

BRIEF REPORT

Laboratory-Acquired Severe Acute Respiratory Syndrome

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THE OUTBREAK OF SEVERE ACUTE RESPIRATORY SYNDROME (SARS) IN Singapore ended in late May 2003.¹ The Centers for Disease Control and Prevention (CDC) removed its travel alerts for Toronto, Hong Kong, China, and Taiwan shortly thereafter.² We report the first case of SARS to occur in Singapore after the initial worldwide outbreak ended. Our report documents the transmission of SARS in a laboratory setting.

CASE REPORT

A 27-year-old graduate student in microbiology at a local university was admitted to Singapore General Hospital on September 3, 2003, with fever. In July and August 2003, he had worked with a nonattenuated strain of West Nile virus in a biosafety level 3 (BSL-3) laboratory at Institute A, where research on SARS-associated coronavirus (SARS-CoV), dengue virus, and Kunjin virus was also conducted. The patient's illness had begun on August 26, 2003, with fever, headache, and polyarthralgia. Three days later, evaluation at the emergency department at Singapore General Hospital revealed apyrexia and a normal complete blood count and chest radiograph, so he was discharged home. He returned with persistent fever on September 3 and was admitted. A review of systems was notable only for a dry cough of two days' duration. He reported no history of exposure to SARS and no clinically significant travel history.

On admission, the patient had a temperature of 37.6°C. Oxygen saturation was 98 percent while he was breathing ambient air. The physical examination was unremarkable. Laboratory evaluation showed mild leukopenia, thrombocytopenia, transaminitis, and an elevated lactate dehydrogenase level of 709 U per liter (normal range, 180 to 380). His chest radiograph was normal. Investigations included routine blood cultures and testing for various pathogens. Sputum, blood, stool, and conjunctival swab specimens obtained on September 3, 4, and 8 were sent for SARS-CoV testing (Table 1).

The patient was initially admitted to a general ward. The following day, he was transferred to an isolation room because of concern regarding the remote possibility of exposure to SARS. On September 8, a polymerase-chain-reaction (PCR) assay of a sputum sample and a serologic test of a blood sample were reported to be positive for SARS-CoV. The Singapore Ministry of Health was notified, and the patient was transferred to the designated isolation facility at Tan Tock Seng Hospital. Chest radiographs on September 11 showed a left midzone pulmonary infiltrate. Computed tomography of the thorax on September 13 (Fig. 1) confirmed the presence of the pulmonary infiltrate in

Table 1. Diagnostic Test Results.*

Pathogen	Test	Type of Sample	Date of Collection	Result
Bacteria	Blood culture	Blood	Sept. 3 Sept. 9	Negative Negative
Coronavirus				
OC43	PCR	Sputum	Sept. 4	Negative
229E	PCR	Sputum	Sept. 4	Negative
SARS	IgM (IFA)	Serum	Sept. 3	Negative
SARS	IgG (IFA)	Serum	Sept. 8 Sept. 3	Weakly positive Negative
SARS	EIA	Serum	Sept. 8 Sept. 3	Positive (titer, 160) Negative
SARS	RT-PCR (Artus, Roche, and in-house primers)	Sputum	Sept. 8 Sept. 4	Positive (titer, ≥6400) Positive
SARS	RT-PCR (Artus, Roche, and in-house primers)	Stool	Sept. 4	Positive
SARS	RT-PCR (Artus, Roche, and in-house primers)	Conjunctiva	Sept. 4	Negative
SARS	RT-PCR (Artus, Roche, and in-house primers)	EDTA-treated blood	Sept. 4	Negative
SARS	RT-PCR (Artus, Roche, and in-house primers) and conventional PCR†	West Nile virus sample (cryovial)	Sept. 19	Positive
Cytomegalovirus	IgM IgG	Serum Serum	Sept. 9 Sept. 9	Negative Positive
Dengue virus	IgM IgM, rapid screen IgG, rapid screen RT-PCR	Serum Serum Serum Serum	Sept. 3 Sept. 9 Sept. 9 Sept. 3 or 5‡	Negative Negative Negative Negative
Epstein–Barr virus	VCA IgM (IFA)	Serum	Sept. 9	Negative
Malaria	Blood film Blood film	Blood Blood	Sept. 3 Sept. 3	Negative Negative
Parvovirus B19	IgM IgG	Serum Serum	Sept. 9 Sept. 9	Negative Negative
Toxoplasma	IgM	Serum	Sept. 9	Negative
West Nile virus	RT-PCR	Serum	Sept. 5	Negative
West Nile virus	RT-PCR	West Nile virus sample (cryovial)	Sept. 19	Positive

* PCR denotes polymerase chain reaction, IFA immunofluorescence assay, EIA enzyme immunoassay, RT reverse transcriptase, and VCA viral capsid antigen.

† The assays were performed as described by Ng et al.³

‡ The exact date could not be verified.

the apical segment of the left lower lobe. The patient's respiratory status remained stable, requiring no supplemental oxygen. His fever resolved 12 days after onset, and his dry cough persisted for another week. He was discharged on September 16 and spent 14 days in home quarantine.

METHODS

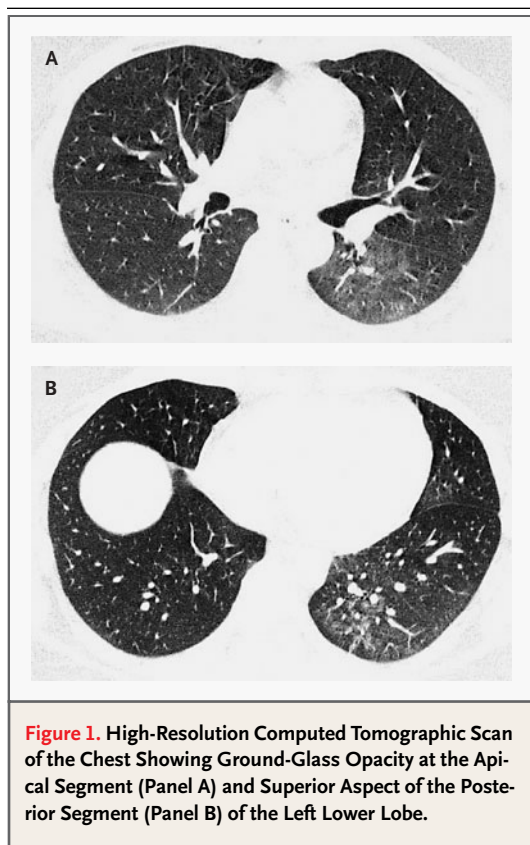
EPIDEMIOLOGIC INVESTIGATIONS

We reviewed medical records and interviewed the patient, his family, and work contacts. We conduct-

ed a thorough review of laboratory biosafety procedures, including written procedures, inspected the facility, and interviewed personnel about training and practice procedures.

LABORATORY INVESTIGATIONS

Sputum, blood, stool, and conjunctival swab specimens from the patient were sent for SARS-CoV testing. Testing for additional pathogens included blood cultures for bacteria, blood smears for malaria, serologic tests for dengue, cytomegalovirus, Epstein–Barr virus, parvovirus B19, and toxoplasma,



as well as reverse-transcriptase PCR (RT-PCR) for dengue, West Nile virus, and OC43 and 229E coronaviruses. The sample of West Nile virus that had been handled by the patient was sent to two different laboratories for RT-PCR for West Nile virus and SARS-CoV.

NUCLEIC ACID EXTRACTION

The QIAamp DNA Stool Mini Kit (Qiagen) was used on the stool samples. RNA from the other clinical samples and the sample of West Nile virus was extracted with the use of the QIAamp Viral RNA Mini Kit.

RT-PCR

Clinical samples were tested for SARS-CoV by means of three PCR assays: RealArt HPA-Coronavirus LC RT PCR (Artus), LightCycler SARS-CoV Quantification Kit (Roche), and an in-house one-step RT-PCR that used primers targeted at the nucleocapsid gene,⁴ SANS1 5'TGGACCCACAGAT-TCAACTGA3' and SANPA2 5'GCTGTGAACCAAGACGCAGTAT3', and the probe 5'FAM-TAACCAG-AATGGAGGACGCAATGG-TAMRA3'. The sample

of West Nile virus was tested for SARS-CoV by means of the same assays, as well as conventional nested PCR.³ RT-PCR for West Nile virus was performed according to the methods of Shi et al.⁵ and Scaramozzino et al.⁶

SEROLOGIC ANALYSIS

Enzyme immunoassay was performed according to the CDC protocol; the lysate of SARS-CoV-infected Vero E6 cells and lysate of uninfected Vero E6 cells were supplied by the CDC. Immunofluorescence assays for the detection of IgM and IgG were carried out on SARS-CoV-infected Vero E6 cells that had been placed onto the top row of 12-well Teflon-coated slides and uninfected Vero E6 cells that had been placed on the bottom row. Rabbit antihuman IgM and IgG conjugated to fluorescein isothiocyanate was used (Dako). Serum samples from the patient's work contacts were also tested for the presence of SARS-CoV antibodies by enzyme immunoassay.

VIRAL CULTURE

A sputum sample collected on September 4, 2003, was inoculated onto Vero E6 cells.

SEQUENCE ANALYSIS OF THE SARS-CoV VIRAL GENOME

RNA was extracted from the patient's sputum and the sample of West Nile virus and then converted to complementary DNA by reverse transcription with the use of a set of primers across the SARS-CoV genome. Two rounds of PCR with nested primers were used to amplify the viral genome. Sufficient amplified DNA was obtained to perform DNA sequencing for 15 of the 16 regions of the viral genome. The sequence of the genome for strain Sin0409, which was the patient's isolate, and SinWNV, which was the isolate from the sample of West Nile virus, was derived by two independent methods: sequencing by hybridization with the use of DNA resequencing arrays,⁷ and conventional sequencing with the use of automated capillary-based instrumentation.⁸

RESULTS

EPIDEMIOLOGIC INVESTIGATIONS

The university and the laboratory where the patient worked do not maintain stocks of live SARS-CoV. The patient reported no prior BSL-3 laboratory training; his previous experience was with the attenuated Sarafend strain of West Nile virus, a biosafety level 2 agent. An interest in comparing this strain with

the more pathogenic New York strain of West Nile virus necessitated his working in a BSL-3 laboratory. An agreement was made for him to do this work at the BSL-3 facility at Institute A. Institute A had been heavily involved in SARS-CoV work during the outbreak and continued this work through August 2003.

The patient visited Institute A three times in late July. He entered the BSL-3 laboratory on only one of these visits, during which he was trained for 20 minutes in BSL-3 procedures. He then proceeded to inoculate cells with West Nile virus. The flasks subsequently showed evidence of bacterial contamination and were destroyed. A laboratory technician at Institute A performed the second inoculation without the patient present. During the next few weeks, the technician maintained the cells while simultaneously continuing routine work on SARS-CoV.

The patient's fourth and final visit to Institute A was on August 23, 2003, three days before the onset of illness. That morning, before the patient's arrival, the technician combined two T75 flasks containing the previously inoculated West Nile virus into one 50-ml tube and centrifuged it. The patient entered the BSL-3 laboratory three times that day. The first time, he went in with the technician, wearing only street clothes, and did not engage in any work. The technician removed the centrifuge tube from the protective container in the open laboratory, placed it under a biosafety hood, and provided the patient with further instructions. Both then exited the laboratory. The patient reported putting on a gown and two sets of gloves before reentering the laboratory alone, where he spent 20 minutes unsupervised, transferring the cell supernatant into prelabeled cryovials using plastic, disposable Pasteur pipettes. He added bleach to the used tube before discarding it. All tasks were completed under the biosafety hood. After completion, he exited the laboratory to meet the technician. Together, both reentered the laboratory and transferred the cryovials to a -70°C freezer located in the biosafety level 2 facility. The cryovials remained in the freezer; no further work on them was reported.

Interviews with the patient's family and work contacts did not identify any other possible source of infection with SARS-CoV. One work contact had had a gastrointestinal illness a week before the patient became ill; however, his clinical symptoms differed from those characteristic of SARS, and he had no antibodies against SARS-CoV 27 days after the onset of illness. None of the contacts reported a clin-

ically significant history of travel or contact with a patient with SARS during the Singapore outbreak between March and May 2003.

A total of 8 household contacts, 2 community contacts, 32 hospital contacts, and 42 work contacts were identified, of whom 25 were placed under home quarantine. Both laboratories where the patient had worked were closed as a precautionary measure. Environmental samples were not obtained because the laboratory had been extensively disinfected.

PCR, SEROLOGIC ANALYSES, AND VIRAL CULTURE

The patient's sputum and stool samples collected on September 4 were positive for SARS-CoV on all three PCR assays. Conjunctival and blood samples were negative for SARS-CoV on PCR. These clinical samples were reextracted; repeated PCR with the use of the Artus kit yielded the same results. However, no SARS-CoV was cultured from sputum after 15 days of incubation.

PCR testing for West Nile virus was positive on the sample of West Nile virus from the cryovial handled by the patient. In addition, however, high levels of SARS-CoV RNA (4.625×10^6 copies per milliliter) were detected in the same cryovial.

The patient's serum sample collected on September 3 was negative for SARS-CoV antibody on enzyme immunoassay and immunofluorescence assays, whereas that collected on September 8 had a titer of at least 6400 on enzyme immunoassay and was positive for IgM and IgG (titer, 160) on immunofluorescence assays. The patient's samples were negative for all other pathogens except cytomegalovirus, for which he had a positive IgG titer (Table 1).

Serologic tests of samples obtained from 24 quarantined contacts four weeks after their last exposure to the patient were negative for SARS-CoV.⁹ Work contacts were tested a median of 20 days (range, 16 to 24) after their last contact with the patient. None of them had been exposed to the patient after he became ill. Serologic analyses of blood samples obtained from the technician on September 10 and September 16, 18 and 24 days, respectively, after her last possible exposure to the patient, were negative for SARS-CoV. She was not tested for West Nile virus infection.

GENOME SEQUENCE ANALYSIS OF SARS-CoV FROM THE PATIENT

A partial sequence (87 percent complete) was determined for the SARS viral genome isolated from the

patient. The sequence of this strain, Sin0409, was compared with 44 SARS-CoV genomes determined for strains collected from patients around the world. The Sin0409 sequence was inspected at 13 key positions that varied among previously sequenced SARS-CoV genomes. The presence or absence of these variant nucleotides represents signatures within the viral genome and has proved useful to establish links in SARS-transmission histories.¹⁰ These signature sequences were used to determine the most likely origin of the patient's illness. The sequence of the 13 signature positions within Sin0409 showed that it was most similar to Sin2774 (Fig. 2). Sin2774 was isolated early in the epidemic from a primary contact of the first index patient (Sin2500) in Singapore. This strain has become the predominant laboratory strain used in SARS research in Singapore. The close sequence similarity of the patient's SARS virus with the laboratory strain provides additional evidence of laboratory transmission.

The SARS-CoV strain (SinWNV) isolated from the sample of West Nile virus was partially (91 per-

cent) sequenced and compared with the sequence of the patient's sample and of all other SARS genome sequences (Fig. 2). The SinWNV sequence was identical to Sin2774 at the 13 key positions and thus confirmed that this sample of West Nile virus was contaminated with the laboratory strain of SARS. The sequence of Sin0409 had a deletion of 47 bases that had not been seen in any other SARS strain, including previously sequenced isolates of the laboratory strain Sin2774. The identical deletion was found in the SinWNV isolate, which strongly implicates this laboratory stock as the source of the SARS-CoV that infected the patient.

DISCUSSION

This case report is clinically significant because it documents the occurrence of SARS since the worldwide epidemic ended in July 2003. The clinical features and incubation period are consistent with those of previously described cases of SARS,^{11,12} the positive results for SARS-CoV on PCR of clinical specimens were borne out by different assays, and the patient had seroconversion to SARS-CoV.

This case is scientifically important in that it documents laboratory-acquired SARS-CoV infection. Other, nonlaboratory sources of infection were investigated and ruled out. The patient could have been infected earlier and had a latent SARS-CoV infection. However, the presence of SARS-CoV nucleic acid in sputum and stool samples and the documented seroconversion, which coincided with a clinical illness compatible with SARS, indicate that he had an acute infection. Alternatively, the patient could have been exposed to another person with unrecognized SARS infection. However, the findings of the epidemiologic investigation do not provide support for this theory.

The most likely explanation is that the patient acquired his infection in the laboratory. The strongest evidence supporting this assertion was the presence of SARS-CoV in the sample of West Nile virus with which he had been working three days before his illness began. Furthermore, the strain isolated from the patient had the same signature sequences as the SARS-CoV found to be contaminating the sample of West Nile virus, and these sequences, in turn, were highly similar to those of the predominant research strain in Institute A and Singapore.

Both West Nile virus and SARS-CoV are grown in Vero E6 cells.^{13,14} Although the precise mechanism of contamination remains unknown in this case, the

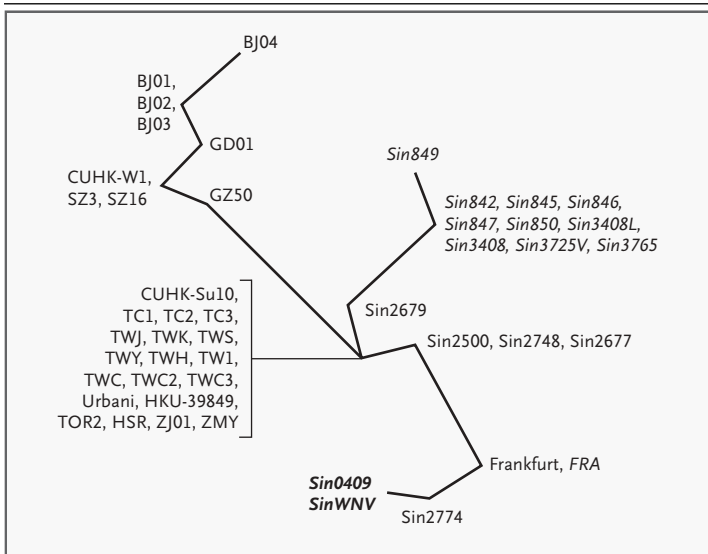


Figure 2. Molecular Relationships among 47 SARS-CoV Genomes.

A phylogenetic tree was constructed by means of a maximum-likelihood method⁹ with the use of sequence information from 13 informative positions (nucleotides 9404, 9854, 17564, 18965, 19084, 19838, 21721, 22222, 22549, 23174, 23735, 23792, and 28268 in the Urbani strain; GenBank accession number AY278741) and 1 deletion (position 27760 to 27807). Sin0409 (the strain isolated from the patient) and SinWNV (the strain isolated from the sample of West Nile virus) appear on the same branch, indicating complete equivalence at all 14 sites. All SARS-CoV sequences were obtained from GenBank except for those indicated in italics, which were from the Genome Institute of Singapore.

high level of SARS-CoV in the sample of West Nile virus suggests that the two viruses were growing simultaneously in the cells and indicates that contamination occurred before August 23. Titers of SARS-CoV range from 0 to 6 log copies per milliliter, depending on the sample; respiratory specimens typically yield higher titers than stool specimens (0 to 1 log copy per milliliter). The moderately high SARS-CoV titer in the contaminated cryovial of West Nile virus relative to that in respiratory and stool samples may well account for the occurrence of this case of laboratory-acquired SARS, despite the fact that large numbers of clinical specimens were processed during the previous epidemic.

Until now, human-to-human spread has been the predominant mode of transmission of SARS.^{11,15} Laboratory-acquired infections have been documented with other pathogens, including West Nile virus.¹⁶ We postulate that transmission occurred as the result of an error made by the technician, the patient, or both. However, interviews with both persons identified no previous, recognized laboratory accidents. We speculate that the patient was not coinfecting with West Nile virus because this virus is transmitted by parenteral exposure.¹⁶ This case of laboratory-acquired SARS indicates that concern regarding the potential risk to laboratory personnel is justified, and the epidemiologic criteria in the cur-

rent case definitions of the World Health Organization (WHO)¹⁷ and CDC⁹ may need to be amended to include laboratory exposure to SARS-CoV as a risk factor for infection. Furthermore, because laboratories handling live SARS-CoV are potential sources of infection, this case highlights the importance of strict adherence to effective biosafety practices.¹⁸

This case underscores the need for unceasing vigilance and for the ability to respond swiftly and comprehensively in order to prevent another outbreak of SARS. The patient's illness was mild and his radiologic findings developed late, making the diagnosis dependent on a high index of suspicion and the availability of reliable laboratory tests. A delay in diagnosis in hospital settings increases the risk that the outbreak will spread and ultimately reach the community. This is the challenge we now face in preparing for the next reemergence of SARS.

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