

## Incidence of respiratory pathogens in persons hospitalized with pneumonia in two provinces in Thailand

S. J. OLSEN<sup>1\*</sup>, S. THAMTHITIWAT<sup>2</sup>, S. CHANTRA<sup>3</sup>, M. CHITTAGANPITCH<sup>4</sup>,  
A. M. FRY<sup>5</sup>, J. M. SIMMERMAN<sup>5</sup>, H. C. BAGGETT<sup>2</sup>, T. C. T. PERET<sup>6</sup>,  
D. ERDMAN<sup>6</sup>, R. BENSON<sup>7</sup>, D. TALKINGTON<sup>8</sup>, L. THACKER<sup>7</sup>,  
M. L. TONDELLA<sup>7</sup>, J. WINCHELL<sup>7</sup>, B. FIELDS<sup>7</sup>, W. L. NICHOLSON<sup>9</sup>,  
S. MALONEY<sup>2</sup>, L. F. PERUSKI<sup>2</sup>, K. UNGCHUSAK<sup>10</sup>, P. SAWANPANYALERT<sup>4</sup>  
AND S. F. DOWELL<sup>11</sup>

<sup>1</sup> Division of Emerging Infections and Surveillance Services, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; <sup>2</sup> International Emerging Infections Program, Thailand MOPH – US CDC Collaboration, Nonthaburi, Thailand; <sup>3</sup> Crown Prince Hospital, Sa Kaeo, Thailand; <sup>4</sup> National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand; <sup>5</sup> Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; <sup>6</sup> Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; <sup>7</sup> Division of Bacterial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; <sup>8</sup> Division of Foodborne, Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; <sup>9</sup> Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; <sup>10</sup> Bureau of Epidemiology, Ministry of Public Health, Thailand; <sup>11</sup> Division of Global Disease Detection and Emergency Response, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

(Accepted 1 March 2010; first published online 31 March 2010)

### SUMMARY

Although pneumonia is a leading cause of death from infectious disease worldwide, comprehensive information about its causes and incidence in low- and middle-income countries is lacking. Active surveillance of hospitalized patients with pneumonia is ongoing in Thailand. Consenting patients are tested for seven bacterial and 14 viral respiratory pathogens by PCR and viral culture on nasopharyngeal swab specimens, serology on acute/convalescent sera, sputum smears and antigen detection tests on urine. Between September 2003 and December 2005, there were 1730 episodes of radiographically confirmed pneumonia (34·6% in children aged <5 years); 66 patients (3·8%) died. A recognized pathogen was identified in 42·5% of episodes. Respiratory syncytial virus (RSV) infection was associated with 16·7% of all pneumonias, 41·2% in children. The viral pathogen with the highest incidence in children aged <5 years was RSV (417·1/100 000 per year) and in persons aged ≥50 years, influenza virus A (38·8/100 000 per year). These data can help guide health policy towards effective prevention strategies.

**Key words:** Aetiology, epidemiology, pneumonia, Thailand.

### INTRODUCTION

Pneumonia remains a major global public health problem, and an important focus for scientific study

[1]. Identifying causative agents can be a challenge because the lung itself is rarely sampled and culturing of blood or respiratory secretions can be insensitive or difficult to interpret [2]. There is still a paucity of data

\* Author for correspondence: S. J. Olsen, Ph.D., Centers for Disease Control and Prevention 1600 Clifton Road, MS C12, Atlanta, GA 30333, USA. (Email: SOlsen@cdc.gov)

from low- and middle-income countries, particularly in tropical and subtropical areas, and it is likely that the causative agents, their seasonal and age distributions, and other aspects of pneumonia epidemiology differ in these settings and require tailored prevention and control strategies.

Beginning in 2002, the Thailand Ministry of Public Health and the U.S. Centers for Disease Control and Prevention (CDC) combined resources to establish active pneumonia surveillance for persons of all ages in two Thai provinces [3]. Global events such as the SARS outbreak, avian influenza A (H5N1) and 2009 influenza A (H1N1) infections in humans fuelled interest in and funding for influenza and other viruses that previously received little attention [4–8]. The threat of emerging pathogens presenting as pneumonia emphasized the importance of effective epidemiological and clinical surveillance integrated with reliable laboratory diagnostics. Here we present results for the first 30 months of active pneumonia surveillance in Thailand, focusing on the incidence of viral and atypical bacterial pathogens.

## METHODS

### Setting

Thailand is a middle-income country with a gross national income of U.S. \$2990 *per capita*, a low infant mortality rate (7 deaths/1000 live births) and an average life expectancy of 70 years [9]. It has a tropical climate with a mid-May to September monsoon season. An enhanced surveillance system to actively identify prospective cases of pneumonia requiring hospitalization began in Sa Kaeo province in 2002 and was expanded to Nakhon Phanom province in 2003. Sa Kaeo (population 514 065 in 2003) is in eastern Thailand and borders Cambodia, and Nakhon Phanom (population 730 659 in 2005) is in northeast Thailand and borders Laos. The combined population of these two provinces accounts for ~2% of the total population of Thailand.

### Study population

Pneumonia surveillance is described in detail elsewhere [3]. Briefly, surveillance officers actively identified patients hospitalized at any of the eight hospitals (one provincial, six district and one military) in Sa Kaeo province and any of the 12 hospitals (one

provincial, ten district and one military) in Nakhon Phanom province. There are no private hospitals in these two provinces and through community surveys we know that the hospitalized surveillance system captures 58–80% of all pneumonias in these two provinces [10, 11]. Surveillance identified patients with active infection (either reported fever, reported chills, measured temperature  $>38.2$  °C or  $<35.5$  °C, or an abnormal white blood cell count or differential), and lower respiratory tract disease (either abnormal breath sounds, documented tachypnoea, observed cough, sputum production, or dyspnoea). Basic demographic, clinical and laboratory findings were collected on a standard surveillance form. Patients who had a chest radiograph performed within 48 h of admission were approached for enrolment into the aetiological study. Chest radiographs were digitized and sent to Bangkok to be interpreted by a panel of radiologists using WHO criteria as previously described [12]. Here we analysed data from a period when complete viral aetiological information was available – between 1 September 2003 and 31 August 2005 (2 years for Sa Kaeo) and between 1 January and 31 December 2005 (1 year for Nakhon Phanom). This study was approved by a CDC Institutional Review Board (protocol no. 3754) and the Ethical Review Board of the Thailand Ministry of Public Health.

### Specimen collection

Consenting patients provided nasopharyngeal swab, urine, and acute and convalescent serum specimens [13]. Briefly, within 48 h of admission and after informed consent, one Dacron<sup>®</sup> swab was placed into one nostril to the nasopharynx and rotated once to collect epithelial tissue and absorb secretions. Swabs were placed in viral transport media, refrigerated at 2–8 °C for up to 48 h, and then the media was aliquoted and stored at –70 °C. A blood sample was collected at the time of enrolment and again 3 weeks later (5 ml of blood from children aged  $<3$  years and 10 ml for all others); serum was aliquoted and stored at –70 °C in Thailand and –20 °C after transfer to the USA. A 10-ml urine sample was collected and refrigerated at 2–8 °C at each hospital until it was transported to the provincial hospitals each day and frozen at –70 °C. Specimens were transported on dry ice once weekly to the laboratory in Bangkok for specimen processing and shipping to CDC in Atlanta, GA, USA.

Table 1. Laboratory methods used to identify respiratory pathogens

Respiratory pathogen	Assay	Specimen type	Ages tested	Dates tested (if limited)
<i>Chlamydia pneumoniae</i>	Focus Diagnostics <sup>®</sup> microimmunofluorescence assay (IgG and IgM)	Serum	All	Sept. 2003–Aug. 2004
<i>Coxiella burnetii</i>	PCR	NP swab	All	Sept. 2003–Aug. 2005
<i>Legionella longbeachae</i>	ELISA (IgG and IgM)	Serum	All	Sept. 2003–Aug. 2004
<i>Legionella pneumophila</i> serogroup 1	Indirect immunofluorescence assay (IgG, IgA, IgM)	Serum	≥18 yr	Sept. 2003–Aug. 2005
<i>Mycoplasma pneumoniae</i>	BinaxNOW <sup>®</sup> rapid immunochromatographic assay	Urine	≥18 yr	Sept. 2003–Aug. 2004
<i>Mycobacterium tuberculosis</i>	Remel <sup>®</sup> ELISA (IgG and IgM)	Serum	All	Sept. 2003–Aug. 2004
<i>Streptococcus pneumoniae</i>	PCR	NP swab	All	Sept. 2003–Aug. 2005
	Acid fast bacilli smear	Sputum	All	
Adenoviruses	BinaxNOW <sup>®</sup> rapid immunochromatographic assay	Urine	≥18 yr	
Bocavirus	Culture, RT–PCR	NP swab	All	
Coronaviruses (229E, HKU1, NL63, OC43)	ELISA (IgG)	Serum	All	Sept. 2003–Aug. 2004
Influenza viruses (A and B)	RT–PCR	NP swab	All	Sept. 2004–Aug. 2005
Metapneumovirus	RT–PCR	NP swab	All	Sept. 2003–Aug. 2005
Parainfluenza viruses 1–3	Culture, RT–PCR	NP swab	All	
Respiratory syncytial virus	ELISA (IgG)	Serum	All	Sept. 2003–Apr. 2005
Rhinoviruses	Culture, RT–PCR	NP swab	All	Sept. 2003–Aug. 2004
	ELISA (IgG)	Serum	All	Sept. 2003–Apr. 2005
	RT–PCR	NP swab	All	Sept. 2003–Aug. 2005

NP, Nasopharyngeal; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; RT–PCR, reverse transcriptase–polymerase chain reaction.

### Laboratory tests

The summary of laboratory testing methods is shown in Table 1. Methods used include currently available diagnostic assays for use in the hospital setting in rural Thailand, commercially available assays, as well as research assays developed by CDC.

#### Hospital tests

Testing for *Mycobacterium tuberculosis* was performed by acid fast bacilli (AFB) smear of patient sputum in the hospital laboratory at the discretion of the attending physician. HIV testing was performed using the local hospital's routine method which included ELISA, Western blot, radioimmunoprecipitation, immunofluorescence assay (IFA) or gelatin particle agglutination. HIV testing was performed at the discretion of the doctor until January 2005, when we began to actively approach patients for their

consent. Positive tests were reported to the patient's physician and counselling was provided by the hospital according to established local practices. HIV-positive patients were referred into the existing Thailand health system for management. Hospitals did not routinely perform blood cultures.

#### Rapid antigen tests

BinaxNOW<sup>®</sup> rapid immunochromatographic assays (Binax Inc., USA) were used to detect *Streptococcus pneumoniae* and *Legionella pneumophila* serogroup 1 antigen in patients' urine. Laboratory technicians followed the manufacturer's instructions. Only patients aged ≥18 years were tested for *S. pneumoniae* and *L. pneumophila*.

#### Viral cultures

Culture for influenza viruses, respiratory syncytial virus (RSV), adenovirus, parainfluenza viruses and

metapneumovirus was performed at the Thailand National Institute of Health laboratories. The supernatant from nasopharyngeal swabs was inoculated in Madin–Darby canine kidney cells (MDCK), Hep-2 human epithelial cells (HEp-2) and *Macaca mulatta* (rhesus monkey) kidney cells (LLC-MK2). Cells were examined daily for cytopathic effect (CPE). Cells showing CPE were tested immediately by commercial IFA (Chemicon International Inc., USA) for the five groups of viruses being investigated. Cultures showing no CPE after 7–10 days' incubation were passed into fresh cells and monitored as above. If no CPE developed after the second passage, all cells were harvested and reconfirmed by IFA using pooled monoclonal antibodies; positives were further tested with specific monoclonal antibodies.

#### *Polymerase chain reaction (PCR) assays*

PCR was performed only on nasopharyngeal samples. Reverse transcriptase–polymerase chain reaction (RT–PCR) assays were developed at CDC to detect RSV, parainfluenza viruses 1, 2 and 3 (HPIV1, 2, 3), influenza viruses types A and B, rhinoviruses, metapneumovirus, adenoviruses, bocavirus and coronaviruses. Samples collected during the 2 years in Sa Kaeo and first 6 months in Nakhon Phanom were tested by GeneScan RT–PCR assays as previously described [14]. Samples collected during the last 6 months in Nakhon Phanom were tested by newly developed real-time RT–PCR assays. In direct comparisons with GenScan RT–PCR assays, the real-time RT–PCR assays showed comparable sensitivity with serially diluted viral nucleic acid and gave identical results with a sample of GenScan RT–PCR positive and negative study samples (D. Erdman, CDC, personal communication). To identify rhinoviruses, picornavirus-positive samples were subjected to a second round of semi-nested PCR using primers specific for rhinovirus [15]. PCR assays were recently developed for bocavirus [16] and coronaviruses 229E, OC43, HKU1, and NL63 [17]. Real-time PCR assays for the detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* were developed at CDC [18, 19].

#### *Serology*

*Chlamydia pneumoniae* serology (IgG and IgM) was performed using a microimmunofluorescence assay (Focus Diagnostics, USA). *Legionella longbeachae* combined IgG, IgA and IgM titres were measured

using an indirect IFA (only patients aged  $\geq 18$  years) [20]. *Mycoplasma pneumoniae* serology (IgG and IgM) was performed using a semi-quantitative ELISA (Remel, USA). *Coxiella burnetii* serology (IgM and IgG) was measured by ELISAs [21, 22]. Influenza serology was performed with the haemagglutinin inhibition test. RSV, HPIV 1, 2 and 3, adenovirus and metapneumovirus IgG antibodies were tested by ELISA [23–25].

#### **Analysis and definitions**

Mid-year population estimates for Sa Kaeo and Nakhon Phanom were obtained using data from the National Economic and Social Development Board of Thailand [26]. Crude incidence was calculated as a population-weighted average for the 6 months when both sites had data (January–July 2005). We adjusted the incidence for enrolment by multiplying the number of patients eligible by the proportion that tested positive for a given pathogen in those patients enrolled. Monthly unadjusted incidence is shown only for viruses with a monthly incidence above 1/100 000 (excludes parainfluenzaviruses 2 and 3, and the coronaviruses). Recurrent pneumonia was defined as two episodes of pneumonia separated by at least 14 days. Viral infections were determined by a positive RT–PCR result, positive viral culture or fourfold rise in antibody titre. HIV was considered positive if any of the hospital tests were reported as positive. *Legionella pneumophila* infections were determined by urine antigen test positivity and *L. longbeachae* by a fourfold rise in antibodies or PCR positivity. *C. pneumoniae* infections were determined by a positive PCR or serology result (fourfold rise in IgG titre and/or IgM  $\geq 16$ ) [27]. *M. pneumoniae* infections were defined as a seroconversion from negative in acute serum to positive in convalescent serum, or PCR positivity. Tuberculosis was defined using the WHO definition of at least two AFB sputum smear-positive results or one positive smear with an abnormal chest radiograph [28]. To distinguish recognized pathogens from agents with an unclear pathogenic role in pneumonia, we defined a category named 'recognized pneumonia aetiologies' which included any of the respiratory pathogens except bocavirus, coronaviruses and rhinoviruses. Proportions were compared using  $\chi^2$  and differences in means using the *t* test. All analyses were performed using SPSS version 15.0 (SPSS Inc., USA).

## RESULTS

Between September 2003 and December 2005, surveillance officers identified 15 403 patient admissions with active infection and lower respiratory tract disease. Of these, 8351 (54%) received a chest radiograph and were approached for consent and enrolment into the aetiological study; 3910 (46.8%) were enrolled. Enrolled patients were more likely to be older (median age 32 vs. 24 years,  $P=0.001$ ) than those who were not enrolled [3, 29].

A chest radiograph was available for reading by the radiologist panel for 2444 (62.5%) of pneumonia episodes. Of those read, 1730 radiographs in 1689 patients (70.8%) had evidence of pneumonia; 1293 (74.7%) of the abnormal radiographs showed interstitial infiltrates, 1047 (60.5%) showed alveolar infiltrates (675 showed evidence of both interstitial and alveolar infiltrates) and 65 (3.8%) had other evidence of pneumonia. Of the 1730 episodes of radiographically confirmed pneumonia, 599 (34.6%) were in children aged <5 years and 1013 (58.6%) were in males. The median length of hospital stay was 5 days (range 1–139). A total of 114 (6.6%) patients had evidence of HIV infection [34/695 (4.9%) patients were tested as part of the study and an additional 80 had evidence of previous testing or clinical evidence in the medical chart]. Of the 114 with any evidence of having HIV, four were aged <5 years, five were aged 5–17, 92 were aged 18–49 and 13 were aged  $\geq 50$  years.

### Aetiological agents and incidence

Of the 1730 episodes of radiographically confirmed pneumonia, 231 (13.4%) had a bacterial agent identified, 746 (43.1%) had a viral agent identified, and 176 (10.2%) had mixed infections (Table 2). Of pneumonia episodes in children aged <5 years, 2.2% had a bacterial agent identified, 75.6% a viral agent and 15.2% had mixed infections. Children aged <5 years had a higher proportion of pathogens identified compared to all other ages; the difference was statistically significant ( $P<0.001$ ).

Crude and adjusted pathogen-specific incidence in radiographically confirmed pneumonia is shown in Table 3 [Supplementary Table S1 (available online) presents incidence in all patients enrolled]. Of children aged <5 years, RSV had the highest adjusted weighted average annual incidence (all values are per 100 000) (417.1), followed by rhinoviruses (240.9),

bocavirus (114.6), and influenza A (90.2). In persons aged 5–49 years, rhinovirus incidence was highest (7.2), followed by *M. tuberculosis* (5.7), influenza A (3.9) and RSV (3.2). Of persons aged  $\geq 50$  years, influenza A incidence was the highest (38.8) followed by rhinoviruses (37.1), *S. pneumoniae* (34.7) and *M. tuberculosis* (30.5).

### Mixed viral infections

Overall, 5.0% (87/1730) of all episodes of radiographically confirmed pneumonia (11.7% of episodes in children aged <5 years) had more than one virus identified (Table 4). A rhinovirus was identified in 54.0% (47/97) of all mixed infections.

### Seasonality

The monthly incidence of radiographically confirmed pneumonia peaked twice each year, in January–March and June–September (Fig. 1). The peak in January–March included pneumonia patients with parainfluenza virus 3 and bocavirus. In contrast, the June–September peak included pneumonia patients with RSV, metapneumovirus and adenovirus. Influenza viruses A and B and bocavirus had peaks in both pneumonia seasons. June–August 2005 had the highest incidence of RSV infection; the all-age monthly incidence peaked in July at 9.0/100 000. Rhinoviruses were identified throughout the study period with no distinct seasonality.

### Pneumonia mortality

Overall, 66 (3.8%) pneumonia episodes were fatal, but this varied by year and site [28/478 (5.9%) in Sa Kaeo year 1, 29/512 (5.7%) in Sa Kaeo year 2 and 9/740 (1.2%) in Nakhon Phanom]. Of the 66 pneumonia deaths, 40 (60.6%) were male and the median age was 69 years (range 4 months to 90 years). Three deaths (4.5%) were in children aged <5 years. Fifteen (22.7%) persons had evidence of HIV infection. Fifteen (22.7%) of the patients who died had at least one respiratory pathogen identified: four had evidence of rhinoviruses (one also with HIV), five had evidence of tuberculosis (two also with HIV, one with coronaviruses 229 and OC43), four of pneumococcus (one also with HIV) and two of influenza A (one also with *C. pneumoniae*). There was no identified aetiology in the three deaths in young children. Of patients who died, the median length of hospital stay

Table 2. Number and percent of respiratory pathogens by age group detected in specimens from 30 months of pneumonia surveillance in Thailand, 1 September 2003 to 31 December 2005. Denominators are listed in cells when testing was not complete for the 30 months

Respiratory pathogen	Number (%) positive in patients with pneumonia									
	Total		< 5 yr		5–17 yr		18–49 yr		≥ 50 yr	
	Enrolled (n=3910)	CXR pos. (n=1730)	Enrolled (n=1325)	CXR pos. (n=599)	Enrolled (n=408)	CXR pos. (n=108)	Enrolled (n=705)	CXR pos. (n=322)	Enrolled (n=1472)	CXR pos. (n=701)
<i>Chlamydia pneumoniae</i>	92/3417 (2.7)	57/1583 (3.6)	13/1152 (1.1)	6/547 (1.1)	3/365 (0.8)	1/97 (1.0)	24/622 (3.9)	13/296 (4.4)	52/1278 (4.1)	37/643 (5.8)
<i>Coxiella burnetii</i>	3/755 (0.4)	1/478 (0.2)	1/150 (0.7)	0/87	0/49	0/17	1/201 (0.5)	0/120	1/355 (0.3)	0/245
<i>Legionella longbeachae</i>	20/755 (2.6)	14/478 (2.9)	n.t.	n.t.	n.t.	n.t.	7/201 (3.5)	4/129 (3.1)	13/355 (3.7)	10/245 (4.1)
<i>Legionella pneumophila</i> serogroup 1	0/755	0/478	n.t.	n.t.	n.t.	n.t.	0/201	0/129	0/355	0/245
<i>Mycoplasma pneumoniae</i>	38/3417 (1.1)	23/1583 (1.5)	11/1152 (1.0)	7/547 (1.3)	12/365 (3.3)	8/97 (8.2)	8/622 (1.3)	5/296 (1.7)	7/1278 (0.5)	3/643 (0.5)
<i>Mycobacterium tuberculosis</i>	92 (2.4)	81 (4.7)	0	0	3 (0.7)	1 (0.9)	38 (5.4)	33 (10.2)	52 (3.5)	47 (6.7)
<i>Streptococcus pneumoniae</i>	131 (3.4)	72 (4.2)	n.t.	n.t.	n.t.	n.t.	32 (4.5)	18 (5.6)	99 (6.7)	54 (7.7)
Adenoviruses	100 (2.6)	43 (2.5)	70 (5.3)	31 (5.2)	8 (2.0)	1 (0.9)	6 (0.9)	2 (0.6)	16 (1.1)	9 (1.3)
Bocavirus	53/1165 (4.5)	21/512 (4.1)	44/379 (11.6)	19/190 (10.0)	4/118 (3.4)	0/30	2/215 (0.9)	1/91 (1.1)	3/453 (0.7)	1/201 (0.5)
Coronavirus 229E	10/1920 (0.5)	5/990 (0.5)	3/529 (0.6)	2/277 (0.7)	1/167 (0.6)	0/47	2/416 (0.5)	1/220 (0.5)	4/808 (0.5)	2/446 (0.4)
Coronavirus HKU1	11/1920 (0.6)	7/990 (0.7)	3/529 (0.6)	2/277 (0.7)	0/167	0/47	4/416 (1.0)	2/220 (0.9)	4/808 (0.5)	3/446 (0.7)
Coronavirus NL63	8/1920 (0.4)	5/990 (0.5)	1/529 (0.2)	0/277	1/167 (0.6)	1/47 (2.1)	2/416 (0.5)	1/220 (0.5)	4/808 (0.5)	3/446 (0.7)
Coronavirus OC43	35/1920 (1.8)	16/990 (1.6)	8/529 (1.5)	7/277 (2.5)	2/167(1.2)	0/47	9/416 (2.2)	3/220 (1.4)	16/808 (2.0)	6/446 (1.3)
Influenza virus A	436 (11.2)	137 (7.9)	117 (8.8)	51 (8.5)	85 (20.8)	10 (9.3)	62 (8.8)	15 (4.7)	172 (11.7)	61 (8.7)
Influenza virus B	150 (3.8)	39 (2.3)	39 (2.9)	13 (2.2)	52 (12.7)	11 (10.2)	28 (4.0)	6 (1.9)	31 (2.1)	9 (1.3)
Metapneumovirus	60 (1.5)	31 (1.8)	38 (2.9)	23 (3.8)	5 (1.2)	2 (1.9)	7 (1.0)	3 (0.9)	10 (0.7)	3 (0.4)
Parainfluenza virus 1	67 (1.7)	5 (0.3)	35 (2.6)	2 (0.3)	9 (2.2)	1 (0.9)	8 (1.1)	1 (0.3)	15 (1.0)	1 (0.1)
Parainfluenza virus 2	36 (0.9)	6 (0.3)	17 (1.3)	3 (0.5)	8 (2.0)	1 (0.9)	3 (0.4)	0	8 (0.5)	2 (0.3)
Parainfluenza virus 3	164 (4.2)	37 (2.1)	107 (8.1)	29 (4.8)	10 (2.5)	3 (2.8)	13 (1.8)	1 (0.3)	34 (2.3)	4 (0.6)
Respiratory syncytial virus	597 (15.3)	289 (16.7)	498 (37.6)	247 (41.2)	36 (8.8)	13 (12.0)	21 (3.0)	9 (2.8)	42 (2.9)	20 (2.9)
Rhinoviruses	470/3417 (13.8)	219/1583 (13.8)	242/1152 (21.0)	119/547 (21.8)	55/365 (15.1)	16/97 (16.5)	64/622 (10.3)	27/296 (9.1)	109/1278 (8.5)	57/643 (8.9)
Any bacteria	357 (9.1)	231 (13.4)	26 (2.0)	13 (2.2)	18 (4.4)	10 (9.3)	105 (14.9)	70 (21.7)	208 (14.1)	138 (19.7)
Any virus	1871 (47.9)	746 (43.1)	991 (74.8)	453 (75.6)	246 (60.3)	57 (52.8)	210 (29.8)	66 (20.5)	424 (28.8)	170 (24.3)
Any mixed viral infection*	253 (6.5)	87 (5.0)	172 (13.0)	70 (11.7)	24 (5.9)	2 (1.9)	20 (2.8)	6 (1.9)	37 (2.5)	9 (1.3)
Any potential pneumonia aetiology	2109 (53.9)	913 (52.8)	1002 (75.6)	458 (76.5)	257 (63.0)	64 (59.3)	282 (40.0)	119 (37.0)	568 (38.6)	272 (38.8)
Any recognized pathogen†	1744 (44.6)	735 (42.5)	845 (63.8)	374 (62.4)	213 (52.2)	50 (46.3)	219 (31.1)	93 (28.9)	467 (31.7)	218 (31.1)

CXR, Chest X-ray; n.t., not tested.

\* Excluding bocavirus.

† Excluding bocavirus, coronaviruses and rhinoviruses.

Table 3. Population-weighted crude and adjusted annual incidence of radiographically confirmed pneumonia per 100 000 by age group

Respiratory pathogen	Crude annual incidence/100 000 persons			Adjusted* annual incidence/100 000		
	< 5 yr	5–49 yr	≥ 50 yr	< 5 yr	5–49 yr	≥ 50 yr
<i>Chlamydia pneumoniae</i>	4·6	1·1	11·5	14·6	2·6	25·5
<i>Coxiella burnetii</i>	0	0	1·1	0	0	2·5
<i>Legionella longbeachae</i>	n.t.	1·0	11·1	n.t.	2·5	24·6
<i>Legionella pneumophila</i> sg 1	n.t.	0	0	n.t.	0	0
<i>Mycoplasma pneumoniae</i>	5·3	1·0	0·9	18·3	2·3	2·0
<i>Mycobacterium tuberculosis</i>	0	2·6	14·7	0	5·7	30·5
<i>Streptococcus pneumoniae</i>	n.t.	1·4	16·8	n.t.	3·1	34·7
Adenoviruses	23·6	0·2	2·8	55·6	0·5	5·8
Bocavirus	50·3	0·3	1·1	114·6	0·6	2·2
Coronaviruses (229E, HKU1, NL63, OC43)	14·1	0·9	6·5	45·0	2·2	14·3
Influenza virus A	38·8	1·9	19·0	90·2	3·9	38·8
Influenza virus B	9·9	1·3	2·8	24·7	2·5	5·9
Metapneumovirus	17·5	0·4	0·9	56·4	0·9	2·0
Parainfluenza virus 1	1·5	0·2	0·3	3·0	0·3	0·6
Parainfluenza virus 2	2·3	0·1	0·6	4·5	0·1	1·2
Parainfluenza virus 3	22·1	0·3	1·2	43·7	0·5	2·4
Respiratory syncytial virus	188·0	1·7	6·2	417·1	3·2	12·3
Rhinoviruses	90·6	3·3	17·8	240·9	7·2	37·1

n.t., Not tested.

\* Adjusted for enrolment.

was 7 days (range 1–43). One elderly patient with influenza who died had an extended length of stay (139 days) due to several pre-existing medical comorbidities [13].

## DISCUSSION

This pneumonia study includes patients of all ages from a well-described population and integrates epidemiological and clinical surveillance with thorough viral laboratory diagnostics to expand the understanding of the viral causes of pneumonia in a middle-income country in a tropical area. It also adds to valuable information about pneumonia derived largely from wealthy temperate countries in studies over the past 30 years [30–37]. The population-based design, capturing all hospitalized pneumonia cases in the 1·2 million population of the two provinces allows for rates of disease to be calculated and compared, a feature of only a few previous studies [30, 32, 36, 37]. Overall, the study provides data that may help to guide empirical treatment, target pneumonia prevention strategies and direct future research in Thailand.

Viral pathogens, especially RSV, caused a substantial proportion of pneumonias in young children, a

finding that is consistent with other studies around the world [38, 39]. The adjusted incidence of RSV-associated pneumonia in children was 417·1/100 000. Viruses were also important in hospitalized adult pneumonias, perhaps an under-appreciated finding [23, 25, 32, 37]; 8·7% of patients aged ≥ 50 years had influenza A infection and 2·9% had RSV infection. Improved prevention strategies, such as vaccination or improved hand hygiene, to combat common viruses such as influenza and RSV might greatly reduce hospitalized pneumonia in Thailand.

In Thailand, there appears to be two distinct pneumonia seasons with different pathogens contributing to each. Viral activity is known to vary from year to year. For example, in the USA, RSV has an annual peak but year-to-year and geographic variability is quite great [40]; and the alternating year pattern of parainfluenza virus activity is well recognized [41]. In the tropics, RSV has been associated with the rainy season [38], a finding consistent with the pattern we found in Thailand. In the current study, we believe surveillance artifact (i.e. a nurse strike in one province) may have contributed to the lower RSV peak observed in 2004. Several more years of data will help distinguish recurrent seasonal patterns from

Table 4. *Mixed viral infections in episodes of hospitalized, radiographically confirmed pneumonia by age group (bocavirus is excluded)*

	Total	<5 yr	5–17 yr	18–49 yr	≥ 50 yr
Mixed viral infections	87	70	2	6	9
Two pathogens	77	62	2	6	7
Rhinovirus and					
Influenza A		4	1		
Influenza B		1			
Adenovirus		4			2
RSV		24			
Parainfluenzavirus 3		3			
Coronavirus hku1		1			
Coronavirus OC43		1			
Metapneumovirus		2			
RSV and					
Adenovirus		8			
Metapneumovirus		5		1	
Parainfluenzavirus 3		1			
Parainfluenzavirus 1				1	
Influenza A		5	1		1
Influenza B				1	
Influenza A and					
Influenza B				1	1
Coronavirus hku1				1	
Adenovirus					1
Parainfluenzavirus 3		1			
Influenza B and					
Adenovirus		1		1	
Coronavirus 229e and					
Coronavirus OC43					1
Metapneumovirus					1
Adenovirus and parainfluenzavirus 2		1			
Three pathogens	9	7			2
Rhinovirus and					
RSV and adenovirus		4			
RSV and metapneumovirus		2			
Adenovirus and parainfluenzavirus 3		1			
Influenza A and					
RSV and adenovirus					1
Influenza B and coronavirus n163					1
Four pathogens	1	1			
Coronavirus 229e, influenza B, RSV, metapneumovirus					

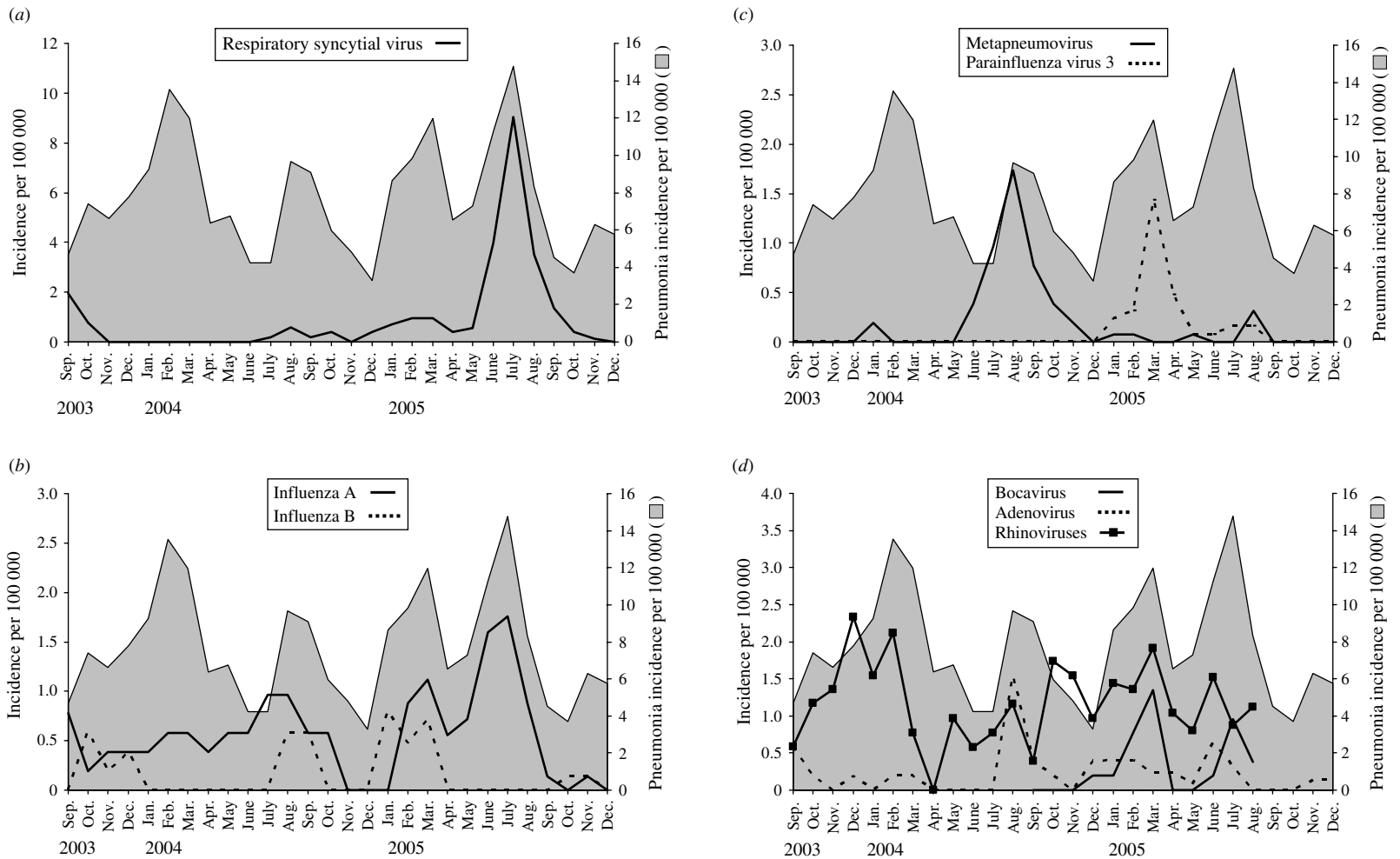
RSV, Respiratory syncytial virus.

temporary aberrations or outbreaks. Seasonality is important to describe since it has implications for timing of preventive measures; the circulation of influenza A and B almost year round would complicate any vaccine strategy.

Incidence is important for understanding the burden of disease, and it is an important tool for policy makers. In this study, patients signed informed

consent before undergoing the extensive diagnostic workup, and enrolment averaged 48% over the 30 months and was lowest in children. Furthermore, only 62.5% of those enrolled had a chest radiograph. To more accurately estimate incidence, we made adjustments (Table 3). Of note, many viral diseases present with pneumonia or another clinical syndrome (e.g. RSV can present as pneumonia or bronchiolitis)





**Fig. 1.** Averaged monthly incidence of radiographically confirmed pneumonia (shaded in grey) and (a) respiratory syncytial virus, (b) influenza viruses A and B, (c) metapneumovirus and parainfluenza virus 3, (d) bocavirus, adenovirus and rhinoviruses.

so our incidence probably underestimates the true burden.

A large limitation of this study is the non-standard use of diagnostic assays to confirm bacterial causes of pneumonia. As a result, we are unable to describe the bacterial aetiology of childhood pneumonia in this setting. Adults aged  $\geq 50$  years had an elevated incidence of tuberculosis and two vaccine-preventable diseases, influenza and pneumococcal pneumonia. The incidence of tuberculosis is likely to be a gross underestimate since AFB smears were used at the discretion of the physicians. In addition, atypical bacterial agents diagnosed by urine antigen, PCR assay or serology were also common in older adults with pneumonia. The adjusted incidence of *C. pneumoniae* was 25.5/100 000 in adults aged  $\geq 50$  years. However, commercial serological tests for *C. pneumoniae* have not been well validated clinically and therefore sensitivity, specificity and predictive values for these tests are not accurately known. *L. longbeachae* was an interesting finding and deserves more study, as was the notable absence of *L. pneumophila*.

Improved molecular diagnostics have radically changed our understanding of the role of viruses in respiratory disease. However, with improved sensitivity comes a greater challenge in data interpretation, namely how to interpret mixed viral infections. In this study, rhinoviruses were most frequently identified in mixed infections. We also found viral co-infections to be much more frequent in young children than in older children and adults, a finding that probably reflects the increased probability of young children to acquire viral infections. Clinical and epidemiological data, as well a control population, will be critical to proper interpretation of the identified association between selected viruses and pneumonia and to determining causality vs. carriage.

Pneumonia remains a leading infectious killer worldwide, and the increased attention in Asia and tropical countries, where viral respiratory diseases including SARS and H5N1 influenza viruses emerged, is warranted. Importantly, a large proportion of pneumonia is caused by known treatable or preventable infectious agents. At the local level information on the causes of pneumonia will allow clinicians to better understand pneumonia aetiology and make informed treatment decisions. In Thailand, molecular diagnostic capabilities (i.e. RT-PCR) are being added to the provincial hospital laboratories in these surveillance sites. At the national and regional level, these data will allow public health officials to make informed policy

decisions on prevention strategies, such as Thailand's recent decision to publicly provide influenza vaccine to elderly persons. At the global level, comprehensive surveillance which integrates epidemiological, clinical and laboratory information in the context of capacity building and technology transfer will contribute to better preparedness against the next infectious disease threat.

## ACKNOWLEDGEMENTS

We thank Peera Areerat and Denchai Sornkij for their senior leadership in the field; Sununta HENCHAI, Suchada Kaewchana, Prabda Prapasiri, Sathapana Naorat, Possawat Jorakate and Anek Kaewpan for technical assistance in the field; Julie Fisher, Julia Rhodes, Prasong Srisaengchai, Pongpun Sawatwong and Pornpak Khunatorn for assistance in data management; Sona Budhiraja, Vongduraus McClee, George Gallucci, Julita Yarwood and Henrietta Hall for assistance in the laboratory. Funding was provided by the Centers for Disease Control and Prevention and the Thailand Ministry of Public Health. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of CDC.

## NOTE

Supplementary material accompanies this paper on the Journal's website (<http://journals.cambridge.org/hyg>).

## DECLARATION OF INTEREST

None.

## REFERENCES

1. **Rudan I, et al.** Epidemiology and etiology of childhood pneumonia. *Bulletin of the World Health Organization* 2008; **86**: 408–416.
2. **Mizgerd JP.** Acute lower respiratory tract infection. *New England Journal of Medicine* 2008; **358**: 716–727.
3. **Olsen SJ, et al.** The incidence of pneumonia in rural Thailand. *International Journal of Infectious Diseases* 2006; **10**: 439–445.
4. **Centers for Disease Control and Prevention.** Isolation of avian influenza A(H5N1) viruses from humans – Hong Kong, May–December 1997. *Morbidity and Mortality Weekly Report* 1997; **46**: 1204–1207.

5. **Centers for Disease Control and Prevention.** Outbreaks of avian influenza A (H5N1) in Asia and interim recommendations for evaluation and reporting of suspected cases – United States, 2004. *Morbidity and Mortality Weekly Report* 2004; **53**: 97–100.
6. **Drosten C, et al.** Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *New England Journal of Medicine* 2003; **348**: 1967–1976.
7. **Ksiazek TG, et al.** A novel coronavirus associated with severe acute respiratory syndrome. *New England Journal of Medicine* 2003; **348**: 1953–1966.
8. **Dawood FS, et al.** Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *New England Journal of Medicine* 2009; **360**: 2605–2615.
9. **UNICEF.** Thailand Statistics ([http://www.unicef.org/infobycountry/Thailand\\_statistics.html](http://www.unicef.org/infobycountry/Thailand_statistics.html)). Accessed 11 February 2009.
10. **Chamany S, et al.** Assessing the sensitivity of surveillance for pneumonia in rural Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 2008; **39**: 549–556.
11. **Jordan HT, et al.** A comparison of population-based pneumonia surveillance and health-seeking behavior in two provinces in rural Thailand. *International Journal of Infectious Diseases* 2009; **13**: 355–361.
12. **Javadi M, et al.** Diagnosing pneumonia in rural Thailand: Digital cameras versus film digitizers for chest radiograph teleradiology. *International Journal of Infectious Diseases* 2006; **10**: 129–135.
13. **Simmerman JM, et al.** The cost of influenza in Thailand. *Vaccine* 2006; **24**: 4417–4426.
14. **Erdman DD, et al.** GeneScan reverse transcription-PCR assay for detection of six common respiratory viruses in young children hospitalized with acute respiratory illness. *Journal of Clinical Microbiology* 2003; **41**: 4298–4303.
15. **Schrag SJ, et al.** SARS surveillance during emergency public health response, United States, March–July 2003. *Emerging Infectious Diseases Journal* 2004; **10**: 185–194.
16. **Lu X, et al.** Real-time PCR assays for detection of bocavirus in human specimens. *Journal of Clinical Microbiology* 2006; **44**: 3231–3235.
17. **Dare RK, et al.** Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *Journal of Infectious Diseases* 2007; **196**: 1321–1328.
18. **Tondella ML, et al.** Development and evaluation of real-time PCR-based fluorescence assays for detection of *Chlamydia pneumoniae*. *Journal of Clinical Microbiology* 2002; **40**: 575–583.
19. **Zhang M, et al.** Development of TaqMan probe-based PCR with customized internal controls for detecting *Mycoplasma pneumoniae* and *Mycoplasma fermentans*. In: 99th General Meeting of the American Society for Microbiology. Chicago, IL, 1999.
20. **Wilkinson HW, et al.** Reactivity of serum from patients with suspected legionellosis against 29 antigens of *Legionellaceae* and *Legionella*-like organisms by indirect immunofluorescence assay. *Journal of Infectious Diseases* 1983; **147**: 23–31.
21. **Field PR, et al.** Comparison of a commercial enzyme-linked immunosorbent assay with immunofluorescence and complement fixation tests for detection of *Coxiella burnetii* (Q fever) immunoglobulin M. *Journal of Clinical Microbiology* 2000; **38**: 1645–1647.
22. **Field PR, et al.** Evaluation of a novel commercial enzyme-linked immunosorbent assay detecting *Coxiella burnetii*-specific immunoglobulin G for Q fever pre-vaccination screening and diagnosis. *Journal of Clinical Microbiology* 2002; **40**: 3526–3529.
23. **Dowell SF, et al.** Respiratory syncytial virus is an important cause of community-acquired lower respiratory infection among hospitalized adults. *Journal of Infectious Diseases* 1996; **174**: 456–462.
24. **Falsey AR, et al.** Human metapneumovirus infections in young and elderly adults. *Journal of Infectious Diseases* 2003; **187**: 785–790.
25. **Marx A, et al.** Parainfluenza virus infection among adults hospitalized for lower respiratory tract infection. *Clinical Infectious Diseases* 1999; **29**: 134–140.
26. **National Economic and Social Development Board of Thailand.** Population projections of Thailand 2000–2030 ([http://www.nesdb.go.th/temp\\_social/pop.zip](http://www.nesdb.go.th/temp_social/pop.zip)). Accessed 20 May 2008.
27. **Dowell SF, et al.** Standardizing *Chlamydia pneumoniae* assays: recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). *Clinical Infectious Diseases* 2001; **33**: 492–503.
28. **World Health Organization.** WHO recommended surveillance standards, Second edition. WHO/CDS/CSR/ISR/99/2/EN Geneva: World Health Organization, 1999 ([http://www.who.int/csr/resources/publications/surveillance/WHO\\_CDS\\_CSR\\_ISR\\_99\\_2\\_EN/e/](http://www.who.int/csr/resources/publications/surveillance/WHO_CDS_CSR_ISR_99_2_EN/e/)). Accessed 12 September 2005.
29. **Phares CR, et al.** Epidemiology of severe pneumonia caused by *Legionella longbeachae*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*: 1-year, population-based surveillance for severe pneumonia in Thailand. *Clinical Infectious Diseases* 2007; **45**: e147–155.
30. **Filice GA, et al.** Bacteremia in Charleston County, South Carolina. *American Journal of Epidemiology* 1986; **123**: 128–136.
31. **Selwyn BJ.** The epidemiology of acute respiratory tract infection in young children: comparison of findings from several developing countries. Coordinated Data Group of BOSTID Researchers. *Reviews of Infectious Diseases* 1990; **12**: S870–888.
32. **Marston BJ, et al.** Incidence of community-acquired pneumonia requiring hospitalization. Results of a population-based active surveillance Study in Ohio. The Community-Based Pneumonia Incidence Study Group. *Archives of Internal Medicine* 1997; **157**: 1709–1718.
33. **Wubbel L, et al.** Etiology and treatment of community-acquired pneumonia in ambulatory children. *Pediatric Infectious Disease Journal* 1999; **18**: 98–104.
34. **Juven T, et al.** Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatric Infectious Disease Journal* 2000; **19**: 293–298.

35. **Tsolia MN, et al.** Etiology of community-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. *Clinical Infectious Diseases* 2004; **39**: 681–686.
36. **Jackson ML, et al.** The burden of community-acquired pneumonia in seniors: results of a population-based study. *Clinical Infectious Diseases* 2004; **39**: 1642–1650.
37. **Jennings LC, et al.** Incidence and characteristics of viral community-acquired pneumonia in adults. *Thorax* 2008; **63**: 42–48.
38. **Weber MW, Mulholland EK, Greenwood BM.** Respiratory syncytial virus infection in tropical and developing countries. *Tropical Medicine & International Health* 1998; **3**: 268–280.
39. **Stensballe LG, Devasundaram JK, Simoes EA.** Respiratory syncytial virus epidemics: the ups and downs of a seasonal virus. *Pediatric Infectious Disease Journal* 2003; **22**: S21–32.
40. **Mullins JA, et al.** Substantial variability in community respiratory syncytial virus season timing. *Pediatric Infectious Disease Journal* 2003; **22**: 857–862.
41. **Fry AM, et al.** Seasonal trends of human parainfluenza viral infections: United States, 1990–2004. *Clinical Infectious Diseases* 2006; **43**: 1016–1022.