A Nationally Coordinated Laboratory System for Human Avian Influenza A (H5N1) in Thailand: Program Design, Analysis, and Evaluation

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Background. The first phase of national surveillance for avian influenza (H5N1) human disease in Thailand occurred over a 4-month period that began on 1 December 2003. Subsequently, a nationally coordinated laboratory system (NCLS) for avian influenza (H5N1) was created to assess population-based surveillance, specimen procurement, case detection, and reporting at the national level.

Methods. We conducted a pre- and postintervention study to evaluate the NCLS designed during the 6-week interval from 1 April through 15 May 2004. During the pre-NCLS period (1 December 2003 through 31 March 2004), 12 cases of human avian influenza (H5N1) were confirmed. During the post-NCLS period (16 May 2004 through 31 December 2006), interventions were implemented for human avian influenza (H5N1) surveillance, case detection, and expedited, computer-based reporting.

Results. During the pre- and post-NCLS periods, 777 (85%) of 915 and 10,434 (95%) of 11,042 clinical respiratory specimens, respectively, were adequate for confirmatory testing (P < .001), the median time from procurement to results decreased from 17 days (range, 14–24 days) to 1.8 days (range, 0.25–4 days; P < .001), and the duration of specimen shipment decreased from 46.5 h to 21.1 h (P < .001). Thirteen cases of avian influenza (H5N1) were detected during the 31-month postintervention period. H5N1 reverse-transcriptase polymerase chain reaction and real-time reverse-transcriptase polymerase chain reaction sensitivity was 100% and specificity was 99.8%.

Conclusions. The NCLS exemplifies a systematic approach to national surveillance for avian influenza A (H5N1). This NCLS program in Thailand serves as a model for human avian influenza (H5N1) preparedness that can be adopted or modified for use in other countries.

The public health threat of avian influenza A (H5N1) transmission among poultry and from birds to humans has prompted global preparedness plans for this disease [1, 2]. Although human-to-human transmission of avian influenza A (H5N1) has not yet been efficient the theoretical risk exists and is compounded by recent isolation of a strain with potential for human-to-human transmission [3–5]. Key preparedness strategies include designated interdisciplinary planning committees, committee liaisons to external health entities, linkages with regional emergency preparedness groups, written pandemic influenza protocols, and plans for facility access, occupational health, mass distributions of vaccine and antiviral agents, and surge capacity. The development of new rapid diagnostic techniques for avian influenza A (H5N1) have emerged [6, 7]; however, limited data are available for optimizing inclusion of these tests in laboratory-based avian influenza A (H5N1) surveillance.

In Thailand, the first wave of the human avian influenza A (H5N1) pandemic began on 1 December 2003, when the first human case of H5N1 was detected by the Ministry of Public Health, and lasted 4 months.

Received 14 November 2007; accepted 18 December 2007; electronically published 26 March 2008.

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Clinical Infectious Diseases 2008;46:1394–1400

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DOI: 10.1086/586752

1394 • CID 2008;46 (1 May) • Kitphati et al.
A rapid surge in clinical specimens (blood, nasopharyngeal, and throat swab specimens) submitted to the Thai National Institute of Health (NIH) for H5N1 testing prompted the Thai NIH to establish the National Coordinating Laboratory System (NCLS) over a 6-week period. The mission of the NCLS was to standardize and improve administrative and laboratory-based efforts to screen respiratory specimens, optimize laboratory case detection, and report results. We conducted a pre- and postintervention study of the Thai NCLS for key laboratory-based surveillance, quality assurance, and outcome parameters for avian influenza (H5N1) preparedness.

METHODS

Preintervention interval. Nationwide surveillance to detect influenza A (H5N1) was initiated on 1 December 2003, after outbreaks of sudden death in poultry were reported in central Thailand [8]. Surveillance focused on all medical encounters for putative pneumonia or influenza-like illness with self-report of exposure to ill or dead poultry [8]. Patients at risk of infection were reported through the provincial public health office to (1) the regional disease prevention and control centers and (2) the Bureau of Epidemiology at the Ministry of Public Health. Throat and nasopharyngeal (NP) swab specimens or aspirate specimens were sent for viral culture to the Thai NIH of the Department of Medical Sciences. Initial screening, if possible, was performed as point-of-care rapid tests for H5N1 (SD BIOLINE Influenza Antigen A/B [MT Promedt Consulting] and QuickVue Influenza A+B test [Quidel]) at the referring hospitals and/or clinics. All specimens were confi med for the presence of H5N1 by viral culture. Field-based investigation occurred for all suspected and con fi med cases of avian influenza (H5N1) via face-to-face interviews by staff members from the provincial health office to assess putative exposures and the household environments.

The NCLS Program Design (1 April–15 May 2004). The Ministry of Public Health assembled an NCLS organizing committee composed of 5 scientists, 3 physicians, 2 technicians, 7 health care–affilate personnel, and 4 epidemiologists. The committee first convened with the objectives of optimized specimen collection and accountable reporting of results (figure 1). The committee met weekly—if not every other day—over the 6-week interval and identifi ed the 5 following core NCLS program components: (1) 1 national, centralized laboratory; (2) trained, dedicated personnel; (3) H5N1 laboratory surveillance of throat and NP specimens using RT-PCR, real-time RT-PCR, and viral culture; (4) laboratory reporting algorithms; and (5) a national, secured Web site to provide education regarding proper clinical specimen collection and for reporting the results [9]. The goals for the centralized laboratory were to improve processing of a high volume of clinical specimens, streamline specimen collection and transportation, evaluate rapid and con-

fi matory tests, and create systematic, expedited, computer-based reporting. According to the program protocol, the initial rapid tests (SD BIOLINE Influenza Antigen A/B [MT Promedt Consulting] and QuickVue Influenza A+B test [Quidel]) for avian influenza (H5N1) were point-of-care tests. Standard respiratory specimens were submitted to the Thai NIH and 13 Regional Medical Sciences Centers of the Department of Medical Sciences for H5 identification according to World Health Organization reference laboratory standards [10]. The committee determined that specimen specimens, if collected, would be stored but would not be a routine component of the surveillance program because of the real-time limitations for collection and testing of acute- and convalescent-phase paired serum specimens.

Preliminary evaluation of the rapid influenza A test after the first wave of the Thai H5N1 outbreak suggested low sensitivity (28%) [11]. This test performance prompted the Thai NIH to implement molecular diagnostic techniques to facilitate early diagnoses of avian influenza (H5N1). All influenza A clinical respiratory specimens (throat and NP swab specimens or aspirate specimens) positive for subtypes H1, H3, and H5 by specifi c PCR primers were further tested using RT-PCR and real-time RT-PCR primers specifi c to H5N1 (fi gure 1) [8, 12–14]. Viral isolation was also performed using standard methods [15]. Throat and NP specimens were considered to be positive for avian influenza virus if the viral culture result was positive and was confi med by immunofluorescent antibody with H5-specific monoclonal antibody (provided by the World Health Organization), if epithelial cells in clinical specimens were immunofluorescent esent antibody positive for H5-specific monoclonal antibody, if epithelial cells in clinical specimens were immunofluorescent antibody positive for H5, or if the RT-PCR (RT-PCR or real-time RT-PCR; using H5-specifi c primers) result was positive. A specimen was negative for avian influenza virus if the results of immunofluorescent antibody, RT-PCR or real-time RT-PCR, and viral isolation (second passage) were negative. If specimens were positive by RT-PCR and real-time RT-PCR and subsequently negative by viral culture, additional viral sequence analyses were performed for the specimen. If viral sequencing identifi ed H5, the specimen was determined to be H5N1 positive.

Surveillance and case definitions data collection, and measurement of outcomes. Patients with confi med cases of H5N1 were defi ned as patients with laboratory evidence of influenza A (H5N1) infection [8]. Patients with suspected cases were defi ned as patients with severe pneumonia or an influenza-like illness, reported exposure to ill or dead poultry, and laboratory evidence of influenza A not confi med as H5N1 [8, 16]. Excluded patients were those reported through the system who did not meet the defi nition for patients with confi med or suspected cases, including those with infections caused by influenza A H1 or H3 or other laboratory-confi med pneumonia pathogens. Data collection included demographic and
clinical data, specimen quality (e.g., inappropriate obtainment, incorrect containers, and total specimen shipment duration), number of specimens submitted per patient, sensitivity and specificity of the rapid test and RT-PCR, total laboratory turnaround time, specimen processing time, and estimated annual cost for program establishment (e.g., training personnel, establishing computer programs and the computerized network, computers, administration fees, and conference expenses), and maintenance of the NCLS program (e.g., salaries, logistics and equipment, field investigations for specimen obtainment, Web site maintenance, administration fees, and conference expenses). The outcomes measured included the quality, accuracy, and time from specimen obtainment to availability of the final report. The measurements of specimen quality were evaluated externally and internally. The external evaluation included assessment of specimens by type, containment, and adequacy for
further testing. The internal evaluation included assessment of the quality of the specimens with use of the human β-actin gene [17]. The sensitivity and specificity of the rapid tests were compared with the those of viral culture (the gold standard). Time-motion studies were measured at the 3 following points: (1) period from specimen obtainment to arrival at the central laboratory (pretest period), (2) period from specimen arrival to completion of testing (testing period), and (3) period after completion of testing to availability of the final report in the computer-based reporting system (posttest period). To optimize case detection and reporting, an NCLS expert panel was established to review the clinical, epidemiological, and laboratory data before the reporting of a confirmed avian influenza (H5N1) case. The panel membership included 2 virologists, an infectious diseases specialist, a senior epidemiologist, a representative from the Thai NIH, and a representative from the Ministry of Public Health.

**Data analysis.** Categorical variables were presented as absolute values, and percentages were compared using the χ² test or Fisher’s exact test, as appropriate. Continuous variables were expressed as median values. The Wilcoxon rank sum test was used to compare continuous variables. All tests were 2-tailed. P < .05 was considered to be statistically significant Cost estimates were based on a conversion value, with 35 bahts being equivalent to US$1.

**RESULTS**

**Pre-NCLS phase.** During the initial 4-month phase of national surveillance for human avian influenza (H5N1), 915 throat and NP specimens from 610 patients (range, 1–3 specimens per patient) were submitted (table 1). The epidemiological investigation is reported elsewhere [8]. Among 580 patients with suspected avian influenza (H5N1) and 30 with probable avian influenza (H5N1), 12 (2%) had H5N1 infection confirmed by RT-PCR, real-time RT-PCR, and/or viral culture. All 12 patients with confirmed cases had positive results of culture for avian influenza (H5N1). Seven (58%) of these 12 patients had both NP rapid tests and viral culture performed, and 2 (28.5%) of these 7 patients had positive NP rapid test results.

**Post-NCLS phase.** After the establishment of the NCLS, 11,042 specimens from 4417 patients were submitted for avian influenza (H5N1) testing during the 31-month period (table 1). Thirteen patients (0.3%) had H5N1 infection confirmed by RT-PCR, real-time RT-PCR, and/or viral culture. All 13 patients with confirmed cases had positive avian influenza (H5N1) viral culture results; no temporal or geospatial cluster patterns were noted in the epidemiological investigations of the confirmed and suspected cases. Ten (77%) of the 13 patients with confirmed H5N1 infection had specimens submitted for both NP rapid tests and viral culture, and 3 (23%) of these patients had positive H5N1 NP rapid test results. Of note, 1 patient with H5N1 infection received a neuraminidase inhibitor >48 h before specimen collection and had a negative rapid NP test result.

**Program evaluation of rapid tests and NCLS quality indicators.** The sensitivity and specificity of combined RT-PCR and real-time RT-PCR testing of the respiratory specimens for avian influenza (H5N1) were 100% and 99.8%, respectively (table 2). During the pre- and postintervention periods, 777 (85%) of 915 and 10,434 (95%) of 11,042 clinical specimens, respectively, were adequate for confirmatory testing (P < .001), the median time from specimen collection to results decreased from 17 days (range, 14–24 days) to 1.8 days (range, 0.25–4 days; P < .001), and the duration of specimen shipment decreased from 46.5 h to 21.1 h (P < .001). The total laboratory turnaround time was 1.8 days (range, 0.25–7 days). The laboratory turnaround time was 0.4 days (range, 0.25–1 days) during the pretest period, 1.2 days (range, 0.6–5 days) during the posttest period, and 0.2 days (range, 0.1–0.8 days) during the posttest period. The estimated total annual cost for establishment of the NCLS was $17,113 (training personnel accounted for $4285, establishing computer programs and computerized networks cost $4300, computers cost $5715, and administrative fees and conference expenses accounted for $2813), with an estimated annual maintenance cost of $14,256 (salaries accounted for $5850, logistics and equipment cost $2850, field investigations for specimen collection cost $2070, Web site maintenance accounted for $1550, and administrative fees and conference expenses accounted for $1936). The perceived benefit of laboratory-based surveillance, compared with the traditional epidemiological surveillance methods and resources, included reduction in community-based assessments, improved specimen collection, and optimized administrative and laboratory algorithms.

**DISCUSSION**

The development and implementation of the Thai NCLS for avian influenza (H5N1) provides a model program of laboratory-based surveillance, case detection, and expedited reporting of human disease. This program can be readily adopted or modified to resource-adequate and resource-limited settings to enhance avian influenza (H5N1) global preparedness plans. Initially, this program was created after a high rate of poultry deaths prompted a national surveillance effort for human disease that identified avian influenza (H5N1) in 5 adults and 7 children [8]. Notably, the study linked human disease to direct contact with sick poultry, young age, pneumonia, lymphopenia, and rapid progression to respiratory failure [8] and was followed by several clinical investigations of avian influenza (H5N1) in Southeast Asia [3–5, 11, 16, 18–21].

The design and implementation of the NCLS program now characterizes the foundation of a systematic, efficient and rel-
Table 1. Demographic and clinical characteristics of 5027 persons screened for avian influenza (H5N1) before and after implementation of the Thai nationally coordinated laboratory system.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Preintervention period&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Postintervention period&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 915)</td>
<td>(n = 11,042)</td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td>14 (1–65)</td>
<td>21 (2–70)</td>
</tr>
<tr>
<td>Male sex, proportion (%) of patients</td>
<td>366/610 (60)</td>
<td>2739/4417 (62)</td>
</tr>
<tr>
<td>Index of suspicion for avian influenza (H5N1), no. (%) of specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspected</td>
<td>823 (90)</td>
<td>10,950 (99)</td>
</tr>
<tr>
<td>Probable</td>
<td>60 (7)</td>
<td>52 (0.5)</td>
</tr>
<tr>
<td>Confirmed</td>
<td>12 (1.3)</td>
<td>13 (0.1)</td>
</tr>
<tr>
<td>Specimen collection, no. of specimens per day (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimens submitted for testing</td>
<td>40 (10–140)</td>
<td>45 (20–145)</td>
</tr>
<tr>
<td>Specimens tested for avian influenza (H5N1)</td>
<td>35 (8–120)</td>
<td>39 (14–128)</td>
</tr>
<tr>
<td>Results that need to be reported</td>
<td>32 (6–115)</td>
<td>35 (7–110)</td>
</tr>
<tr>
<td>Telephone call for laboratory results</td>
<td>Not applicable</td>
<td>25 (5–150)</td>
</tr>
<tr>
<td>Median no. of specimens per patient with a suspected case (range)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.2 (1–3)</td>
<td>1.8 (1–6)</td>
</tr>
<tr>
<td>No. of mortalities</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

<sup>a</sup> From 1 January 1 through 30 April 2004.

<sup>b</sup> From 16 May 2004 through 31 December 2006.

<sup>c</sup> \( P<.05 \).

atively cost-efficient program. Notably, 12 confirmed cases were identified during the 121-day pre-NCLS period (99 cases per 1000 days), compared with 13 confirmed cases identified during the 959-day post-NCLS period (13.6 cases per 1000 days). Mortality among patients with confirmed cases was similar for both periods (67% and 69%, respectively) (table 1). Contributions to the significant decrease in case detection during the post-NCLS period likely reflect a multitude of interventions within and beyond the NCLS program. Inherent in the NCLS design, expanded surveillance, case detection, medical education, and public awareness have integrated roles, providing an invaluable infrastructure if a human avian influenza (H5N1) pandemic were to emerge. All countries involved in such a pandemic would need to rapidly engage such a program, with coordinated mass screening and case containment as critical components of global avian influenza (H5N1) preparedness.

As with all studies, recognized limitations to the assessment of the NCLS program exist. First, laboratory surveillance for human avian influenza (H5N1) was restricted to throat and NP swab specimens and tracheal aspirate specimens. Blood specimens, although sometimes submitted, were not prospectively evaluated because of the limitations of serologic testing of paired acute- and convalescent-phase specimens. There are no current data to suggest that case detection has been underestimated by the laboratory-based algorithms. Second, routine use of the rapid tests at the point-of-care visit remained inconsistent across the country, exemplifying the continued need for ongoing field-based education and use of the national Web site to optimize appropriate specimen collection. Third, we have limited data on the empirical and preemptive use of antiviral therapy and the potential impact of such practices on specimen collection and on the use of diagnostic tests. A noted trend has been for physicians to prescribe antiviral therapy to the index patient before specimens are obtained [11].

From the laboratory perspective, rapid diagnostic tests for influenza A and avian influenza (H5N1) have technical limitations [11, 22, 23]. In general, these limitations include variation in rapid diagnostic test sensitivity, specimen collection techniques, and consistent containment and transportation of specimens. Furthermore, several reports also suggested the low sensitivity of rapid influenza tests to detect H5N1 [5, 11, 22]. Therefore, the use of molecular techniques with shorter turnaround times can facilitate early diagnosis [24], proper management and early control strategies [21], and expanded surveillance. In addition, obtaining multiple specimens per patient was used as an NCLS strategy to enhance case detection. Three major modifications to enhance NCLS program capacity have occurred since program inception. First, an expert review panel was created to monitor core administrative and laboratory-based policies and changes over time. Second, 50 surveillance and rapid response team trainees were hired to promote public awareness of human and avian influenza (H5N1), assess appropriate specimen collection, and have readily accessible skills in outbreak investigations and epidemiological assessments. Lastly, 4 additional mobile laboratory units opened during 2006–2007 to maintain high-volume surveillance. Although the NCLS surveillance definition of a probable case has low specificity, the laboratory algorithm and programmatic goals remain focused on extensive national surveillance and early case detection. The estimated costs for the maintenance
Table 2. Comparison of specimen collection and laboratory processes before and after implementation of the Thai nationally coordinated laboratory system for avian influenza (H5N1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preintervention period&lt;sup&gt;a&lt;/sup&gt; (n = 915)</th>
<th>Postintervention period&lt;sup&gt;b&lt;/sup&gt; (n = 11,042)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen quality, no. (%) of specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inappropriate specimen collection&lt;sup&gt;c&lt;/sup&gt;</td>
<td>128 (14)</td>
<td>552 (5)</td>
</tr>
<tr>
<td>Incorrect specimen collection&lt;sup&gt;e&lt;/sup&gt;</td>
<td>92 (10)</td>
<td>110 (1)</td>
</tr>
<tr>
<td>Specimen transportation time,&lt;sup&gt;c,d&lt;/sup&gt; median h (range)</td>
<td>46 (6–72)</td>
<td>21.5 (2–24)</td>
</tr>
<tr>
<td>RT-PCR or real-time RT-PCR testing of NP specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>NA</td>
<td>100</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>NA</td>
<td>99.8</td>
</tr>
<tr>
<td>Total laboratory turnaround time,&lt;sup&gt;c&lt;/sup&gt; median days (range)</td>
<td>17 (14–24)</td>
<td>1.8 (0.25–7)</td>
</tr>
<tr>
<td>Processing time, median days (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretest period&lt;sup&gt;e&lt;/sup&gt;</td>
<td>NA</td>
<td>0.4 (0.25–1)</td>
</tr>
<tr>
<td>Testing period&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
<td>1.2 (0.6–5)</td>
</tr>
<tr>
<td>Posttest period&lt;sup&gt;g&lt;/sup&gt;</td>
<td>NA</td>
<td>0.4 (0.1–0.8)</td>
</tr>
</tbody>
</table>

NOTE. NA, not applicable; NP, nasopharyngeal.

<sup>a</sup> From 1 January through 30 April 2004.
<sup>b</sup> From 16 May 2004 through 31 December 2006.
<sup>c</sup> P<sub>c</sub>.05.
<sup>d</sup> Defined as duration >24 h after specimen collection.
<sup>e</sup> Period from specimen collection to arrival at the laboratory coordinating center.
<sup>f</sup> Period from specimen arrival until testing was completed.
<sup>g</sup> Period from testing completion until reporting into the computer-based system.

of the NCLS program are $14,256 annually. Because Thailand is a middle-income country where the overall estimated costs are inexpensive, compared with those in developed countries, our estimated annual costs for establishment and maintenance of the NCLS program may not be generalizable to other developed nations.

Current efforts are being directed toward integrating the NCLS with a geographic information system to help monitor and distinguish avian influenza A (H5N1) rates among poultry and humans. It is anticipated that such a system will aid geospatial and temporal tracking of human and animal disease, permit modeling of disease transmission and real-time surveillance, and serve as a resource for other emerging infectious diseases. In conclusion, the NCLS program in Thailand plays an integrated role in Southeast Asian avian influenza (H5N1) pandemic preparedness. Despite recognized program limitations, the relative costs and benefit associated with this program justify national surveillance in this middle-income country. This system offers flexibility for capacity building and application to both infectious and noninfectious preparedness and serves as a model for avian influenza laboratory-based preparedness.

**Acknowledgments**

We thank Dr. Paijit Warachit, Director General of the Ministry of Public Health, for his thoughtful suggestions and support during the avian influenza outbreaks.

**Financial support.** National Center and Genetic Engineering and Biotechnology, National Science and Technology Development Agency (BT-B-01-MG-13-5019 to A.A.).

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