.ru

Human Metapneumovirus and Chronic Obstructive Pulmonary Disease

To the Editor: We read with interest an article, Human Metapneumovirus Detection in Patients with Severe Acute Respiratory Syndrome, in your journal (1). In the report, Chan et al., did not question that SARS-CoV is the etiologic agent of severe acute respiratory syndrome (SARS); however, human metapneumovirus (HMPV) was found in 25 (52%) of 48 probable SARS cases that were investigated, and SARS-CoV was detected in 11 (22.9%) of them. Another recent article reported HMPV in five of six patients in whom SARS was diagnosed in Canada (2); four of the six were coinfected with
SARS-CoV. The prevalence of HMPV infection in SARS patients validates the interest in HMPV’s possible role in SARS etiology.

From November 2001 to February 2002, 1 year before the first cases of SARS appeared, we tested the sputum of patients >64 years of age who had experienced exacerbation of chronic obstructive pulmonary disease, for HMPV. Investigations were conducted on 90 episodes in 89 elderly patients, 62 males and 27 females, in which we found no other microorganisms that could have been related to the exacerbation of chronic obstructive pulmonary disease. RNA was extracted from the sputum samples and amplified by reverse transcriptase–polymerase chain reaction (RT-PCR) to detect HMPV as previously described (3). Results of bacterial culture and culture and PCR to detect respiratory syncytial virus and influenza virus types A and B were negative, whereas HMPV was found in the sputum of five (three men and two women) immunocompetent patients, 77–87 years of age. The prevalence of HMPV infection was 5.5%, similar to the percentage obtained by Chan et al., when HMPV RT-PCR was conducted on the respiratory samples. Fever (temperature >38°C) was not present in any of the five patients infected with HMPV. Two patients were admitted to a hospital. Both patients had bronchial infection and cough with bronchospasm and moderate respiratory insufficiency (oxygen saturation rate: 90.3% and 88%, respectively) for >1 week. Sputum samples from an additional 70 elderly patients with exacerbation of chronic obstructive pulmonary disease with positive detection for influenza virus (n = 50) or respiratory syncytial virus (n = 20) were tested for HMPV infection. None of the samples showed HMPV infection.

Sequence analysis of amplicons from the five samples positive for HMPV infection showed >95% similarity with HMPV sequences found in other parts of the world (4,5). Additional studies should be conducted to confirm that HMPV exacerbates chronic obstructive pulmonary disease. However, by performing an RT-PCR directly on the sample instead of the more efficient RT-PCR after viral culture used by Chan et al., these findings suggest that HMPV is a frequently undetected agent in acute respiratory infection unrelated to SARS. The important questions are whether HMPV and SARS-CoV coinfection would facilitate more severe SARS, or whether HMPV infection would facilitate a more efficient transmission of SARS-CoV.

Diego Vicente,* Milagrosa Montes,* Gustavo Cilla,* and Emilio Pérez-Trallero†

*Hospital Donostia, San Sebastián, Spain; †Basque Country University, San Sebastián, Spain

References

Address for correspondence: Diego Vicente, Servicio de Microbiología, Hospital Donostia, Paseo Dr. Beguiristain s/n 20014 San Sebastián (Gipuzkoa), Spain; fax: +34-943-00-70-63; email: ludvirol@chdo.osakidetza.net

Integrons in Salmonella Keurmassar, Senegal

To the Editor: Infections caused by Salmonella are the primary cause of foodborne diseases; multidrug resistance to Salmonella enterica subsp. enterica is increasing. The selective pressure created by the widespread use of antimicrobial agents in animals and humans as prophylactic and therapeutic agents may have contributed to the dissemination of resistant bacterial strains. In 2000, the new serovar Keurmassar (35:c:1,2) of S. enterica, was described in Senegal (1). Integrons are efficient gene-capture systems by site-specific recombination and are involved in antimicrobial-drug resistance in gram-negative bacteria (2). Three classes of integrons are well characterized and are involved in antimicrobial resistance. Integrons have been found in different nontyphoidal serovars of S. enterica and recently in serovar Typhi (3).

We evaluated the contribution of integrons to the antimicrobial drug resistance of eight isolates of S. enterica serovar Keurmassar sent to the Senegalese National Salmonella and Shigella Reference Laboratory at the Pasteur Institute in Dakar from March to May 2000. One strain was isolated from poultry flesh, and seven strains were isolated from human stool or blood samples. Susceptibility testing was performed by disk diffusion method on Mueller-Hinton agar according to the Comité de l’antibio-gramme, Société Française de Microbiologie, recommendations. The eight strains expressed an extended-spectrum β-lactamase, which was previously identified as SHV-12 (1). The strains were also resistant to aminoglycosides (amikacin, gentamicin, netilmicin, spectinomycin, streptomycin, and
tobramycin), chloramphenicol, sulfamethoxazole, tetracycline, and trimethoprim. Genomic diversity was studied by pulsed-field gel electrophoresis (PFGE) and analysis of XbaI restriction fragments as described previously (3). The eight strains isolated from poultry and humans specimens showed identical PFGE patterns, which suggested that all strains of *S. enterica* serovar Keurmassar isolated until May 2000 in Senegal belonged to the same clone.

Strains were screened for the integrons by polymerase chain reaction by using three sets of primers specific for the *intI1, intI2,* and *intI3* genes coding for the integrase as described previously (3). The *intI1* gene was detected in all strains. Class 2 or 3 integrons were not detected. Cassette assortment in class 1 integrons was determined by using the primers 5′CS and 3′CS complementary to the 5′ and 3′ segments as described previously (3). With these primers, we obtained two amplification products of 1 kb and 1.7 kb for each strain, which suggested that all strains contained at least two class 1 integrons. Sequencing of these amplification products showed that the first product of 1 kb contained the *aadA2* cassette, which confers resistance to streptomycin and spectinomycin. The second amplicon of 1.7 kb carried a new arrangement of two cassettes: *aac(6′)-Ile*, which confers resistance to gentamicin, netilmicin, and tobramycin; and *ereA2*, which encodes resistance to erythromycin. Class 1 integrons were previously found in strains of *S. enterica* of different serovars: Agona, Albany, Brandenburg, Enteritidis, Goldcoast, Hadar, Infantis, Ohio, Panama, Poona, Saintpaul, Typhi, Typhimurium, Virchow, and Worthington (3–7). All these integrons, except that of serovar Infantis, contained a streptomycin-spectinomycin resistance determinant, *aadA2* or mostly *aadA1*, alone or in combination with other gene cassettes. The cassette *aac(6′)-Ile* was previously described in a single class 1 integron in *Pseudomonas aeruginosa* (AF162771). The *ereA2* cassette was first described in *Providencia stuartii* (8) in a class 1 integron. This cassette was since described in class 1 integrons of clinical gram-negative isolates and recently in a class 2 integron in *Escherichia coli* (9). To determine whether the resistance determinants carried by the integrons were transferable, we performed a conjugation experiment from *S. enterica* serovar Keurmassar to an *E. coli* strain resistant to nalidixic acid. We first used a selective medium containing nalidixic acid 50 µg/mL plus 25 µg/mL of streptomycin, one of the two integrons carrying the *aadA2* cassette. All antimicrobial drug resistances were transferred at once from each strain to *E. coli*. The analysis of plasmid content prepared by alkaline lysis method from all transconjugants showed a single plasmid of >30 kb. The polymerase chain reaction analysis of the plasmid DNA confirmed the transfer of the two integrons, which suggested that the integrons were borne by a conjugative plasmid.

The multidrug resistance of these strains could be explained by the fact that antimicrobial agents are used extensively in the poultry industry in Senegal to reduce deaths and to increase productivity (1). Moreover, in Senegal, as in many countries in Africa, antimicrobial agents are sold over the counter, which leads to self-medication, thus increasing the selective pressure.

This is the first finding of integrons in the newly discovered serovar Keurmassar of *S. enterica*. One integron contained two cassettes, *aac(6′)-Ile* and *ereA2*. This was also the first finding of such an integron with a new arrangement of these two cassettes in a clonal strain of *S. enterica* serovar Keurmassar that had recently emerged. Indeed, the *aac(6′)-Ile* cassette was described only once in *P. aeruginosa*. Moreover, aminoglycosides are not used extensively in Africa because they are very expensive. Therefore, determining how this cassette combination was selected is difficult. The strains studied were resistant to multiple antimicrobial agents, including broad-spectrum cephalosporins by production of the extended-spectrum β-lactamase SHV-12. The two class 1 integrons described here could account for the resistance to only a few drugs. The *blaSHV-12* gene was not carried by an integron. Otherwise, ampicillin, trimethoprim, and tetracycline are the antimicrobial agents commonly used to treat diarrheal diseases in Africa. All of the strains studied were resistant to these antimicrobials agents. Trimethoprim resistance *dfr* genes are frequently found in integrons (2). However, in this study, we were not able to detect integrons containing *dfr* cassettes.

The presence of a conjugative plasmid and integrons in this serovar is of clinical importance. Indeed, the spread of the multiple antimicrobial agent resistance to other *Salmonella* serovars or gram-negative bacteria might easily occur by the transfer of such a plasmid. Moreover, integrons could allow the acquisition of new genes.

Amy Gassama-Sow,*† Awa Aidara-Kane,† Nabil Raked,* François Denis,* and Marie-Cécile Ploy*

*Laboratoire de Bactériologie-Virologie-Hygienne, Limoges, France; and †Institut Pasteur, Dakar, Sénégal

References


Address for correspondence: Marie-Cécile Ploy, Laboratoire de Bactériologie-Virologie-Hygienè CHU Dupuytren, 2, avenue Martin Luther King, 87042 Limoges Cedex; fax: 33-5-55-05-67-22; email: marie-cecile.ploy@unilim.fr

Hepatitis B Infection, Eastern India

To the Editor: The National Institute of Cholera and Enteric Diseases (ICMR), Kolkata, India, conducted a serologic study in July 2003 to determine the rate of hepatitis B virus (HBV) infection of brothel-based commercial sex workers. These study participants worked in the South-24 Parganas district of West Bengal, one of the eastern states of India. Routine immunization to prevent HBV infection is not a practice in India, and chronic HBV infection is endemic (1). The nature of their work makes commercial sex workers more vulnerable to HBV infection, which could accelerate the infection’s spread into the general community, particularly in areas with low literacy rates and socioeconomic status.

The study participants were 167 commercial sex workers from three prominent brothels, which were located in small towns and along the national highway of the district. Blood samples from the participants were tested by using the HBsAg unlinked anonymous method. The results showed that 23.3% (unpub. data, National Institute of Cholera and Enteric Diseases) of the commercial sex workers were infected with HBV. The Figure shows the distribution of commercial sex workers and prevalence of HBV infection by age group.

To ascertain a possible route of transmission other than sexual activity, all study participants were questioned about their medical history, including jaundice, injury requiring blood transfusion, surgical events, and tattooing. The evidence indicated that HBV infection in the study participants was not acquired by any route other than sexual activity. Since the carrier rate of chronic HBV in the general population is approximately 5% in eastern India (2), this increase in chronic HBV infection can be attributed to sexual transmission.

The Figure indicates that HBV infection is not correlated to age or duration of commercial sex work; the rate of infection is distributed almost equally in all age groups except the 26- to 30-year-old group in which it was slightly lower. Most of the HBV-infected commercial sex workers likely were infected early in their profession and remained infected throughout their lives, which led to higher rate of chronic infection as compared with the general population.

This higher rate of chronic HBV infection among commercial sex workers (23.3%) is of concern, particularly in a country with an estimated 4.58 million persons infected with HIV. In India, >80% of HIV infection is transmitted sexually (3). HIV infection can affect the natural course of HBV; sexual activity between HIV- and HBV-infected persons could prolong the infected status of those infected with HBV, as was shown in a previous study (4). This sexual activity could facilitate HBV transmission, particularly in areas that have few resources or where the rate of condom use is low or questionable.

After this study concluded, all study participants were notified of their infection status and advised to use condoms when engaging in sexual activity. The commercial sex work-
letters and their family members were advised that they should be tested for HBV infection and receive HBV vaccination if test results were negative. Local health authorities were advised that commercial sex workers and their clients should be vaccinated to prevent HBV infection.

Kamalesh Sarkar,* Dwijendra N. Ganguly,† Baisali Bal,* Malay K. Saha,* and Sujit K. Bhattacharya*

*National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, India; and†Kolkata National Medical College, Kolkata, West Bengal, India

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Address for correspondence: Kamalesh Sarkar, P-33 CIT Road Scheme XM, Beliaghata, Kolkata-10, India; fax: 91-33-2350-5066; email: Kamal412496@yahoo.com

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**Ehrlichia Prevalence in Amblyomma americanum, Central Texas**

To the Editor: Ehrlichia chaffeensis and E. ewingii, agents of human monocytic ehrlichiosis and ehrlichiosis ewingii, respectively, are transmitted by the lone star tick, *Amblyomma americanum*, which is found from west-central Texas northward to Iowa, and southeastward to the Atlantic Coast (1). In *A. americanum*, *E. chaffeensis* has been found in several states, while *E. ewingii* has only been found in North Carolina, Florida, and Missouri (1,2). *E. ewingii* infection in white-tailed deer (*Odocoileus virginianus*), a potential reservoir, has been found in the states mentioned previously as well as in Kentucky, Georgia, and South Carolina (3,4).

Human ehrlichioses are underdiagnosed in the United States and may be as prevalent as Rocky Mountain spotted fever in some areas (1). Ehrlichioses are prevalent in Texas, and fatal cases have been reported (1,5). This study was conducted to examine ticks from central Texas for *Ehrlichia* and provide information to increase public health awareness of this problem. Adult *A. americanum* ticks were collected from a 38.8-hectare game fenced-pasture (Plot #8) in the Kerr Wildlife Management Area, Kerr County, Texas. Ticks were trapped by using blocks (approximately 85 g) of dry ice centered on smooth, white, nylon cloths measuring approximately 1 m². These traps were placed on the ground in the brush or in areas under tree canopies for approximately 1 h.

Trapped adult *A. americanum* were frozen in liquid nitrogen and then bisected with a sterile scalpel. Halves of the bisected ticks were stored at –80°C. The other halves were pooled in groups of six. DNA was extracted from these pools by using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA), and evaluated by using a nested species-specific 16S rRNA gene polymerase chain reaction (PCR) for *E. chaffeensis* and *E. ewingii*, with *E. canis* as a negative control. The first-round primers were genus-specific for *Ehrlichia* (ECC and ECB). The forward primers of the nested PCR were HE1, EE72, and Ecan, which were specific to *E. chaffeensis*, *E. ewingii*, and *E. canis*, respectively. The reverse primer is a common primer (HE3) for all species (6–8). An aliquot of the negative control reaction containing no DNA template was carried through both rounds of the nested PCR with every reaction set. A dilution series of stock *E. chaffeensis* DNA mixed with tick DNA showed no substantial inhibition of the PCR, even with DNA concentrations as low as 0.2 ng/mL.

Tick pools positive for *E. chaffeensis* or *E. ewingii* by PCR were examined by using DNA from the individual tick halves. DNA was extracted by using the Nucleobond DNA/RNA Isolation Kit (BD Biosciences Clontech, Palo Alto, CA). To confirm positive PCR results for individual ticks, first-round amplicons (primers ECB and ECC) were separated by electrophoresis. The 478-bp band was recovered using the QIAquick Gel Extraction Kit, then cloned into the pCR2.1-TOPO vector with the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). DNA sequences were obtained from both directions of the insert in the recombinant plasmids by using PE Applied Biosystems (Foster City, CA) 373XL automated DNA sequencers in the UTMB Sequencing Core.

Of the 66 adult *A. americanum* ticks examined, 5 were positive for *E. ewingii* (7.6%). The 16S rRNA gene sequences from these five positive samples were most similar to the *E. ewingii* 16S rRNA gene sequence (GenBank accession no.U96436). Sequence variations are summarized in the Table. These mutations may result from polymerase errors prior to cloning. *E. ewingii* has never been cultured or handled by our laboratory, and all negative controls for the nested PCR were negative, minimizing the possibility of false-positive results.

This is the first report of ticks infected with *E. ewingii* in states other than North Carolina, Florida, or Missouri. Ticks are found in damp...
wooded areas (1,9). Seasonal population changes have been associated with climatic factors, including precipitation, temperature, and day length (9–11). These ticks were collected during August, one of the hottest months of the year in Texas, with temperatures averaging 33°C. Adult ticks are more abundant earlier in the summer, and the actual prevalence of E. ewingii infection may be higher. August is a dry month in Texas, averaging 2.32 inches of an annual rainfall of 26 to 30 inches (12). Although cases of E. ewingii infection have not been reported from Texas, this study shows the presence of ticks infected with E. ewingii in Texas.

No ticks infected with E. chaffeensis were found in this sample. The prevalence of E. chaffeensis may be so low that it was not detected in the small sample size. Also, E. chaffeensis may not survive well at this extreme of the host range. Infection exclusion may occur in the tick or reservoir hosts (or both), such that an established population of one ehrlichial species prevents another ehrlichial species from establishing itself. This phenomenon has been noted in the related rickettsial organisms Rickettsia peacockii and R. rickettsii in the Rocky Mountain wood tick, Dermacentor andersoni (13).

Another finding involves using nested 16S rRNA PCR to identify ehrlichial infection. These primers are not as specific as thought previously. Arthropods should be carefully cleaned to prevent contamination by Shigella and other soil contaminants. A single positive-nested PCR reaction should not be considered sufficient for positive identification of the organism. Sequencing of the outer PCR product, or another confirming method, should be used to positively identify the organism. Primers directed to more divergent sequences, such as the dsb gene, should be utilized in place of, or in addition to, 16S rRNA gene PCR (14).

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Scott Wesley Long,* J. Mathews Pound,† and Xue-jie Yu*

*University of Texas Medical Branch, Galveston, Texas, USA; and †United States Department of Agriculture – Agricultural Research Service, Kerrville, Texas, USA

References

Address for correspondence: S. Wesley Long, Department of Pathology, The University of Texas Medical Branch, 301 University Boulevard, Galveston, TX, USA; fax: 409-747-2415; email: swlong@utmb.edu
Echinococcus multilocularis in Northern Hungary

To the Editor: Echinococcus multilocularis infection is one of the most dangerous zoonoses in the Northern Hemisphere and causes more human death than rabies in Europe. Recent data indicate that E. multilocularis infection is spreading geographically and is being transmitted at an increasing rate in Europe (Figure). Since 1995, the parasite has been found in Poland, the Czech Republic, the Slovak Republic, Belarus, Hungary, and Romania; infections in humans have been increasing in frequency in central eastern Europe since the late 1990s (1–4). Since the 1990s, similar infection trends in foxes and humans have been observed in central western European countries, including eastern Austria, northern Germany, Denmark, the Netherlands, Luxembourg, and Belgium (1,2,5). Despite the increasing prevalence of E. multilocularis infection in foxes, the number of human cases did not vary in the historically known area (eastern France, Switzerland, southern Germany, and western Austria) during the 1990s (1), probably because of increased public awareness and control measures. In our previous study (2), the parasite was detected only in foxes in the Hungarian-Slovak border area in northeastern Hungary. In the current study, we found the parasite distributed along the watershed area of the River Danube in northern Hungary.

In the second half of 2003, carcasses of red foxes were sent to the Central Veterinary Institute in Budapest for examination in connection with the rabies immunization and control program in seven counties (Vas, Gyor-Sopron, Komárom-Esztergom, Pest, Nógrád, Heves, and Borsod-Abáuj-Zemplén) that bordered or were near the border of Austria and the Slovak Republic. These foxes were included in the current study. Methods of transporting and storing the carcasses, examining the intestinal tracts, and identifying parasites have been described previously (2).

Of 150 foxes examined, 19 animals from four counties (Gyor-Sopron, Komárom-Esztergom, Pest, and Nógrád) harbored 2, 3, 4, 6, 7, 14, 22, 31, 51, 54, 114, 130, 200, 250, 300, 400, 800, and 1,300, and 5,500 mature worms of Echinococcus. On the basis of the most important morphometric guidelines and the results of the species-specific polymerase chain reaction (PCR) assay, the parasites were identified as E. multilocularis. The examined foxes were found from 5 to 70 km from the known endemic areas of the Slovak Republic, Austria, and Hungary, and from 5 to 50 km from the northern border of the country. On the basis of this information and the previous study (2), the overall prevalence rate of infection was 16% (24/156) in the five northern counties of Hungary (Gyor-Sopron 30%, Nógrád 26%, Komárom-Esztergom 7%, Pest 6%, and Borsod-Abáuj-Zemplén 5%). This prevalence rate is similar to those observed in Poland, Belgium, and the Slovak Republic in recent years. In these countries, a total of 30 human cases have been reported since 1995 (3–6).

In Germany, infected foxes were more frequently found near water (7), which indicates a water-related natural cycle of the parasite. The spatial aggregation analysis of the parasite in intermediate hosts demonstrated that areas with humid conditions are at high risk for human exposure (8). In Europe, the most important water-related intermediate host of E. multilocularis is the water vole (Arvicol a terrestris) (9). The prevalence of E. multilocularis in water voles can be as high as 39% in disease-endemic areas (10). Areas with high water-vole densities yielded a 10-fold higher risk for alveolar echinococcosis in humans compared to areas with low densities (10). These data indicate that water voles may play an important role in the epidemiology of E. multilocularis. All infected foxes included in this and the previous study (2) were found near permanent natural waters, i.e., in those areas where water vole populations exist, such as Lake Ferto, the River Danube, the River Ipoly, the River Rába, and several streams connected to the watershed area of the River Danube. E. multilocularis might have spread in the northern part of Hungary along the watershed area of the River Danube, coming from the known disease-endemic areas of Austria and the Slovak Republic. Similar spreading of the parasite along waterways was also observed in the Slovak Republic (11).

In the historically known E. multilocularis–endemic mountain areas, both fossorial and aquatic water voles exist (12). The density of these populations can be 10-fold greater than that of aquatic populations in other European countries (12). On the basis of the long incubation period of the parasite in humans (5–15 years) and the dates of the first human cases reported outside the historically known area (Figure), foxes might have reached the population density needed (13) to maintain the parasite cycle in low water-vole density areas in Europe from the 1980s (Figure). Although the parasite crossed the border of several countries that surrounded the known area, further spreading was not observed in those countries where A. terrestris is an endangered species (the Netherlands, northern Italy) or where water voles are absent from the fauna (western and southern France) (12).

The River Danube and several small streams crossing Budapest, the capital of Hungary with a population of 2 million, create ideal circumstances for urbanization of the life cycle of a parasite that involves water voles and red foxes. Urbanization of
the life cycle of *E. multilocularis* was recently observed in Prague, the capital of the Czech Republic with a population of 1 million and similar hydrographic features (14); therefore, occurrences of this zoonosis should be continuously monitored in Budapest. Further studies are necessary to monitor the possible spread of the parasite in other regions that are thought to be currently free of the infection.

The regulatory, veterinary, and public health authorities of the European Union mobilized considerable financial and human resources to control rabies and paid less attention to alveolar echinococcosis in the 1990s, although incidence data indicate that alveolar echinococcosis is increasing and became an emerging infectious disease in Europe. In the Directive 2003/99/EC of the European Parliament and of the Council repealing Council Directive 92/117/EEC, echinococcosis has been added to the list of zoonoses to be monitored in the European Union countries. Effective methods to control *E. multilocularis* are unavailable; however, the zoonosis should be monitored and evaluated, and development of control programs should be intensified.

**Acknowledgments**

We thank Lajos Tekes and Vilmos Pálfi for supporting our studies and Károly Andi and Zsolt Tóth for their help in sample collection.

**Tamás Sréter,* Zoltán Széll,* Zsuzsanna Sréter-Lancz,† and István Varga‡**

*Central Veterinary Institute, Budapest, Hungary; †National Food Investigation Institute, Budapest, Hungary; and ‡Szent István University, Budapest, Hungary

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**Figure.** Distribution of *Echinococcus multilocularis* in Europe (1,2,4, this study). Black areas: Infection was reported in men, foxes and or rodents. Dark gray areas: Infection was described only in foxes and or rodents. Light gray areas: Only human cases were noted. White areas: *E. multilocularis* free territories. Question marks: The presence or appearance of the parasite is projected. Note: The prevalence of infection in foxes is similar in the majority of the affected countries.
Antimicrobial Resistance in Campylobacter

To the Editor: We wish to rectify several errors in our commentary, Antibiotics in Animal Feed and Spread of Resistant Campylobacter from Poultry to Humans (1). The fluoroquinolone enrofloxacin was approved in 1996 for therapeutic use by addition to drinking water upon the decision of a licensed veterinarian “for the control of mortality in chickens associated with Escherichia coli organisms and control of mortality in turkeys associated with *E. coli* and *Pasteurella multocida* organisms” (2). This therapeutic use was withdrawn (3) but is now under appeal. Initial approval and subsequent efforts to withdraw use of enrofloxacin in the United States parallel the earlier trend in Europe and specifically Denmark, where the use of antimicrobial agents as growth promoters has been banned (4).

Enrofloxacin is not approved for prophylactic or growth promotion use in poultry feed as stated in our commentary and in the first section of the flowchart (1). However, when enrofloxacin is added to the drinking water of poultry, large numbers of both ill and healthy animals are exposed to the agent (5). Although extra-label use of enrofloxacin is prohibited, microbiologic culture of either of the cited bacteria is not required before administration (2). Despite the restrictions on enrofloxacin use, emergence of fluoroquinolone-resistant *Campylobacter* species, with poultry as an important source, has been documented in the United States (5,6). Thus the decision to withdraw therapeutic use of enrofloxacin (3) was warranted. Therefore, our conclusion remains: use of enrofloxacin in poultry materialistically contributed to increase in human infection by fluoroquinolone-resistant *Campylobacter* species. Given the above, our commentary should have been entitled Use of Antibiotics in the Poultry Industry and Spread of Resistant *Campylobacter* to Humans. We regret the errors and hope we have clarified this issue.

Nicole M. Iovine* and Martin J. Blaser†

*New York University School of Medicine, New York, New York, USA; †New York Harbor Department of Veterans Affairs Medical Center, New York, New York, USA

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Address for correspondence: Martin J. Blaser, Department of Medicine, NYU School of Medicine, 550 First Avenue, OBV-606, New York, NY 10016, USA; fax: 212-263-3969; email: martin.blaser@med.nyu.edu
Manual of Travel Medicine and Health

Robert Steffen, Herbert L. DuPont, and Annelies Wilder-Smith

BC Decker Inc, 2003


Pages: 628, Price: US $59.95

Although the field of travel medicine is relatively young, the number of textbooks about the field is growing. This international trio of authors from Switzerland, the United States, and Singapore, recently published the second edition of their textbook, which adds new chapters and updates epidemiologic information. Because of the complexity of travel medicine, good resources for clinicians and travelers are needed. This edition represents a welcome addition to the library of travel medicine.

The main audience for this textbook is travel medicine physicians. Like the first edition, it is designed to be a reference book. Although small enough to fit in a pocket of a white coat, this paperback is very readable and comes with an easy-to-use CD.

Part 1 of the book, Basics, provides an overview of general topics for physicians to discuss with their traveling patients. The authors encourage a comprehensive strategy, one that discusses prevention measures such as vaccines and their appropriate uses. Appendix C is an excellent table that lists the required and recommended vaccinations for each country. The text also provides excellent information for travelers in varied situations, such as pilgrims to the Hajj, migrants, pregnant women, international adoptees, athletes, and persons who are immunocompromised.

In addition to providing current information on immunizations, the authors provide thorough information on malaria, including some individual country maps displaying areas of risk. Although the malaria review is comprehensive, caution should be exercised when deciding not to provide prophylaxis for travelers to a country where malaria is endemic.

Part 2, Infectious Health Risks and Their Prevention, is the familiar chronicle of travel-related infectious diseases. This section includes numerous maps and tables describing the epidemiology of the diseases. The authors have updated this part by adding several diseases, including severe acute respiratory syndrome.

The book provides pertinent information on travelers’ medical kits, water disinfection, and noninfectious health risks such as high altitude, arctic travel, diving, jet lag, and ultraviolet radiation. New for this edition are informative chapters on deep vein thrombosis and pulmonary embolism and in-flight accidents.

Another strength of this work is the section on posttravel medical treatment. This chapter presents concise guidelines for the clinician who is treating posttravel patients (with diarrhea, fever, malaria, dermatologic disorders, eosinophilia, sexually transmitted diseases) or screening expatriates after prolonged stays in tropical regions. A particularly useful feature is the dosing recommendations, many of which are for infrequently used drugs.

In conclusion, the Manual of Travel Medicine and Health, Second Edition, should be a useful textbook for travel medicine physicians and those in training who want to learn more about the field. While the traditional topics are covered in customary detail, the strength of the book is its comprehensiveness and portability, providing a convenient reference.

The Vaccine Book

Barry R. Bloom and Paul-Henri Lambert


ISBN: 0121072584

Pages: 436, Price: US $59.95; UK £37.50

Few fields in medical science involve as wide a range of specialties and expertise as vaccinology. It encompasses the research, development, and manufacturing processes of vaccines, their incorporation into immunization programs, and the logistic and clinical aspects of their use. Commissioning experts to write chapters with a minimum of jargon, minutiae, and redundancy, for a book with a target audience of immunologists, microbiologists, clinical trial specialists, epidemiologists, economists, policy-setting public health officials, and practitioners who administer the resulting products and provide follow-up care, is challenging. But the experienced editors of this book have achieved this goal. Dr.
Bloom was previously a *Mycobacterium* immunologist at the Albert Einstein School of Medicine and is now dean of the Harvard School of Public Health. Dr. Lambert is a vaccine immunologist at the University of Geneva.

The Vaccine Book first covers the impact of disease, including chapters on vaccine economics and finance policy, and the potential for widespread vaccination to change the epidemiology of the target disease. One example is the herd effect of childhood rubella vaccination, which postpones infection in nonimmunized women into their childbearing years. The next section reviews the immune system, and here lies the book’s greatest disappointment. Its chapter on basic immunology is confusing and presumes familiarity with terms and concepts without antecedent explanation. It lacks a logical flow in describing what is yet known of the (infinitely?) complex immune system and its many "up-" and "down-regulating" feedback loops. Readers hoping for a chapter-length "Immunology 101" course would be advised to turn elsewhere (1,2).

The phased stages of clinical trials are covered in excellent chapters by accomplished authors with practical insights. Another section shows how knowledge of microbial pathogenesis can affect vaccine design, including Rolf Zinkernagel's well-written chapter on immunologic memory. Another chapter on parasite pathogenesis, however, delves too deeply into the immunity of *Leishmania* as a case study.

Stanley Plotkin's thoughtful overview of the 11 disease-specific chapters annotates new vaccine technologies as well as current issues of debate, such as replacing the live oral polio vaccine worldwide with injectable, inactivated polio vaccine once the eradication program breaks the chain of wild-virus circulation, to avoid reverse mutations and resulting vaccine-associated paralysis. Plotkin also provides a comprehensive table of vaccine types currently available or in active clinical development.

Remaining sections of The Vaccine Book cover the ethics of research and use of vaccines, their safety and controversies, and their introduction into healthcare systems. The editors conclude with major future challenges, such as circumventing microbial escape, vaccines for chronic and autoimmune diseases, and maintaining public support of immunization in the face of antivaccine movements.

The breadth of vaccinology inevitably requires leaving out some topics. There is no chapter on measles vaccines, used universally for a major cause of childhood death and disability. Manufacturing steps such as fermentation, purification, formulation, fill, and finish are not described. There is little on quality assurance and regulation, such as the investigational new drug application process and current good manufacturing practice, although good clinical practice is mentioned. Despite these gaps, compared to this field's authoritative encyclopaedia (3), at three times The Vaccine Book's mass and four times its pages, this handy 1.1-kg compilation is a more comfortable read, indeed.

Bruce G. Weniger*

*Centers for Disease Control and Prevention, Atlanta, GA, USA

References


Address for correspondence: Bruce G. Weniger, Centers for Disease Control and Prevention, National Immunization Program, Mailstop E61, 1600 Clifton Road, Atlanta, GA 30333, USA; fax: 404-639-8834; email: bgw2@cdc.gov

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WEST NILE VIRUS

Epidemic of West Nile Virus in the United States, 2002

In 2002, West Nile virus (WNV) dramatically expanded its geographic range in the United States and caused the largest recognized epidemic of neuroinvasive arboviral diseases in the Western Hemisphere. That year, an estimated 180,000–1,200,000 cases of WNV infections occurred in humans in the United States. The Centers for Disease Control and Prevention’s ArboNet surveillance system received reports from 39 state health departments, 29 of which reported cases for the first time, and Washington, D.C., of 4,146 WNV cases. Clinical and demographic information indicated that 2,942 (71%) were neuroinvasive (meningitis or encephalitis), 1,157 (28%) were West Nile fever, and 47 (1%) were clinically nonspecific. The fatality-to-case ratio was 9% among patients with neuroinvasive illness and 12% among patients with encephalitis. Neuroinvasive illness and death were strongly associated with advancing age and occurred slightly more frequently among males than females. Neuroinvasive illnesses occurred from mid-May to mid-December with peak incidence during the week of August 24; 84% occurred in 11 midwestern states in the Mississippi and Ohio River basins. Observations during 1999–2002 suggest that, in coming years, WNV will assume an epidemiologic pattern of intense seasonal transmission in the United States, with hundreds to thousands of human cases reported annually. WNV detection in nonhuman species appears to be a sensitive but relatively nonspecific predictor of impending transmission to humans. WNV prevention should emphasize organized, well-funded, sustained mosquito-control programs that stress the reduction of *Culex* mosquitoes, and public education messages that stress personal protection from mosquito exposure and elimination of perresidential mosquito habitats.


West Nile Virus: An Overview of its Spread in Europe and the Mediterranean Basin in Contrast to its Spread in the Americas

West Nile infection was considered a minor arbovirus in the Old World despite several outbreaks with encephalitis cases in the 1950s in Israel. From 1994 to 2003, West Nile outbreaks were reported in humans and horses in Algeria, Romania, Russia, Israel, Tunisia, Morocco, and France with neuroinvasive forms and fatalities mainly in elderly persons. Vectors are mosquitoes principally from the *Culex* genus, but few isolates of West Nile virus have been obtained. Birds are amplifying hosts for the virus and are considered resistant to the disease. However, the occurrence of an abnormal number of deaths in some bird species in Israel in 1998 indicated that a more virulent strain had emerged, which surprisingly reached New York City in 1999 and spread in the New World. Phylogenetic studies have shown two lineages of West Nile strains in sub-Saharan Africa, but only strains from lineage 1 were identified in the Mediterranean region and southern Europe. European authorities are concerned about new modes of transmission through blood donations and organ transplants, which occurred in the United States in 2002. An enhanced surveillance for West Nile infection in humans, horses, birds, and vectors may indicate that the virus is present in different locations, but the occurrence of outbreaks is still unpredictable.


Emerging Vectors in the Culex pipiens Complex

These authors compared New World and Old World populations of *Culex pipiens* due to this taxon’s likely importance as a vector of West Nile virus (WNV): its abundance, high rates of WNV infection, peak biting activity, positive vector competence, and transovarial virus transmission. By using polymorphisms at eight nuclear microsatellite loci, they first demonstrated that American and North European populations of *Cx. pipiens* are genetically different. Also, by using multilocus fingerprinting techniques, they demonstrated that a large proportion of *Cx. pipiens* in the northeastern United States are hybrids of human-biter (autogenous) and bird-biter (anautogenous) forms. These forms are genetically distinct and have been isolated in northern Europe, where they segregate by habitat. The bird-biting mosquitoes live and breed in open areas above-ground and diapause during the winter; human-biting mosquitoes live exclusively in enclosed areas, like the underground rail lines, that are kept warm year-round. Underground human-biting mosquitoes were found to be more closely related to other human-biting mosquitoes from North Africa, the Middle East, Japan, and Australia than to neighboring above-ground specimens. Combined with susceptible migrating birds and highly concentrated human populations, the finding of hybrids in the United States led the authors to hypothesize that hybrid *Cx. pipiens* that may bite both humans and birds have contributed to the unprecedented number of human cases of WNV in North America.
TUBERCULOSIS

Stable Association between Strains of *Mycobacterium tuberculosis* and their Human Host Populations

*Mycobacterium tuberculosis* is a global pathogen that kills two million persons each year. Hirsh et al. investigated whether it is really one and the same *M. tuberculosis* that infects people born in different parts of the world. The evolutionary relationships among 100 *M. tuberculosis* isolates from San Francisco were deduced from the unique genomic sequences deleted from each isolate. For the same 100 isolates, a long-term epidemiologic dataset showed where each isolate’s host had been born, and whether he or she had contracted the infection before or after coming to San Francisco. Together, the evolutionary and epidemiologic data showed that a host’s place of birth was highly predictive of the genetic identity of the *M. tuberculosis* he or she carried. This pattern held true even among hosts who had been infected after arriving in San Francisco. An estimate of the time separating the genetically divergent groups of *M. tuberculosis* that are carried by persons born in different locations suggested that the associations between human populations and their genetically distinctive strains of *M. tuberculosis* have persisted for centuries.

Conference Summary

Emerging Infections: What Have We Learned from SARS?

Given the current size and mobility of the human population, emerging diseases pose a continuing threat to global health. This threat became reality with the outbreak of severe acute respiratory syndrome (SARS). The emergence of a disease requires two steps: introduction into the human population and perpetuated transmission. Although preventing the introduction of a new disease is ideal, containing a zoonosis is a necessity. The lessons that we have learned from SARS were the topic of a meeting of The Royal Society on January 13, 2004, in London, England.

Zoonoses are responsible for most emerging infectious diseases, including infections caused by Ebola virus, West Nile virus, monkeypox, hantavirus, HIV, and new subtypes of influenza A. In the case of SARS coronavirus (SARS-CoV), serologic evidence indicates that the virus was spread through interspecies transmission from wild game markets in Guangdong, China (Malik Peiris, University of Hong Kong). This finding led to bans in the wild meat trade from Nan Shan Zhong (Guangzhou Respiratory Disease Research Institute) similar to the ban on eating wild meat in Britain. Creutzfeldt-Jakob disease emerged in Britain.

Ecologic changes, concomitant with increasing contact between humans and animal disease reservoirs, contribute to zoonoses. The emergence of SARS was facilitated by increased contact between people and animal disease reservoirs as the wild meat industry expanded recently. Global warming will likely contribute to the spread of dengue beyond tropical regions (Tony McMichael, National Centre for Epidemiology and Population Health, Canberra, Australia). Habitat fragmentation by deforestation may increase the contact between people and reservoir species. For example, hemorrhagic fever virus has been linked to deforestation in South America.

Containing an emerging disease depends on rapidly designing and implementing a control strategy appropriate to the epidemiology of the disease. Interdisciplinary and international collaboration occurred with unprecedented rapidity during the SARS outbreak. The network of laboratories in 17 countries organized by the World Health Organization (WHO) coordinated information sharing (David Heymann, WHO) and was instrumental in rapidly identifying the etiologic agent of SARS (1) and in fulfilling Koch’s postulates (2) (Albert Osterhaus, Erasmus University, Rotterdam).

As is typical of an emerging disease, no vaccines or drugs to combat SARS existed, making quarantine, patient isolation, travel restrictions, and contact precautions the only means of limiting transmission. Mathematic models provided a framework for evaluating alternative control measures and making predictions about the course of the epidemic (3,4). Previously, similar models had guided public health policy, for example, in halting an outbreak of hoof and mouth disease in the United Kingdom in 2001 (5,6). One of the complications in setting parameters in an emerging disease model is the difficulty in estimating epidemiologic limits from the initially small sample sizes. Thus, openly sharing data and analysis of key model parameters are vital.

The model must be appropriate to the nature of the disease and the accuracy of the parameter estimates (7). Stochasticity inherent in transmission dynamics will be particularly pronounced when infection prevalence is low. Population heterogeneity and the network structure of human interactions will affect the spread of an emerging disease. In the 2003 SARS outbreak, healthcare workers were at particular risk (8) and acted as bridges carrying the infection from the hospital and causing community-wide epidemics. High-risk “core groups” have been a major focus of HIV/AIDS models for years (9), but the movement of SARS patients into the core (i.e., the hospital) adds a further complication (3).

The two waves of SARS clusters in Toronto (Robert Maunder, Mount Sinai Hospital, Toronto) highlight the need for surveillance even after an outbreak appears extinguished. Management of the SARS epidemic also demonstrated that public service infrastructure, which affords the greatest chance of success (3), is essential to the rapid containment of an outbreak. In areas most affected, contact tracing was important (10). In Guangdong, police departments tracked down contacts of infected persons, who were then followed up for 10 days after exposure. Evaluating the surge capacity of public health services and hospitals is one way to assess the preparedness of a medical system. The case-fatality rate is a key determinant of the public health impact of an emerging disease and was high for SARS at approximately 15% (11). The relationship between infectiousness and onset of symptoms is also important. Patient isolation has greater potential as a control strategy if the illness can be diagnosed before the person becomes infectious (Roy Anderson, Imperial College London). In contrast, persons infected with influenza virus are highly infectious before they become symptomatic.

The rapidity of pathogen turnover means that evolution in pathogen populations can occur on a time scale that
is epidemiologically relevant. Indeed, SARS-CoV evolved during the course of the SARS outbreak in China (12). Similarly, influenza is perpetuated in the human population by the evolution of new antigenic variants every year (Robin Bush, University of California, Irvine) (13). Even if the transmissibility of an emerging disease is initially below the threshold necessary to sustain it in a population, the potential for the organism’s evolution to higher levels may exist (14,15). Thus, one should not become complacent about diseases that are repeatedly introduced through zoonosis, but teeter on the edge of sustainability within the human population.

The success with which WHO coordinated the global collaboration in containing SARS galvanized the World Health Assembly to grant WHO greater authority to verify outbreaks, conduct investigations of outbreak severity, and evaluate the adequacy of control measures. The outcome of this new authority will depend on integrating the expertise of public health officials, medical doctors, and epidemiologists worldwide with guidance from disease transmission models. The SARS outbreak demonstrated that an epidemic in one part of the world is not just an individual nation’s problem but a global problem.

**Alison P. Galvani**

*University of California, Berkeley, California, USA*

**References**


**Address for correspondence:** Alison P. Galvani, Department of Integrative Biology, University of California, Berkeley, CA 94720-3140, USA.

fax: 410-643-6264; email: agalvani@nature.berkeley.edu

**Conference Summary**

Open Access Publishing

An Open Access Publishing Conference was convened in Atlanta, Georgia, on January 7, 2004, by the libraries of the Centers for Disease Control and Prevention (CDC) and Emory University. Open Access is an emerging publishing model for peer-reviewed scientific research in which authors and their publishers grant free access to their work as long as the authors are acknowledged and the publisher ensures that the work is made freely available in a digital archive (1). The conference brought together key stakeholders including scientists, researchers, publishers, and librarians and included approximately 240 participants with 80 offsite registrants connecting through the simultaneous Web cast.

The keynote address, “The Coming Revolution in the Publication of Scientific Papers,” delivered by Harold Varmus, emphasized that 1) in today’s Internet era, the traditional Gutenberg print publishing model is outdated; 2) electronic publishing has the advantages of lower costs, global distribution, content that can be linked to datasets, improved archiving, and full-text searching; and 3) rigorous peer review is possible in electronic and Open Access formats. Open Access publishing challenges include engaging professional societies in this approach, building sustainable business plans, and changing academic culture so that published works are evaluated for content rather than for the journal label. Open Access publishing is typically financed by author fees along with a combination of philanthropic and advertising support. Examples are the Public Library of Science, Journal of Clinical Investigation, and BioMed Central
journals. Recent milestones include the Bethesda Open Access Principles meeting (1), the Wellcome Trust endorsement of Open Access, and support from the Howard Hughes Medical Foundation and a number of leading European scientific societies.

A panel of speakers gave stakeholders’ perspectives. Sheldon Kotzin reviewed the National Library of Medicine’s (NLM) priorities regarding access to, and permanent retention of, the world’s biomedical literature. Reflecting growing concerns about high costs of scientific publications, the U.S. Congress recently directed the NLM to report on the impact of rising journal subscription prices relative to access to medical research information and to identify remedies to ensure that taxpayer-funded research remains in the public domain. NLM’s Open Access initiative is PubMed Central, a digital archive of freely available life sciences journals. After a slow start, the PubMed Central repository includes 137 journal titles. PubMed Central expects publishers to deposit full contents of each journal issue soon after publication. Supplementary data files are also encouraged. The recent addition of a single article from a journal that is not participating in PubMed Central is broadening the definition of this archive. Another Open Access approach was described by John Nickerson, editor of Emory University’s Molecular Vision, which has been freely available on the Internet since its first issue in October 1995. A low-cost operation, Molecular Vision is a refereed open access journal that has achieved scientific recognition in its field.

Publishing trends affecting libraries were discussed by Linda Watson, University of Virginia Health Sciences Library, and included: 1) journal subscription price increases outpacing library budgets, 2) publishers’ bundling of journal subscriptions into large contracts often not well matched with institutional research interests, 3) consolidations in the publishing industry, 4) restrictive licensing terms overriding copyright and fair use practices, 5) long-term archival access to electronic content, and 6) selective deletions of published articles from databases and e-publications. Presenting a scientist’s perspective, CDC’s Marta Gwinn noted that the scientific community’s overarching responsibility is to ensure that research is conducted with integrity and quality and that access to it is fair, maximizes value to users, and protects the public investment and interests.

The open access conference generated discussion about the scientific research dissemination process and the need to strengthen the connections between evidence-based research and healthcare action. With high quality, peer-reviewed scientific research becoming freely available on the Internet, possibilities for more rapid advances in scientific knowledge and ultimately improved public health are important. Collaboration between government and academia is necessary to make progress toward open access to scientific research.

This conference was supported in part by the National Networks of Libraries of Medicine, Southeastern Atlantic Region. Conference presentations are available from: http://ada/healthsci.emory.edu/openaccess

Jocelyn A. Rankin* and Sandra G. Franklin†
*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; and †Emory University, Atlanta, Georgia, USA

Reference


Address for correspondence: Jocelyn A. Rankin, Chief, Information Center, Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop C04, Atlanta, GA 30333 USA; fax: 404-638-5598; email: Jrankin@cdc.gov

Correction Vol. 10, No. 3

In the article “Murine Typhus with Renal Involvement in Canary Islands, Spain” by Michele Hernández-Cabrera et al., errors occurred in the 2nd paragraph under The Study on page 740: 68% should be 6.8%. The corrected sentence appears below:

In Spain, two seroepidemiologic surveys, in Salamanca and Madrid (Central/Western Spain), yielded seroprevalence rates of 12.8% and 6.8%, respectively, in the general population (4,5).

The corrected article appears online at http://www.cdc.gov/ncidod/EID/vol10no4/03-0532.htm

We regret any confusion this error may have caused.

Correction Vol. 10, No. 3

In “Legionella Infection Risk from Domestic Hot Water,” by Paola Borella et al., errors occurred in the abstract. The seventh sentence should read as follows: “Furthermore, zinc levels of <100 µg/L and copper levels of >50 µg/L appeared to be protective against Legionella colonization.”

We regret any confusion these errors may have caused.

Correction Vol. 10, No. 3

In the article entitled “Distribution of Bovine Spongiform Encephalopathy in Greater Kudu (Tragelaphus strepsiceros)” by Andrew A. Cunningham et al., errors occurred in the title. The corrected title appears below:

Bovine Spongiform Encephalopathy Infectivity in Greater Kudu (Tragelaphus strepsiceros)

The corrected article appears online at http://www.cdc.gov/ncidod/EID/vol10no6/03-0615.htm

We regret any confusion this error may have caused.
“I think of my work as an inhabited landscape, never static or empty…. The wind ruffles; ants crawl; a rabbit burrows” (1). A painter of Salish, French-Cree, and Shoshone heritage, Jaune Quick-to-See Smith was born in St. Ignatius, Montana, and raised on the Flathead Reservation. She studied art at the University of New Mexico, but her personal aesthetic and her poetic association with nature go back to her childhood, when under her father’s tutelage, she learned “to see and feel” (2).

Feeling and astute observation characterize Smith’s representational, abstract, and symbolic landscapes (3). A prolific artist, inspired by the formal innovations of Pablo Picasso, Paul Klee, and others, she uses paint, collage, and other media to compose unique forms on tactile surfaces that explore the continuum of life, the connection between living beings and the land, and the fundamental relationship between all things. As artist, curator, and lecturer, she has promoted understanding and reverence of nature, articulated Native aesthetic tradition in a modern art context, and made her mark on the contemporary American art scene.

Native American cultures did not refer to art as a separate discipline before the mid-19th century. Cultural materials with aesthetic value (totem poles, pottery, beadwork) were integrated into everyday life and traditional practices. Artists painted geometric patterns or symbolic representations of figures on readily available media (sand, hides, clay); wood, bone, and stone were carved too for a three-dimensional effect. The iconography and function of the work varied widely with region and tribe, but all objects were imbued with spirituality and were meant to serve the community (4).

Throughout the 20th century, Native American work was influenced by European realism. The early years were dominated by depictions of ceremonial dances and genre scenes painted in linear, decorative style. Later years saw a multiplicity of styles, including pop art and art deco (4). Smith, like many contemporary artists rooted in Native traditions, uses unique forms and nonfigurative visual language to express truths at once deeply personal and profoundly universal.

Rain, on the cover of this month’s Emerging Infectious Diseases, comprises three parts in a nontraditional ensemble depicting one of Smith’s favored themes, the close bond between humanity and nature. The main part of the ensemble is a long visual field painted in somber tones and punctuated with glistening metal spoons, arranged without regularity but with internal vertical symmetry, synchronized as they are by a powerful guiding force, gravity. The colors (grays, yellows, browns, reds) smear and run, blending into each other in alternating flat patches and dark grooves that give the strands of runny paint a three-dimensional effect.

In this abstract but anthropomorphic image of nature, without painting a single human face, Smith captures centuries of sorrow. As if long held in by a natural wall, massive sadness finally seeps through, forming large quiet drops. Emotions of every hue caused by every possible offense meet in a discolored torrent of grievance. Darkness, pain, and injustice meet grime, ignorance, arrogance, and greed. The inner human pain touches the outer pain of nature, dissolving the canvas in a cathartic rain of tears.

The work, which delivers a compelling ecologic and cultural message, has two other parts. To the side of the
main canvas is a black-and-white sketch of fluid, stylized figures engaged in a free-form dance. Below the framed dancers is a silver-and-pink panel whose center is engraved with the initials “C.S.” for Chief Seattle, Suquamish (1786–1866) and an eloquent advocate of the earth.

Smith’s icon of suffering engages the viewer in an empathetic recall of past wrongs, from environmental degradation to cultural annihilation through, among other causes, the spread of disease. When smallpox was introduced on the North American continent, it devastated the immunologically naïve Native population. Later, the human spirit, whose survival is at the heart of 20th century art (5), triumphed over the disease, eradicating it from the planet. Challenges to the bond between humanity and nature continue, emerging unpredictably and without end. Anthrax spores, fully understood and refined, were released into the U.S. postal system, reviving the specter of intentional biologic contamination. Like scientific efforts to anticipate and curtail the threat (6), Smith’s work confronts the pain of human and environmental catastrophe, embracing efforts to prevent it.

References
Look in the August issue for the following topics:

- **West Nile Virus in California**
- **Mycobacterium ulcerans** Disease (Buruli Ulcer) in Rural Hospital, Southern Benin, 1997–2001
- **Bacterial Gene and Protein Expression in the Host**
- **Vibrio vulnificus** in Taiwan
- **SARS in Three Categories of Hospital Workers, Hong Kong**
- **Long-Term Prognosis for Clinical West Nile Virus Infection**
- Serotype III Streptococcus agalactiae from Bovine Milk and Human Neonatal Infections
- Crimean-Congo Hemorrhagic Fever in Turkey
- Thrombocytopenia and Acute Renal Failure in *Puumala hantavirus* Infections
- Pharmacy Data for Tuberculosis Surveillance and Assessment of Patient Management
- Predicting Antigenic Variants of Influenza A/H3N2 Viruses

Complete list of articles in the August issue at http://www.cdc.gov/ncidod/eid/upcoming.htm
Editorial Policy and Call for Articles

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

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

Emerging Infectious Diseases is published in English Chinese, French, and Spanish translations of some articles can be accessed at http://www.cdc.gov/eid/ncidod/EID/trans.htm. The journal features the following types of articles: Perspectives, Synopses, Research Studies, Policy and Historical Reviews, Dispatches, Commentaries, Another Dimension, Lettets, Book Reviews, and News and Notes. The requirements of each type of article are described in detail below and at http://www.cdc.gov/eid/ncidod/EID/instruct.htm. To expedite publication, we post journal articles on the Internet as soon as they are edited.

Instructions to Authors

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

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

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

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

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

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses (not to exceed 15); figures or illustrations (not to exceed two); "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed two); and a brief biographical sketch of first author—both authors if only two.

Policy and Historical Reviews. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author—both authors if only two. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Dispatches. Articles should be 1,000–1,500 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed two); and a brief biographical sketch of first author—both authors if only two. Dispatches are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

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

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

Letters. This section includes letters that present preliminary data or comment on published articles. Letters (500–1,000 words) should not be divided into sections should contain no more than one figure or table. References (no more than 10) may be included.

Synopses. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author—both authors if only two. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Types of Articles

Perspectives. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary, and a brief biographical sketch of first author—both authors if only two. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.