

Influenza Surveillance in Indonesia: 1999–2003

Charmagne G. Beckett,¹ Herman Kosasih,¹ Chairin Ma'roef,¹ Erlin Listyaningsih,¹ Iqbal R. F. Elyazar,¹ Suharyono Wuryadi,¹ Djoko Yuwono,² James L. McArdle,¹ Andrew L. Corwin,¹ and Kevin R. Porter³

¹US Naval Medical Research Unit Two, and ²National Institute of Health Research and Development, Indonesian Ministry of Health, Jakarta, Indonesia; and ³Naval Medical Research Center, Silver Spring, Maryland

Although influenza is recognized for its worldwide importance, little is known about the disease from tropical countries like Indonesia. From August 1999 through January 2003, a surveillance study was conducted in clinics at 6 sentinel locations. Adults (age, >14 years) and children (age, 4–14 years) presenting with respiratory symptoms suggestive of influenza were asked to enroll in the study. Nasal and pharyngeal swabs were examined by virus isolation, polymerase chain reaction, and rapid immunochromatographic tests. A total of 3079 specimens were collected from 1544 participants. Influenza infection was confirmed in 172 volunteers (11.1%) presenting with influenza-like illness. Influenza A (H1N1 and H3N2) and B viruses were detected at all sites. Peak prevalence tended to coincide with the respective rainy seasons, regardless of location. In light of the recent epidemic of severe acute respiratory syndrome, continued influenza surveillance would be useful in strengthening the infrastructure of the Indonesian public health system.

Influenza is a highly contagious acute respiratory illness of global importance. Global epidemics or pandemics have occurred approximately every 10–15 years, whereas localized outbreaks tend to occur at variable intervals [1, 2]. In Indonesia, although influenza epidemics are likely to occur on a periodic basis, only 2 epidemics have been well documented, and few influenza surveillance studies have been published. Three surveillance studies were conducted in Indonesia soon after the Hong Kong epidemic during 1968–1970 [3–5]. There were also 2 studies conducted in the 1980s and 1990s [6, 7].

Hotta et al. [3] measured anti-influenza hemagglutination-inhibiting antibodies in serum samples collected from persons in Surabaya, Indonesia. They found a greater level of seropositivity against older H1 influenza strains than newer H2 strains. The prevalence of

antibodies against influenza A (H2 Hong Kong type) was very low in serum samples collected in 1968.

Gan et al. [4] collected pharyngeal washings from patients from several locations in Indonesia during an apparent influenza epidemic. They successfully isolated A2/Hong Kong/68 as the etiological agent. The same result was reported by Tjajj and Gani [5] using samples collected in Medan. In 1972, Cross et al. [8] conducted a serological survey in Irian Jaya, which identified antibodies to influenza A2/Hong Kong/68 and influenza B in 65% and 78% of the population, respectively.

During the 2 periods of 1982–1986 [6] and 1991–1993, C.M. and coworkers at our institutions isolated influenza type A and B viruses from the throat swabs of patients with respiratory illness in Jakarta and Yogyakarta (unpublished data; table 1). During a respiratory infection outbreak from November 1995 through February 1996 in Kabupaten Jayawijaya, Irian Jaya, investigators reported the circulation of influenza A/Taiwan/1/86 (H1N1), influenza A/Johannesburg/33/94 (H3N2), and influenza B/Harbin/7/94 [7].

Overall, there is a paucity of data from Indonesia regarding the relative importance of influenza in contributing to epidemic and sporadic disease. Understanding the epidemiology of influenza viruses in this region is important to document the potential burden of disease, compared with that of other respiratory ill-

Received 5 November 2003; accepted 7 February 2004; electronically published 22 July 2004.

The opinions and assertions herein are neither to be considered official nor as reflecting the views of the US Navy or the Naval service at large.

Reprints or correspondence: Dr. Charmagne G. Beckett, American Embassy Jakarta, Unit 8132, NAMRU-2, FPO, AP 96520-8132 (beckettcg@namru2.med.navy.mil).

Clinical Infectious Diseases 2004;39:443–9

This article is in the public domain, and no copyright is claimed.
1058-4838/2004/3904-0001

Report Documentation Page

*Form Approved
OMB No. 0704-0188*

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE 2004	2. REPORT TYPE N/A	3. DATES COVERED -	
4. TITLE AND SUBTITLE Influenza Surveillance in Indonesia: 1999-2003		5a. CONTRACT NUMBER	
		5b. GRANT NUMBER	
		5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)		5d. PROJECT NUMBER	
		5e. TASK NUMBER	
		5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Submarine Medical Research Laboratory Naval Submarine Base New London Box 900 Bldg 148, Trout Avenue Groton, CT 06349-5900		8. PERFORMING ORGANIZATION REPORT NUMBER	
		10. SPONSOR/MONITOR'S ACRONYM(S)	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
		12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited	
13. SUPPLEMENTARY NOTES The original document contains color images.			
14. ABSTRACT			
15. SUBJECT TERMS			
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT SAR
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified	
19a. NAME OF RESPONSIBLE PERSON			

nesses, such as bacterial pneumonia. Surveillance activities can provide useful baseline data for measuring local epidemics and can generate critical information regarding the circulating strains that could have an impact on the annual selection of appropriate vaccine strains (i.e. Northern vs. Southern hemisphere formulations). This report summarizes the findings of recent virological surveillance for influenza virus infections in targeted areas of Indonesia from August 1999 to January 2003.

METHODS AND MATERIALS

Study Design and Population

Passive, laboratory-based surveillance was conducted throughout each year of the study at sentinel sites in Indonesia. The study population consisted of outpatients from clinics located in Jakarta, Tangerang, Bandung, Yogyakarta, Makassar, and Bali (figure 1).

The definition of possible influenza illness included symptoms of fever, headache, myalgia, coryza, and/or respiratory complaints (e.g., cough). Eligible participants were children aged >4 years and adults aged >14 years and included both female and male subjects, including pregnant women. With use of cotton Dacron swabs, specimens were collected from both lower anterior nares (lower nasal sample) and the throat (posterior pharynx sample) of each volunteer. As described in detail below, all samples were tested for influenza using standard virus isolation and multinested RT-PCR (mnRT-PCR). Because of the cost, rapid tests were used on aliquots of lower nasal and throat swabs during 1999–2000 and, subsequently, on lower nasal swabs only during 2001–2003. Influenza infection was considered to be confirmed when virus isolation, mnRT-PCR, or the rapid Directigen test (Becton Dickinson) yielded positive results. The diagnosis was excluded if results of all 3 tests were negative.

Participating physicians were asked to complete a brief questionnaire for each study participant. Data from the clinical

history, findings from a physical examination, and demographic data were collected. All volunteers (or guardians) were asked to provide informed written consent before study enrollment. The protocol was approved by the ethical committees of both the US Naval Medical Research Unit 2 (NAMRU-2) and the Indonesian Ministry of Health (DoD Protocol 1999–30849). The results of the questionnaires were analyzed using Epi Info statistical software (Centers for Disease Control and Prevention). The χ^2 test was applied to the clinical and laboratory data. All *P* values were 2-tailed, and *P* < .05 was considered statistically significant. A performance evaluation for the assays was conducted. Assay agreement was defined using the κ coefficient, and the κ value was interpreted as follows: <2, poor; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, good; and 0.81–1.0, very good.

Laboratory Evaluation

Virus isolation. Throat and lower nasal swab samples were collected by study physicians or nurses and then placed into 1 mL of Hanks' Balanced Salt Solution (HBSS) media that contains gelatin, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 25 U/mL mycostatin. The samples were stored at -70°C and transported monthly to NAMRU-2 for analysis. For virus isolation, sterile 24-well plates were seeded with Madin-Darby canine kidney cells suspended in minimum essential medium containing penicillin, streptomycin, L-glutamine, 1 mmol/L HEPES, and 10% fetal calf serum. Two days after seeding, when a confluent monolayer formed, the medium was aspirated and each well inoculated with 0.2 mL of specimen. Plates inoculated with samples were centrifuged and incubated with serum-free medium and trypsin for 7 days. These plates were then used for virus identification.

Virus identification. An immunofluorescence assay (IFA) with type-specific monoclonal antibodies and hemagglutination inhibition (HI) were used to serotype influenza isolates. For

Table 1. Previous influenza surveillance data from Indonesia, 1983–1993.

Year	Influenza virus A H1N1		Influenza virus A H3N2		Influenza virus B	
	No. of isolates	Strain(s) (no.)	No. of isolates	Strain(s) (no.)	No. of isolates	Strain(s) (no.)
1983	23	Bangkok/1/79; Philippines/2/82 ^a	50	Texas/1/84; Norway/1/84 ^a
1984	4	USSR/90/77 (1); England/333/80 (3)	6	Mississippi/1/85	6	Hong Kong/5/72 (1); Singapore/222/79 (5)
1985	43	ND	22	ND
1991	9	Taiwan/1/86	11	Shanghai/6/89 (9); England/333/80 (1); Washington/15/91 (1)	4	Victoria/2/87
1992	10	Washington/15/91 (6); Beijing/353/89 (3); Sichuan/2/87 (1)	10	Qingdao/102/91
1993	4	Texas/36/91 (1); Taiwan/1/86 (3)	7	Sichuan/2/87 (1); Beijing/353/89 (4); Shanghai/6/89 (2)	12	Panama/45/90 (11); Yamagata/16/88 (1)

NOTE. Adapted from [6]. ND, not done.

^a Exact number of isolates not recorded.



Figure 1. Map of influenza surveillance sentinel sites. Jakarta is the capital city of Indonesia and is located on the island of Java. Tangerang, Bandung, and Yogyakarta are also major urban cities. Bali is a nearby resort island, and Makassar is a city of moderate size in South Sulawesi.

IFA, after a 7-day incubation, plates were washed and centrifuged. One drop of precipitated cells was placed into each well of 12-well Teflon slides (Cel-Line; Erie Scientific) and reacted with the type-specific monoclonal antibodies. The cultures were stained with fluorescein isothiocyanate-conjugated goat anti-mouse IgG and viewed with an epifluorescence microscope to find ≥ 1 cell containing positive fluorescing intracytoplasmic and/or intranuclear inclusions.

HI assays were performed using turkey or chicken RBCs to identify influenza virus serotypes. This was achieved by testing the isolates against standard known reference antisera (provided by the World Health Organization [WHO] Regional Collaborating Centre for Reference and Research on Influenza; Melbourne, Australia) raised against specific influenza A or B serotypes. The full strain designations for all reference viruses and antisera supplied in the annual WHO kits from 1999–2003 are available at <http://www.influenzacentre.org>. When possible, positive virus isolates were sent to the WHO Collaborating Influenza Reference Centre in Melbourne for confirmation.

Influenza virus detection using mnRT-PCR. Lower nasal and throat swab samples preserved in HBSS media were assayed using mnRT-PCR. The QIAamp Viral RNA kit (Qiagen) was used to extract viral RNA from prepared samples. In the mnRT-PCR reaction, viral RNA was amplified using cocktails of oligonucleotide primers designed by Zhang and Evans [9] that are directed against conserved regions of the matrix protein, hemagglutinin, and neuraminidase genes of reference virus strains. The influenza virus strains were A/NWS/33, A/Puerto Rico/8/34, A New Jersey (for influenza A H1N1), A/Japan/305/57 (for influenza A H2N2), A/Victoria/3/75, A/Port Chalmers/1/73 (for influenza A H3N2), B/Lee/40, and B/Maryland/1/59 (for influenza B). The mixture of different primer sets in the mnRT-PCR reaction allows for the rapid simultaneous detection of influenza type B virus together with type A subtyping. Viral RNA was reverse-transcribed to cDNA and then concomitantly amplified in the same tube using Access RT/PCR (Promega) and the outer set of primers. Standard PCR reagents (Applied Biosystems) and the set of inner primers were used

for the nested amplification. The final amplicons were separated by electrophoresis on 2% agarose gel containing ethidium bromide for virus type and subtype identification.

Rapid antigen detection. Commercially available rapid immunochromatography tests (Directigen Flu A and Directigen Flu A + B; Becton Dickinson) to detect influenza A and B antigens were used to identify influenza viruses. These tests were performed in accordance with the manufacturer's instructions.

RESULTS

From August 1999 through January 2003, a total of 1544 volunteers from 6 areas in Indonesia participated in influenza surveillance. Table 2 summarizes the participant's demographic characteristics and the overall results of laboratory analyses. The ratio of male to female participants was 1:2.4, with an

Table 2. Demographic characteristics and summary of results for participants in influenza surveillance in Indonesia

Characteristic	Value
No. of subjects	1544
Sex	
Female	1083 (70.1)
Male	461 (29.9)
Age, mean years ^a	28.8
Age category	
Adults (age, >14 years)	1313
Children (age, 4–14 years)	43
Influenza-like illness	1372 (88.9)
Confirmed influenza	172 (11.1)
Positive test result	
Virus isolation	130 (75.6)
Multinested RT-PCR	144 (83.7)
Directigen Flu test ^b	75 (43.6)

NOTE. Data are no. (%) of subjects, unless otherwise indicated.

^a Age unknown for 188 participants.

^b Manufactured by Becton Dickinson. Quantity of sample not sufficient for 13 participants.

Table 3. Positive results of assays used to confirm influenza illness among 172 confirmed cases of influenza

Assay(s)	No. (%) of positive results
Virus isolation, mnRT-PCR, and Directigen test	60 (34.9)
Virus isolation and mnRT-PCR	43 (25)
Virus isolation and Directigen test	8 (4.7)
mnRT-PCR and Directigen test	6 (3.5)
Virus isolation alone	19 (11)
mnRT-PCR alone	35 (20.3)
Directigen test alone	1 (0.06)

NOTE. Samples collected during 1999–2000 were tested using the Directigen Flu A test (Becton Dickinson). Samples collected during 2001–2003 were tested using BD Directigen Flu A+B test (Becton Dickinson). mnRT-PCR, multinested RT-PCR.

overall age range of 4–79 years. All participants were Indonesian and residents of each sentinel city of surveillance. A total of 3079 specimens were collected from participants; the specimens consisted of 1539 lower nasal swabs and 1540 throat swabs.

Influenza infection was confirmed in 172 volunteers (11.1%) presenting with influenza-like illness (ILI). Virus isolation results were positive for 75.6% of persons with influenza, mnRT-PCR results were positive for 83.7%, and Directigen rapid test results were positive for 43.6%. The mean age of persons with confirmed influenza was 28.8 years. Ten pediatric cases were identified among 43 enrolled children (23.3%), and the majority of adult cases occurred in female participants (ratio of cases in male participants to cases in female participants, 1: 1.77).

Table 3 summarizes the number of influenza cases identified using the 3 assays, either individually or in combination. Table 4 shows the performance of the mnRT-PCR and the rapid tests, compared with virus isolation as the reference assay. For this analysis, because virus isolation was used as the reference assay, positive results for samples tested using either mnRT-PCR or the Directigen test alone were considered to be false-positive

results. Given that mnRT-PCR may be more sensitive than culture, sensitivity calculations based on culture as the standard may not accurately reflect the true performance of this test.

Overall, the mnRT-PCR was significantly more sensitive than that of the Directigen rapid tests (78.4% vs. 54.8%; $P < .0001$). The sensitivity of the Directigen Flu A rapid test was, however, greater than that of mnRT-PCR. The rapid tests correctly differentiated influenza A and B viruses in most cases (specificity, 98.6%–100%; data not shown). The sensitivity of the rapid tests varied, depending on the source of the specimen and the test used. The sensitivity of the Directigen Flu A + B test was higher when lower nasal samples were used than when lower nasal/throat samples were used ($P =$ not statistically significant). For mnRT-PCR, the sensitivity and specificity were higher when using lower nasal samples than for throat samples, but the differences were not statistically significant.

Influenza virus was recovered from 130 participants. Thus, the virus isolation rate was 8.4% of the total population enrolled. Influenza virus was isolated most often 1–3 days after the onset of symptoms (average, 2.2 days after symptom onset; maximum, 6 days after symptom onset). In all locations, influenza infections were identified year-round, with a tendency to peak during the months of the rainy season (generally during December and January) (figure 2). Both influenza A and B viruses circulated in the areas of surveillance, with influenza A H3N2 predominating (table 5). Influenza A H1N1 was identified in fewer patients, compared with the H3N2 strain. All H1N1 strains identified were New Caledonia-like; Moscow-like strains were most common among H3N2 subtypes, but in 2002, H3N2 Fujian-like strains emerged. For influenza B virus, 9 Hong Kong-like strains were detected in 2002. No age-specific patterns of the virus subtypes or strains were obvious; children aged <4 years were excluded from the study for ethical reasons, and only 4.5% of the participants were aged >50 years. Seventy-seven percent of persons with ILI were young or middle-aged

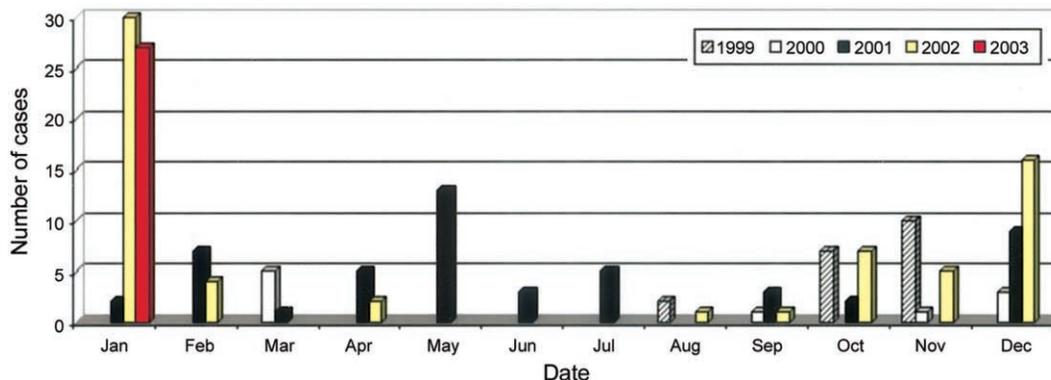


Figure 2. Influenza monthly surveillance, August 1999 through January 2003. Indonesia’s annual rainy season usually begins in early December and ends in late May. Decreased ambient temperatures (mean, 27°C) mark the rainy season, along with heavy rains often associated with flooding.

Table 4. Performance of rapid tests and multinested RT-PCR (mnRT-PCR) for detection of influenza viruses, compared with virus isolation using cell culture.

Test, swab specimen	No. of specimens tested	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, %	NPV, %	κ coefficient (95% CI)
Directigen test						
Flu A test, ^a lower nasal/throat specimen	376	93.3 (70.2–98.8)	98.6 (96.8–99.4)	73.7	99.7	0.82 (0.67–0.96)
Flu A+B test ^{a,b}						
Lower nasal/throat specimen	966	46.7 (35.8–57.8)	99.9 (99.4–100)	97.2	95.7	0.61 (0.49–0.73)
Lower nasal specimen	188	55.9 (39.4–71.1)	99.4 (96.4–99.9)	95.0	91.1	0.66 (0.50–0.82)
Overall ^a	1530	54.8 (46.1–63.3)	99.5 (99–100)	90.7	96.2	0.66 (0.58–0.74)
mnRT-PCR						
Throat specimen	1526	74 (63.3–82.5)	96.6 (95.5–97.4)	53.3	98.6	0.60 (0.50–0.69)
Lower nasal specimen	1520	81.8 (73.1–88.2)	98.2 (97.4–98.8)	76.4	98.7	0.78 (0.71–0.84)
Overall	3046	78.4 (71.8–83.9)	97.4 (96.7–97.9)	64.8	98.7	0.69 (0.63–0.75)

NOTE. Lower nasal and throat swabs were combined and tested as an individual specimen when indicated. NPV, negative predictive value; PPV, positive predictive value.

^a Manufactured by Becton Dickinson.

^b This replaced the Directigen Flu A kit in January 2001.

adults (i.e., age of 21–49 years), and the majority of confirmed infections were due to influenza A virus in all age groups.

There was a significant difference in the occurrence of some clinical signs and symptoms between confirmed influenza cases and ILI cases (table 6). Systemic symptoms, such as fever, chills, malaise, headache, and anorexia, were significantly more common in the influenza case group ($P < .01$). As noted elsewhere [10], volunteers with confirmed influenza A appeared to be more symptomatic than did volunteers infected with influenza B.

DISCUSSION

To our knowledge, this is the only comprehensive influenza surveillance activity in the archipelago of Indonesia. Our study documents that acute respiratory illness in various locations, including tourist destinations (Bali and Yogyakarta), can in part be attributed to influenza. Approximately 11% (172 of 1544) of persons presenting with an acute respiratory illness were found to be infected with either influenza A or B virus. Considering the large population of Indonesia (>200 million people) and its widely dispersed geography (>13,000 islands over 5000 km), one could argue that our sampling may not be representative. However, more than one-half of Indonesia's population (128 million) is concentrated on the island of Java (including Bali), where 4 of our sentinel sites were located. Despite possible sampling bias, our data provide information regarding circulating influenza viruses in a region of the tropics that is not available elsewhere. In other regional countries, such as Singapore and Malaysia, current data are also sparse, compared with Thailand, Australia, and New Zealand [11].

In many tropical countries, including Indonesia, the burden of illness due specifically to influenza is largely unknown, be-

cause the diagnosis is rarely considered and because there is a strong perception among clinicians and laypersons that influenza primarily occurs in temperate climates. For example, the study sites in Jakarta (2 outpatient clinics at a large community hospital) only collect data on visits for upper respiratory tract infections (URTIs) and admissions for pneumonia without microbiological information. From 2000 to 2002, the raw average annual numbers of visits for URTI in the pediatric and internal medicine clinics were 1319 and 1007, respectively. The average annual number of admissions for pneumonia was 267 for the same period. No microbiological or virological data on the causes of these infections are available. Therefore, estimates of the contributions of influenza to URTI occurring at the study sites relative to other infectious causes cannot be made. The monthly trends for both URTI and pneumonia show that cases peak during the months of December, February, and March, suggesting the possibility of a correlation between the rainy seasons and increased transmission of influenza viruses. However, our data are insufficient to make any definite conclusions regarding a seasonal occurrence of influenza in Indonesia.

One of the goals of our study was to provide baseline data, which health authorities could consider in plans for long-term surveillance and the need for vaccination programs. In particular, because Indonesia straddles the equator, virological surveillance data could be used to assist in the annual choice between vaccines distributed to the Northern versus Southern Hemispheres. In the past 4 years, no clear pattern has been detected, but we speculate that a mixture of the northern and southern strains may emerge with each continued year of surveillance.

Antiviral therapy has not been a mainstay of treatment in Indonesia, because clinicians may not readily recognize cases

Table 5. Influenza viruses isolated from sentinel sites in Indonesia

Site, year	Influenza virus A H1N1		Influenza virus A H3N2		Influenza virus type B	
	No. of isolates	Strain(s) (no.) ^a	No. of isolates	Strain(s) (no.) ^a	No. of isolates	Strain(s) (no.) ^a
Jakarta						
2000	1	New Caledonia/20/99
2001	2	New Caledonia/20/99	2	Moscow/10/99	2	Johannesburg/5/99 (1); Sichuan/379/99 (1)
2002	1	Fujian/411/02
Bandung						
1999	1	New Caledonia/20/99	11	Moscow/10/99 (8); Sydney/5/97 (3)
2000	2	New Caledonia/20/99	3	Sichuan/379/99 (1); Beijing/184/93 (2)
2001	5	Moscow/10/99	5	Johannesburg/5/99
2002	16	Moscow/10/99 (10); Fujian/411/02 (2); NI (4)	4	Johannesburg/5/99 (2); Hong Kong/330/01 (1); NI (1)
2003	4	Moscow/10/99 (2); Fujian/411/02 (1); NI (1)
Tangerang						
2001	8	New Caledonia/20/99 (6); NI (2)	8	Moscow/10/99 (6); NI (2)	9	Sichuan/379/99 (3); Shangdong/7/97 (2); Johannesburg/5/99 (3); Yamanashi/166/98 (1)
2002	2	New Caledonia/20/99	23	Moscow/10/99 (21); Fujian/411/02 (2)	5	Johannesburg/5/99 (1); Yamanashi/166/98 (1); Hong Kong/330/01 (3)
2003	6	Fujian/411/02 (4); NI (2)
Other sites (Yogyakarta, Bali, and Makassar)						
2002	2	Fujian/411/02 (1); Sydney/5/97 (1)	5	Hong Kong/330/01
2003	1	New Caledonia/20/99	2	Fujian/411/02 (1); Sydney/5/97 (1)

NOTE. NI, not identified.

^a Reference strain that was closest to the Indonesian isolate.

or epidemics caused by influenza. This is primarily a result of the lack of accessible and affordable testing modalities for confirmation in the clinical setting. On the basis of our findings, mnRT-PCR would provide the timeliest modality for influenza virus detection and serotype identification. However, mnRT-PCR is not a practical procedure for point-of-care diagnostic analysis in rural clinic settings. Although the Directigen test did not provide optimal performance, there is still a role for its use until more sensitive and improved rapid assays become available. If mnRT-PCR becomes the mainstay of influenza surveillance, it is vital to vaccine formulation efforts that selected positive samples be processed to attempt virus isolation and strain identification.

Of interest, the rapid test (Directigen Flu) demonstrated an overall low sensitivity (54.8%; 95% CI, 46%–63%) but high specificity and positive predictive values (table 4). However, the test performed well with regard to the detection of influenza A using the original manufactured version (Directigen Flu A; sensitivity, 93.3%; $\kappa = 0.82$). Reina et al. [12] showed that the test's ability to detect influenza B virus was poor, and the addition of influenza B virus antigens could explain the lower sensitivity of the newer kit (Directigen Flu A + B). However, when we analyzed our data for the ability of the rapid test to detect influenza A and B viruses, we found that the sensitivity for influenza A virus was 44.6% and for influenza B virus was 63% (data not shown).

The source of the specimen was also considered as a possible contributor to decreased performance of the rapid test in our study. The manufacturer reports higher sensitivity levels with various specimens (i.e., 95.7% for nasopharyngeal aspirates, 88.5% for nasopharyngeal washes and/or nasopharyngeal swabs, and 76.7% for throat and/or lower nasal swabs for influenza A). Although nasopharyngeal aspirates and washes are generally considered to be the optimal specimens, these procedures were not acceptable to most participants and are not considered to be a standard of practice in the care of outpatients by Indonesian clinicians; thus, they were not performed.

Because we selected outpatient clinics as sentinel sites, it was expected that no severe cases requiring hospitalization would be observed. Most participants displayed symptoms typical of those seen in temperate climates, with fever (97.8%), rhinorrhea (97%), and chills (94.1%) as the leading manifestations. However, fever, chills, malaise, headache, and anorexia were significantly more common among persons with influenza than among those with ILI ($P < .01$) (table 6). Several international case definitions of influenza include fever as an essential sign and variably include other symptoms (e.g., chills, cough, sore throat, headache, and myalgia), thereby making means of diagnosing influenza less universal [13]. Recently, Thursky et al. [13] proposed a simplified case definition consisting of cough, history of fever, and fatigue; their case definition had a higher positive predictive value in Western Australia than did defi-

Table 6. Signs and symptoms among study subjects, as assessed by questionnaire.

Sign or symptom	Subjects with influenza-like illness, %	Subjects with confirmed influenza, %	<i>P</i>
Fever	86.8	97.8	<.01
Chills	83.1	94.1	<.01
Headache	39.4	85.2	<.01
Myalgia	71.7	77.4	.16 ^a
Arthralgia	52.5	60.3	.08 ^a
Malaise	39.3	70.8	<.01
Anorexia	26.9	43.1	<.01
Cough	91.1	89.0	.41 ^a
Cough with sputum	73.7	64.7	<.05
Pharyngitis	53.3	56.7	.45 ^a
Rhinorrhea	93.1	97.0	.08 ^a

NOTE. Only 1103 questionnaires were completed among 1544 participants.

^a Not significant.

nitions published by the US Centers for Disease Control and Prevention and 3 other Australian surveillance systems. From our data, application of this proposed case definition in Indonesia could likely exclude up to 30% of influenza cases. No published standardized case definition specific for Indonesia exists. This study could be the framework for creating—or the impetus for adopting—a national case definition and possibly subsequent policies regarding treatment and planning for pandemics.

In summary, our surveillance conducted in Indonesia over a 4-year period provides valuable data and characterization of influenza viruses circulating at sentinel locations. The seasonality and circulation of respiratory viruses was suggested to occur year-round, similar to what has been observed in other tropical regions [14]. Both the influenza A (H1N1 and H3N2) and B viruses were present at all sites, and there was a tendency for peak prevalence to occur during the rainy seasons. Continued influenza surveillance (and surveillance for infections due to other respiratory pathogens), which also incorporates a measure of the burden of illness, will be useful and can help to strengthen the infrastructure of the Indonesian public health system, particularly in light of the recent epidemic of severe acute respiratory syndrome affecting many countries in Asia.

Acknowledgments

We would like to thank the staff of the Viral Diseases Program (in particular Ida Farida, Rismalia, and Shinta Pangaribuan) for their contributions to the identification of influenza virus, and we thank all the Indonesian physicians and nurses who supported this surveillance activity. We also wish to thank Mr. Andrew Whitehurst for his editorial assistance, Haditya L. Mukri and Kanti Laras for their assistance with graphics, and Dr. Curtis Hayes for his suggestions and comments regarding this manuscript.

Financial support. US Naval Medical Research Center (work unit 847705.82000.25GB.D0016 [GEIS]).

Conflict of interest. All authors: No conflict.

References

1. Wright PF, Webster RG. Orthomyxoviruses. In: Fields virology. 4th ed. Lippincott Williams & Wilkins, 2002:1533–79.
2. Horimoto T, Kawaoka Y. Pandemic threat posed by avian influenza A viruses. *Clin Micro Rev* 2001; 14:129–49.
3. Hotta S, Yamamoto M, Tokuchi M, Sakakibara S, Noerjasin B. Virologic-epidemiological studies on Indonesia IV. *Kobe J Med Sci* 1970; 16:251–9.
4. Gan KH, Gani KS, Hansen AL, Suharto, Sulastri D. Observations on the A2 (Hong Kong) 68-influenza epidemic of 1969/70 in Indonesia and Aruba (Netherlands-Antilles). *J Hyg Epidemiol Microbiol Immunol* 1971; 15:267–70.
5. Tjajik JK, Gani KS. An influenza epidemic in Medan. *Paediatrica Indonesiana* 1972; 12:510–4.
6. Ma'roef C, Sarbini S, Tan R, Bartz CR. Isolasi virus influenza dalam kultur sel dan embrio ayam [abstract]. In: Program abstracts of the Kongres dan Pertemuan Ilmiah Mikrobiologi dan Parasitologi Kedokteran Indonesia (Yogyakarta, Indonesia). 30 September 1986.
7. Corwin AL, Simanjuntak CH, Ingkokusumo G, et al. Impact of epidemic influenza A-like acute respiratory illness in a remote jungle highland population in Irian Jaya, Indonesia. *Clin Infect Dis* 1998; 26: 880–8.
8. Cross JH, Irving GS, Anderson KE, Gunawan S, Saroso JS. Biomedical survey in Irian Jaya (West Irian), Indonesia. *Southeast Asian J Trop Med Public Health* 1977; 8:532–6.
9. Zhang WD, Evans DH. Detection and identification of human influenza viruses by polymerase chain reaction. *J Virol Methods* 1991; 33: 165–89.
10. Blaine WB, Luby JP, Martin SM. Severe illness with influenza B. *Am J Med* 1980; 68:181–9.
11. World Health Organization Collaborating Centre for Reference and Research on Influenza. Annual report. Melbourne, Australia. 2002. Available at: <http://www.influenzacentre.org>. Accessed on 26 May 2003.
12. Reina J, Padilla E, Alonso F, Ruiz de Gopegui E, Munar M, Mari M. Evaluation of a new dot blot enzyme immunoassay (Directigen Flu A+B) for simultaneous and differential detection of influenza A and B virus antigens from respiratory samples. *J Clin Microbiol* 2002; 40: 3515–7.
13. Thursky K, Cordova SP, Smith D, Kelly H. Working towards a simple case definition for influenza surveillance. *J Clin Virol* 2003; 27:170–9.
14. Hampson AW. Epidemiological data in Asian countries. *Vaccine* 1999; 17(Suppl 1):S19–23.