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Respiratory viruses and influenza-like illness: Epidemiology and outcomes in children aged 6 months to 10 years in a multi-country population sample

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Summary *Background:* Better population data on respiratory viruses in children in tropical and southern hemisphere countries is needed.
Methods: The epidemiology of respiratory viruses among healthy children (6 months to <10 years) with influenza-like illness (ILI) was determined in a population sample derived from an influenza vaccine trial (NCT01051661) in 17 centers in eight countries (Australia, South East Asia and Latin America). Active surveillance for ILI was conducted for approximately 1 year (between February 2010 and August 2011), with PCR analysis of nasal and throat swabs.
Results: 6266 children were included, of whom 2421 experienced 3717 ILI episodes. Rhinovirus/enterovirus had the highest prevalence (41.5%), followed by influenza (15.8%), adenovirus (9.8%), parainfluenza and respiratory syncytial virus (RSV) (both 9.7%), coronavirus (5.6%), human metapneumovirus (5.5%) and human bocavirus (HBov) (2.0%). Corresponding incidence per 100 person-years was 29.78, 11.34, 7.03, 6.96, 6.94, 4.00, 3.98 and 1.41. Except for influenza, respiratory virus prevalence declined with age. The incidence of medically-attended ILI associated with viral infection ranged from 1.03 (HBov) to 23.69 (rhinovirus/enterovirus). The percentage of children missing school or daycare ranged from 21.4% (HBov) to 52.1% (influenza).
Conclusions: Active surveillance of healthy children provided evidence of respiratory illness burden associated with several viruses, with a substantial burden in older children.

Introduction

Acute respiratory tract infections (ARTIs) comprise the most common illnesses worldwide, and children often experience several episodes a year.[1] In children, clinical presentation can range from mild, uncomplicated upper respiratory tract illness to severe lower respiratory tract infection (LRTI) including pneumonia, bronchiolitis, croup and exacerbations of asthma or wheezing.[2] The World Health Organization estimated that 1.9 million children died from ARTI in 2000, 70% in Africa and South East Asia.[3] In 2011, pneumonia alone led to 1.3 million deaths worldwide in children less than 5 years of age.[4]

Most ARTIs are caused by viruses, but until the advent of multiplex polymerase chain reaction (PCR) techniques, it was often not possible to identify accurately specific viral infections in clinical cases.[5,6] The pathogens considered responsible for most ARTI are respiratory syncytial virus (RSV), influenza A and B, parainfluenza viruses types 1, 2 and 3, and adenovirus.[2] Several new pathogens associated with ARTI have been identified more recently, including human metapneumovirus (hMPV), rhinovirus, coronavirus, human bocavirus (HBov) and parainfluenza 4.[2,7]

Vaccine efficacy trials provide intensive, active follow-up of a well-defined population and can be used to evaluate viral epidemiology. As part of a trial of pandemic influenza vaccines, which included 1 year of prospective, active, community-based surveillance for influenza-like illness (ILI) in 17 centers in eight countries,[8] we evaluated the prevalence and incidence of respiratory viruses in children 6 months to less than 10 years of age at first vaccination.

Methods

Samples were obtained from an efficacy trial of two pandemic influenza H1N1 vaccines (NCT01051661 sponsored by GSK Vaccines).[8] An analysis estimating the prevalence of RSV in ILI has been previously reported.[9] Here, we report data on all respiratory viruses evaluated, a secondary analysis objective.

Design and conduct of the clinical trial

Healthy children 6 months to <10 years of age were enrolled in a randomized, observer-blind, parallel group, multi-country trial of AS03-adjuvanted versus non-adjuvanted monovalent pandemic H1N1 vaccines.[8] The trial was conducted in 17 centers in Australia, Brazil, Colombia, Costa Rica, Mexico, the Philippines, Singapore, and Thailand between 15 February 2010 and 19 August 2011; enrollment took up to 6 months and timing varied by country. The trial was approved by an Institutional Review Board for each center and written informed consent was obtained from parents/guardians.

ILI surveillance and sample analysis

Parents were instructed to contact the study center within 24 h if the child became ill. Active surveillance via scripted telephone contact was conducted from 2 weeks after first vaccination, and contact made every 1–2 weeks to day 385 for

each child, regardless of time of enrollment. ILI was defined as temperature ≥38.0 °C by any route and at least one of: new/worsening cough, sore throat, stuffy nose, or runny nose.

Study staff visited the child's home to collect one anterior nasal swab and one throat swab, ideally within 24 h of ILI onset, and at most within 7 days. Collection could also take place at a study center or hospital if necessary. Swabs were transported in a single tube of M4RT transport medium, stored at −70 °C and maintained on dry ice during transport. A 7-day symptom-free period was required between new ILI episodes. Sample testing was performed by standard multiplex PCR techniques.[8,10]

Analysis of viral epidemiology

The viruses evaluated were influenza (subtypes A-H1, A-H3 and B), parainfluenza (subtypes 1, 2, 3 and 4), RSV (subtypes A and B), hMPV, rhinovirus/enterovirus (the assay did not distinguish between them), adenovirus, coronavirus (subtypes 229E, OC43, NL63 and HKU1) and HBov. First analyzed separately, influenza, parainfluenza and coronavirus subtypes were grouped in a post-hoc analysis.

Outcome variables

The main outcome variable was PCR-confirmed infection with the stated viruses in nasal/throat swabs in children with ILI. This included infection with the virus under consideration alone (single infection) or with the virus under consideration plus one or more of the other viruses (co-infection). Single and co-infections were also recorded separately.

Clinical characteristics of ILI episodes were reported by the parents of the children, and any hospitalization or medical attendance (by a doctor or other healthcare professional, not including sample collection by study staff) were recorded. Pneumonia was defined as acute illness (one or more of fever ≥38 °C, new or worsening cough, dyspnea, consistent auscultation findings [rales or diminished breath sounds], pain in the chest or abdomen when breathing, or purulent or blood-stained sputum production) and radiologic findings consistent with pneumonia.

Statistical analysis

The total cohort included all children enrolled in the randomized trial. The total cohort with ILI episodes tested by multiplex PCR included all children enrolled who experienced an ILI and had an adequate nasal/throat sample tested by multiplex PCR. The analysis of prevalence of respiratory viruses in ILI and clinical characteristics associated with ILI was performed in the total cohort with ILI episodes tested by multiplex PCR. The analysis of overall incidence of ILI, medically-attended ILI and hospitalized ILI in which respiratory viruses were detected was performed in the total cohort.

The prevalence of respiratory viruses among ILI episodes was calculated as:

$$\text{Prevalence} = X/N$$

where X is the number of ILI episodes with nasal/throat samples positive for the virus and N is the total number of ILI episodes with samples collected within 7 days and tested. As there was at least 7 days between two ILI episodes, it was assumed that each episode was independent. Exact 95% confidence intervals (CI) were computed.[11] Prevalence was stratified according to country, age at the time of the ILI episode (6–11, 12–23, 24–35, 36–59, 60+ months) and whether the child was medically attended or hospitalized.

The incidence per 100 person-years (PY) of virus-associated ILI in the study population was calculated as:

$$\text{Incidence rate} = \frac{\sum_{i=1}^{n} \varepsilon_i}{\sum_{i=1}^{n} \delta_i} * 100$$

where n is the total number of children enrolled in the trial, ε_i is the total number of virus-positive ILI episodes for subject i, and δ_i is the follow-up period for subject i. Incidence rates were stratified according to country and age group at the time of the ILI episode. Exact 95% Poisson CIs were calculated.[12] Observations with incomplete data for the outcome variable and ILI episodes for which no nasal/throat sample was taken were removed from the analysis. Missing data were accounted for by calculating the missing proportion for each country and age group, then multiplying the PY by (1 minus missing proportion).

Results

The trial included 6266 children (total cohort). After excluding children with no ILI or inadequate samples, 2421 children experienced 3717 ILI episodes (total cohort with ILI episodes tested by multiplex PCR). Participant flow is shown in Supplement Fig. 1. Demographics were similar in both cohorts, except that children in the total cohort were older (median 55 versus 42 months) (Supplement Table 1).

Prevalence and incidence of respiratory viruses in ILI

A respiratory virus was detected in 2958 of 3717 ILI episodes (79.6%). Rhinovirus/enterovirus had the highest overall prevalence (41.5%), followed by influenza (15.8%), adenovirus (9.8%), parainfluenza and RSV (both 9.7%), coronavirus (5.6%), hMPV (5.5%) and HBov (2.0%) (Table 1). Of 1541 ILI episodes in which rhinovirus/enterovirus was detected, 986 (64%) were associated with rhinovirus/enterovirus only and 555 (36%) with co-infection with another respiratory virus (Fig. 1; Supplement Table 2; Supplement Table 3). Single virus infections were identified more often in ILI episodes associated with RSV and influenza (including subtypes A and B [Supplement Table 3]). Co-infection was detected more often with adenovirus and HBov (Fig. 1). Single infections with parainfluenza, hMPV and coronavirus were identified at approximately the same frequency as co-infections (Fig. 1). However, parainfluenza 4 and coronavirus 229E were identified more often as co-infections, whilst coronavirus NL63 was identified more often as a single infection (Supplement Table 3).

In all countries, rhinovirus/enterovirus was the most prevalent (36.9–59.2%), whilst HBov was least prevalent (0.8–4.7%) (Table 1). Influenza prevalence ranged from 6.1% to 18.5%; parainfluenza prevalence was approximately 10% except in Brazil (5.8%) and Singapore (6.1%) (Table 1).

Table 1 Prevalence of ILI episodes in which respiratory viruses were detected, by country (total cohort with ILI episodes tested by multiplex PCR).

Country	ILI episodes	Influenza		Parainfluenza		RSV		hMPV		Rhinovirus/enterovirus		Adenovirus		Coronavirus		HBov	
	N	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
All countries	3717	587	15.8 (14.6–17.0)	360	9.7 (8.8–10.7)	359	9.7 (8.7–10.7)	206	5.5 (4.8–6.3)	1541	41.5 (39.9–43.1)	364	9.8 (8.9–10.8)	207	5.6 (4.9–6.4)	73	2.0 (1.5–2.5)
Australia	111	11	9.9 (5.1–17.0)	10	9.0 (4.4–15.9)	18	16.2 (9.9–24.4)	13	11.7 (6.4–19.2)	41	36.9 (28.0–46.6)	8	7.2 (3.2–13.7)	7	6.3 (2.6–12.6)	2	1.8 (0.2–6.4)
Brazil	710	128	18.0 (15.3–21.1)	41	5.8 (4.2–7.8)	42	5.9 (4.3–7.9)	31	4.4 (3.0–6.1)	292	41.1 (37.5–44.8)	76	10.7 (8.5–13.2)	40	5.6 (4.1–7.6)	22	3.1 (2.0–4.7)
Colombia	584	77	13.2 (10.5–16.2)	68	11.6 (9.2–14.5)	49	8.4 (6.3–10.9)	37	6.3 (4.5–8.6)	252	43.2 (39.1–47.3)	65	11.1 (8.7–14.0)	40	6.8 (4.9–9.2)	9	1.5 (0.7–2.9)
Costa Rica	379	40	10.6 (7.6–14.1)	41	10.8 (7.9–14.4)	16	4.2 (2.4–6.8)	7	1.8 (0.7–3.8)	179	47.2 (42.1–52.4)	34	9.0 (6.3–12.3)	21	5.5 (3.5–8.3)	7	1.8 (0.7–3.8)
Mexico	669	124	18.5 (15.7–21.7)	76	11.4 (9.1–14.0)	51	7.6 (5.7–9.9)	48	7.2 (5.3–9.4)	272	40.7 (36.9–44.5)	68	10.2 (8.0–12.7)	55	8.2 (6.3–10.6)	15	2.2 (1.3–3.7)
Philippines	1045	184	17.6 (15.3–20.1)	102	9.8 (8.0–11.7)	167	16.0 (13.8–18.3)	52	5.0 (3.7–6.5)	401	38.4 (35.4–41.4)	95	9.1 (7.4–11.0)	34	3.3 (2.3–4.5)	8	0.8 (0.3–1.5)
Singapore	49	3	6.1 (1.3–16.9)	3	6.1 (1.3–16.9)	4	8.2 (2.3–19.6)	1	2.0 (0.1–10.9)	29	59.2 (44.2–73.0)	7	14.3 (5.9–27.2)	2	4.1 (0.5–14.0)	2	4.1 (0.5–14.0)
Thailand	170	20	11.8 (7.3–17.6)	19	11.2 (6.9–16.9)	12	7.1 (3.7–12.0)	17	10.0 (5.9–15.5)	75	44.1 (36.5–51.9)	11	6.5 (3.3–11.3)	8	4.7 (2.1–9.1)	8	4.7 (2.1–9.1)

N = number of ILI episodes; n = number of ILI episodes positive for the relevant virus; % = percentage of ILI episodes positive for the relevant virus (n/N).
Influenza: influenza A and B; Parainfluenza: parainfluenza 1, 2, 3 and 4; RSV: RSV A and B; Coronavirus: coronavirus 229E, OC43, NL63, HKU1.
CI: confidence interval; HBov: human bocavirus; hMPV: human metapneumovirus; ILI: influenza-like illness; PCR: polymerase chain reaction; RSV: respiratory syncytial virus.

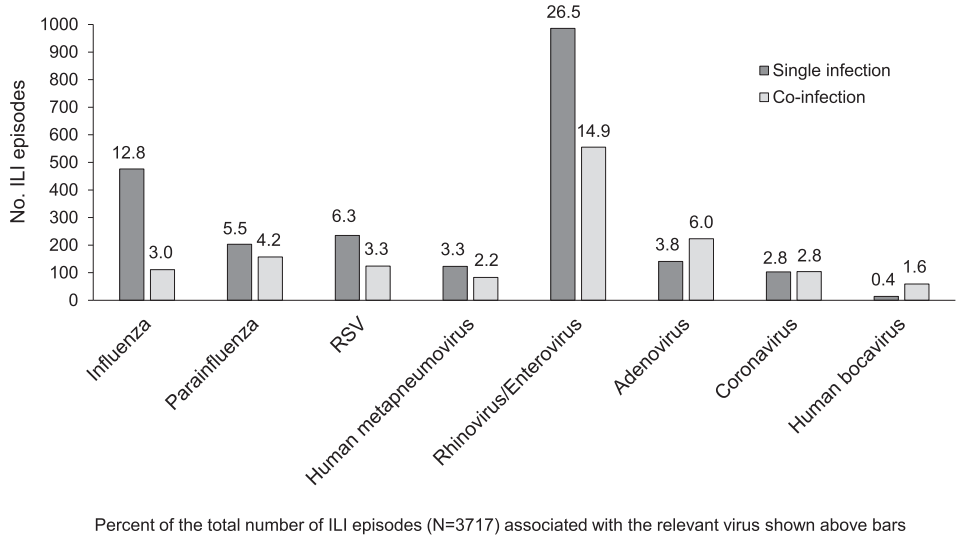

Figure 1 Single infection or co-infection with multiple respiratory viruses among ILI episodes in all ages and all countries (total cohort with ILI episodes tested by multiplex PCR). ILI: influenza-like illness; PCR: polymerase chain reaction; RSV: respiratory syncytial virus.

RSV prevalence ranged from 4.2% in Costa Rica to 16.2% in Australia, hMPV from 1.8% in Costa Rica to 11.7% in Australia, adenovirus from 6.5% in Thailand to 14.3% in Singapore and coronavirus from 3.3% in the Philippines to 8.2% in Mexico (Table 1).

Influenza was most prevalent (21.3%) in the oldest children (60+ months), followed by 36—59 months (15.6%) and the other age groups (9.8—12.3%) (Table 2). All other viruses were least prevalent in the oldest group (Table 2). There was a less obvious pattern in the other age groups, but, in general, prevalence declined with age except for influenza (Table 2).

The incidence of detected respiratory viruses associated with ILI reflected their prevalence. The overall incidence per 100 PY (total cohort, all children randomized) was 29.78 for rhinovirus/enterovirus, 11.34 for influenza, 7.03 for adenovirus, 6.96 for parainfluenza, 6.94 for RSV, 4.00 for coronavirus, 3.98 for hMPV and 1.41 for HBov (Table 3). Australia had the highest incidence of hMPV (5.08) and the second highest of RSV (7.03), but low incidence of the other viruses relative to other countries (Table 3). The Philippines, Singapore and Thailand also had low incidences of most viruses in ILI relative to the Latin American countries (Table 3). Detection of the respiratory viruses at different times during the year was highly variable across countries (Fig. 2a—h).

Hospitalization, medical attendance and clinical characteristics of ILI associated with respiratory viruses

The overall incidence of medically-attended ILI associated with viral infection per 100 PY (total cohort, all children randomized) ranged from 1.03 for HBov to 23.69 for rhinovirus/enterovirus (Table 3). Corresponding values for incidence of hospitalized ILI associated with viral infection were 0 for HBov and 0.81 for rhinovirus/enterovirus (Table 3).

Clinical characteristics of ILI episodes associated with a single respiratory virus (i.e. no co-infection) are shown in Table 4. Median duration of ILI episodes ranged from 8.9 to 13.4 days. Few children were hospitalized but most were medically attended outside study procedures. The percentage of children missing school or daycare was highest with influenza-associated ILI (52.1%), followed by hMPV (41.5%), adenovirus (39.0%), rhinovirus/enterovirus (37.6%), coronavirus (31.1%), RSV (30.2%), parainfluenza (28.1%) and HBov (21.4%) (Table 4). Sore throat was experienced by 25—52% of children, cough by 62—97%, stuffy nose by 40—62%, and runny nose by 66—84% (Table 4). Fever was part of the ILI definition and therefore experienced by all children. Cough was reported in almost all children with influenza, parainfluenza, RSV, hMPV and coronavirus infections, but only in 60—70% of children with rhinovirus/enterovirus, adenovirus and HBov infections. There were no medically important differences in clinical characteristics between children with a single viral infection compared with children with multiple infections (Supplement Table 4).

A total of 58 pneumonia cases were identified among the 6266 children enrolled in the overall clinical trial, corresponding to a detection rate of 0.9%. Of the 58 cases, 32 met the definition of ILI and were therefore eligible for sample collection as per the clinical trial protocol. A sample was collected within 7 days of onset of ILI symptoms for 20 of these 32 cases: one case in Thailand, three in the Philippines, five in Brazil, five in Mexico and six in Colombia (Table 5). No virus was detected in three cases, a single infection was detected in 10 cases (four rhinovirus/enterovirus, two parainfluenza, one influenza, two RSV and one hMPV), and co-infection was detected in seven cases (Table 5). Nine children were hospitalized.

Table 2 Prevalence of ILI episodes in which respiratory viruses were detected, by age group (total cohort with ILI episodes tested by multiplex PCR).

Age (months)[a]	ILI episodes	Influenza		Parainfluenza		RSV		hMPV		Rhinovirus/enterovirus		Adenovirus		Coronavirus		HBov	
	N	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
All ages	3717	587	15.8 (14.6–17.0)	360	9.7 (8.8–10.7)	359	9.7 (8.7–10.7)	206	5.5 (4.8–6.3)	1541	41.5 (39.9–43.1)	364	9.8 (8.9–10.8)	207	5.6 (4.9–6.4)	73	2.0 (1.5–2.5)
6–11	143	14	9.8 (5.5–15.9)	13	9.1 (4.9–15.0)	26	18.2 (12.2–25.5)	6	4.2 (1.6–8.9)	68	47.6 (39.1–56.1)	11	7.7 (3.9–13.3)	10	7.0 (3.4–12.5)	6	4.2 (1.6–8.9)
12–23	676	74	10.9 (8.7–13.5)	105	15.5 (12.9–18.5)	109	16.1 (13.4–19.1)	34	5.0 (3.5–7.0)	345	51.0 (47.2–54.9)	85	12.6 (10.2–15.3)	42	6.2 (4.5–8.3)	26	3.8 (2.5–5.6)
24–35	657	81	12.3 (9.9–15.1)	68	10.4 (8.1–12.9)	92	14.0 (11.4–16.9)	42	6.4 (4.6–8.5)	304	46.3 (42.4–50.2)	89	13.5 (11.0–16.4)	47	7.2 (5.3–9.4)	20	3.0 (1.9–4.7)
36–59	1050	164	15.6 (13.5–18.0)	109	10.4 (8.6–12.4)	88	8.4 (6.8–10.2)	84	8.0 (6.4–9.8)	429	40.9 (37.9–43.9)	102	9.7 (8.0–11.7)	59	5.6 (4.3–7.2)	18	1.7 (1.0–2.7)
60+	1191	254	21.3 (19.0–23.8)	65	5.5 (4.2–6.9)	44	3.7 (2.7–4.9)	40	3.4 (2.4–4.5)	395	33.2 (30.5–35.9)	77	6.5 (5.1–8.0)	49	4.1 (3.1–5.4)	3	0.3 (0.1–0.7)

N = number of ILI episodes; n = number of ILI episodes positive for the relevant virus; % = percentage of ILI episodes positive for the relevant virus (n/N).
Influenza: influenza A and B; Parainfluenza: parainfluenza 1, 2, 3 and 4; RSV: RSV A and B; Coronavirus: coronavirus 229E, OC43, NL63, HKU1.
CI: confidence interval; HBov: human bocavirus; hMPV: human metapneumovirus; ILI: influenza-like illness; PCR: polymerase chain reaction; RSV: respiratory syncytial virus.
[a] At time of ILI episode.

Discussion

Rhinovirus/enterovirus had the highest prevalence and incidence in ILI of all respiratory viruses tested in all countries, followed by influenza, adenovirus, parainfluenza and RSV, coronavirus, hMPV and HBov. The burden of ILI associated with respiratory viruses was considerable, with a high proportion of children being seen by a medical professional and many missing school or daycare.

Our analysis benefited from being part of a clinical trial, as previously described.[9] Most importantly, we conducted 1 year of prospective, active community surveillance of healthy children in tropical and southern hemisphere countries where prospective data are lacking. Most studies of viral epidemiology use hospital-based surveillance because community-based surveillance is difficult and expensive. However, hospital-based surveillance tends to capture only the most severe illness and many cases are missed in developing countries because of limited hospital access. Our analysis avoided these limitations and allowed us to capture the burden of virus-associated ILI in communities. Understanding community epidemiology is essential to implement effective control measures. Other advantages of being part of a clinical trial included a well-characterized population, wide age range up to 10 years, samples taken from a high proportion of children, consistent methodology between countries, and use of sensitive and validated PCR assays.

The trial was conducted in eight countries encompassing Australia, South East Asia and Latin America. The exact timing of enrollment varied somewhat between countries, but was planned so that data collection was performed during the peak 2010—2011 influenza season for each individual country. As stated in the Methods, all children were followed for 385 days, with the complete period of surveillance for the study occurring between 15 February 2010 and 19 August 2011. This allowed us to compare the distribution of viruses across the different countries. There was considerable variation in the incidence and prevalence of the viruses by country, although rhinovirus/enterovirus had by far the highest incidence and prevalence in all countries. HBov had consistently the lowest incidence and prevalence. Several other studies have evaluated the prevalence of viruses in children with respiratory illness. The relative prevalence of the different circulating viruses varied by study. However, the main circulating viruses were similar between studies and with our study, and included picornaviruses (including rhinovirus), adenovirus, RSV, bocavirus, PIVs, hMPV, influenza and coronavirus.[13—17]

Rhinoviruses are classified in the picornavirus family, of the enterovirus genus.[7] A high prevalence of this family has been reported in other studies in different settings.[13,14,18—20] As in our study, an Australian study with active community-based surveillance of healthy preschool-age children with ARTI found that picornaviruses (including rhinoviruses) were the most frequently detected (41.3%).[13] However, other viruses were detected less frequently than in our study: RSV (6.6%), parainfluenza (4.1%), influenza A and hMPV (both 3.7%), adenovirus (3.1%) and coronavirus NL63 (1.5%).[13] In another prospective Australian study in children aged 6 months to 3 years

Table 3 Incidence per 100 person-years (95% CI) of ILI, medically-attended ILI and hospitalized ILI in which respiratory viruses were detected, overall and by country (total cohort).

	No. person-years	Incidence rate (95% CI)							
		Influenza	Parainfluenza	RSV	hMPV	Rhinovirus/ enterovirus	Adenovirus	Coronavirus	HBov
All countries									
ILI overall	5175	11.34 (10.44–12.30)	6.96 (6.26–7.71)	6.94 (6.24–7.69)	3.98 (3.46–4.56)	29.78 (28.31–31.30)	7.03 (6.33–7.80)	4.00 (3.47–4.58)	1.41 (1.11–1.77)
Medically-attended ILI	5175	9.41 (8.59–10.29)	5.70 (5.07–6.39)	6.03 (5.38–6.74)	3.26 (2.78–3.79)	23.69 (22.38–25.06)	5.99 (5.34–6.70)	2.87 (2.43–3.37)	1.03 (0.77–1.34)
Hospitalized ILI	5175	0.25 (0.13–0.43)	0.21 (0.11–0.38)	0.16 (0.07–0.31)	0.17 (0.08–0.33)	0.81 (0.58–1.10)	0.08 (0.02–0.20)	0.06 (0.01–0.17)	0.00 (0.00–0.07)
Individual countries (ILI overall)									
Australia	256	4.30 (2.14–7.69)	3.91 (1.87–7.18)	7.03 (4.17–11.11)	5.08 (2.70–8.68)	16.01 (11.49–21.72)	3.12 (1.35–6.16)	2.73 (1.10–5.63)	0.78 (0.09–2.82)
Brazil	658	19.45 (16.23–23.13)	6.23 (4.47–8.45)	6.38 (4.60–8.63)	4.71 (3.20–6.69)	44.37 (39.43–49.77)	11.55 (9.10–14.46)	6.08 (4.34–8.28)	3.34 (2.10–5.06)
Colombia	733	10.50 (8.28–13.12)	9.27 (7.20–11.75)	6.68 (4.94–8.83)	5.04 (3.55–6.95)	34.36 (30.24–38.87)	8.86 (6.84–11.29)	5.45 (3.90–7.43)	1.23 (0.56–2.33)
Costa Rica	272	14.68 (10.49–19.99)	15.05 (10.8–20.42)	5.87 (3.36–9.54)	2.57 (1.03–5.29)	65.70 (56.43–76.06)	12.48 (8.64–17.44)	7.71 (4.77–11.78)	2.57 (1.03–5.29)
Mexico	1030	12.04 (10.01–14.36)	7.38 (5.81–9.24)	4.95 (3.69–6.51)	4.66 (3.44–6.18)	26.41 (23.37–29.74)	6.60 (5.13–8.37)	5.34 (4.02–6.95)	1.46 (0.82–2.40)
Philippines	1693	10.87 (9.36–12.56)	6.03 (4.91–7.31)	9.87 (8.43–11.48)	3.07 (2.29–4.03)	23.69 (21.43–26.12)	5.61 (4.54–6.86)	2.01 (1.39–2.81)	0.47 (0.20–0.93)
Singapore	113	2.64 (0.55–7.72)	2.64 (0.55–7.72)	3.52 (0.96–9.02)	0.88 (0.02–4.91)	25.55 (17.11–36.70)	6.17 (2.48–12.71)	1.76 (0.21–6.37)	1.76 (0.21–6.37)
Thailand	419	4.78 (2.92–7.38)	4.54 (2.73–7.09)	2.87 (1.48–5.01)	4.06 (2.37–6.50)	17.91 (14.09–22.46)	2.63 (1.31–4.70)	1.91 (0.82–3.77)	1.91 (0.82–3.77)

CI: confidence interval; HBov: human bocavirus; hMPV: human metapneumovirus; ILI: influenza-like illness; RSV: respiratory syncytial virus.

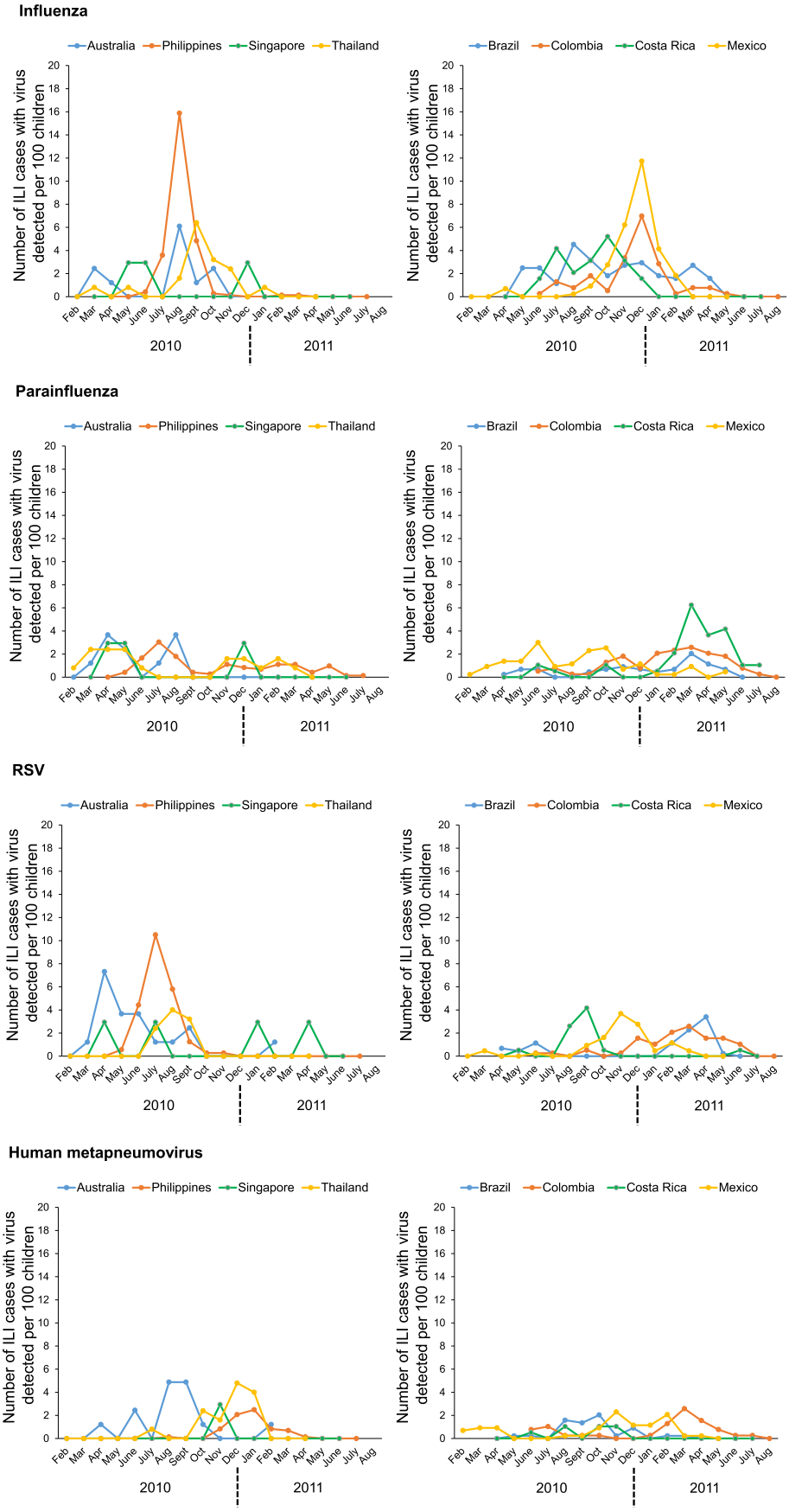

Figure 2 **a-h.** Monthly distribution of ILI episodes in which respiratory viruses were detected. ILI: influenza-like illness; RSV: respiratory syncytial virus.

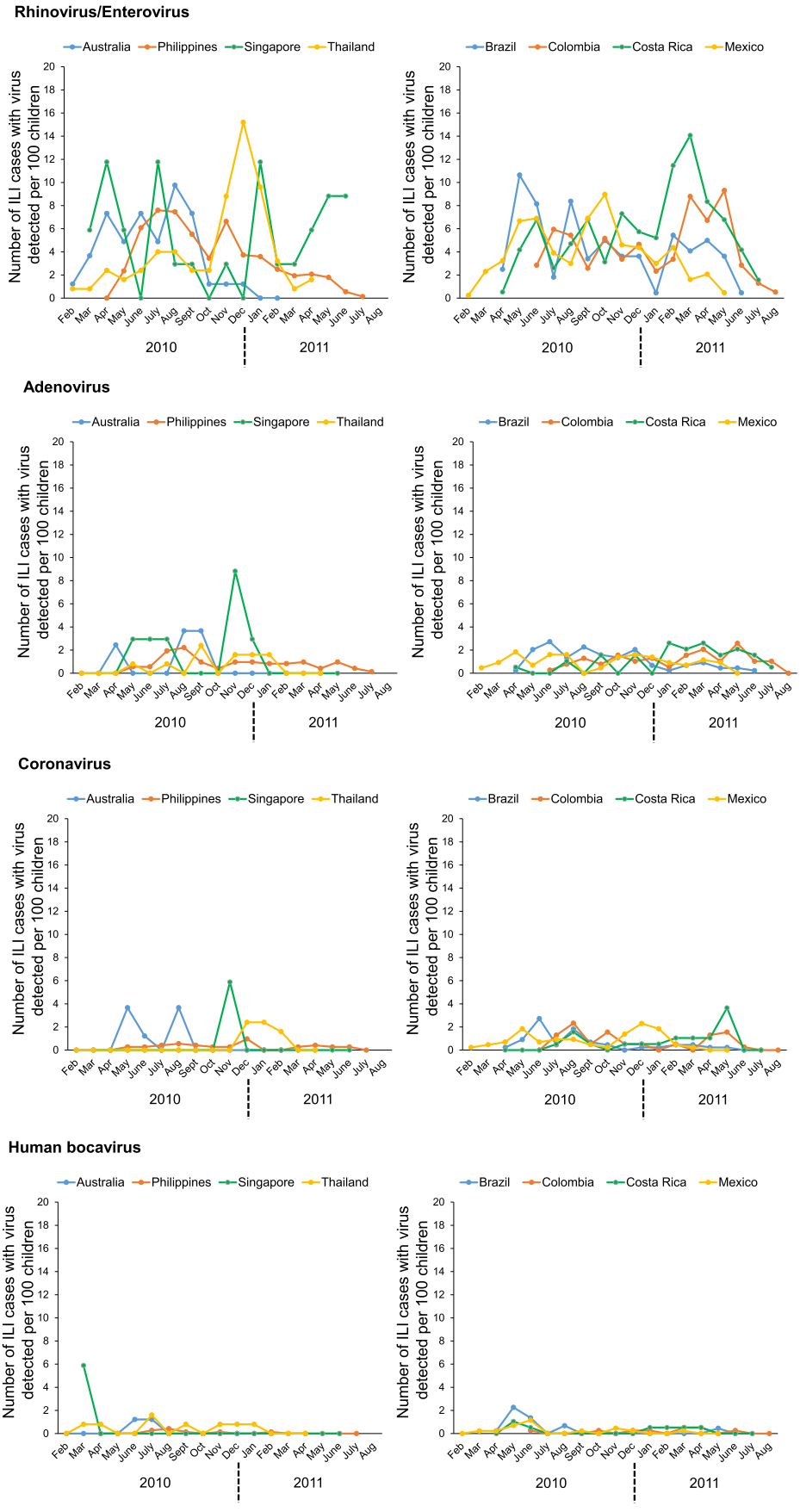

Figure 2 (*continued*).

Table 4 Demographic and clinical characteristics of ILI episodes with a single respiratory virus detected (total cohort with ILI episodes tested by multiplex PCR).

	Influenza	Parainfluenza	RSV	hMPV	Rhinovirus/ enterovirus	Adenovirus	Coronavirus	HBov
Number of cases	476	203	235	123	986	141	103	14
Mean (SD) duration of ILI episode, days	8.9 (5.9)	10.5 (8.6)	9.2 (8.1)	9.8 (7.8)	9.6 (10.8)	9.2 (10.5)	10.1 (10.0)	13.4 (21.3)
Number (%) hospitalized	9 (1.9)	4 (2.0)	5 (2.1)	4 (3.3)	24 (2.4)	2 (1.4)	2 (1.9)	0
Median duration of hospitalization, days	4.0	3.0	6.0	5.0	1.5	4.0	1.5	NA
Number (%) with medical attendance	395 (83.0)	161 (79.3)	207 (88.1)	103 (83.7)	768 (77.9)	121 (85.8)	68 (66.0)	9 (64.3)
Number (%) with pneumonia	1 (0.2)	2 (1.0)	2 (0.9)	1 (0.8)	4 (0.4)	0 (0.0)	0	0
Number (%) with missed school or daycare	248 (52.1)	57 (28.1)	71 (30.2)	51 (41.5)	371 (37.6)	55 (39.0)	32 (31.1)	3 (21.4)
Median duration of missed school or daycare, days	3.0	3.0	3.0	3.0	2.0	3.0	2.5	1.0
Number (%) with sore throat	179 (37.6)	69 (34.0)	59 (25.1)	42 (34.1)	414 (42.0)	69 (48.9)	53 (51.5)	7 (50.0)
Number (%) with cough	408 (85.7)	184 (90.6)	225 (95.7)	119 (96.7)	713 (72.3)	87 (61.7)	90 (87.4)	10 (71.4)
Number (%) with stuffy nose	233 (48.9)	104 (51.2)	95 (40.4)	58 (47.2)	493 (50.0)	63 (44.7)	64 (62.1)	6 (42.9)
Number (%) with runny nose	373 (78.4)	155 (76.4)	188 (80.0)	81 (65.9)	720 (73.0)	95 (67.4)	86 (83.5)	10 (71.4)

HBov: human bocavirus; hMPV: human metapneumovirus; ILI: influenza-like illness; NA: not applicable; PCR: polymerase chain reaction; RSV: respiratory syncytial virus; SD: standard deviation.
All children experienced fever, as it was part of the definition of ILI.

reporting ILI, rhinovirus was again the most commonly detected.[15] However, in contrast to our results, adenovirus was detected at the same frequency as rhinovirus, followed by parainfluenza 3, polyomavirus, hMPV and HBov.[15] Influenza (A/H1N1) and RSV were relatively uncommon; approximately 40% of children were fully or partially vaccinated against influenza.

Rhinovirus is not always the most commonly detected virus in children with respiratory disease. In children under 5 years of age hospitalized for LRTI in Thailand, the most commonly detected viruses were RSV (19.5%), rhinovirus (18.7%), HBov (12.8%) and influenza (8.2%).[16] A study of children aged <3 years hospitalized for LRTI in Brazil found that RSV was most prevalent (53.5% of episodes), followed by hMPV (32.3%), rhinovirus (20.8%), influenza (12.7%), HBov (10.4%), parainfluenza and adenovirus (both 6.5%) and coronavirus (1.2%).[17]

In our analysis, influenza prevalence increased with age. The other viruses showed the opposite trend, with the lowest prevalence observed in the oldest children (60+ months). There was a less obvious pattern in younger ages, but, in general, prevalence of all viruses except influenza declined with age. Despite this, the burden of illness remained considerable in older children.

There was a clear seasonal pattern for influenza, RSV and hMPV in most countries, and to a lesser extent for rhinovirus/enterovirus. A previous study found that, although there was no clear seasonal peak for rhinovirus/enterovirus, onset seemed to correspond with the start of the school year in the USA.[18] A limited one year analysis of human rhinoviruses and enteroviruses in ILI in Latin America showed a year-round temporal distribution throughout Central and South America.[21] However, human rhinovirus C species displayed opposite seasonal trends on either side of the equator, accounting for a higher percentage of ILI cases north of the equator between September and January, while south of the equator detection increased between April and July.[21]

As part of the study, all children received a monovalent influenza A/H1N1 pandemic vaccine; one or two doses of an AS03-adjuvanted vaccine were administered or two doses of an unadjuvanted vaccine. Trivalent seasonal influenza vaccination rate in the present study was approximately 18%. Influenza A subtype H1 was not isolated in any children; influenza A subtype H3 was isolated in 9.0% of children; and influenza B was isolated in 5.7% of children. No difference between the study vaccine groups was observed.

Cases associated with influenza were least likely to be co-infected with other respiratory viruses. Rhinovirus/enterovirus was also more common as a single infection. Adenovirus and HBov were found more often as a co-infection. Bacterial co-infection was not measured as part of this study. In the US-based Influenza Incidence Surveillance Project, which evaluated the most commonly detected viruses in outpatients with ARTI or ILI, three-quarters of all co-infections involved adenovirus and rhinovirus/enterovirus.[18] A UK-based analysis found negative associations between influenza A and hMPV, and between influenza A and rhinovirus.[22] Positive associations were found between parainfluenza and rhinovirus, RSV and rhinovirus, adenovirus and rhinovirus, and parainfluenza and RSV.[22] No correlation was found between co-infection and clinical severity in a study in Brazil evaluating children under 5 years who sought medical care for respiratory tract infections.[23] More research is needed to understand the interaction of respiratory viruses, and the host response to infection.

There were no clear differences between viruses in the severity of illness. Most ILI episodes were medically

Table 5 Characteristics of children with pneumonia and ILI with an available sample.

Country	Age of child at time of the ILI episode (months)	Length of pneumonia episode (days)	Length of hospitalization period (days)	Virus(es) detected
Philippines	32	16	6	Rhinovirus/enterovirus
Philippines	29	19	0	Rhinovirus/enterovirus
Philippines	12	4	6	Parainfluenza 4 Rhinovirus/enterovirus
Thailand	56	22	22	Influenza B Rhinovirus/enterovirus
Brazil	52	11	0	Parainfluenza 4
Brazil	72	11	0	Influenza A
Brazil	29	19	0	Parainfluenza 3 Adenovirus
Brazil	65	6	0	None
Brazil	13	10	0	Rhinovirus/enterovirus
Colombia	47	7	1	hMPV Rhinovirus/enterovirus
Colombia	18	11	0	Rhinovirus/enterovirus Adenovirus
Colombia	14	13	4	RSV
Colombia	54	24	6	Parainfluenza 3
Colombia	26	13	3	Rhinovirus/enterovirus RSV
Colombia	43	13	0	Parainfluenza 1 Rhinovirus/enterovirus Adenovirus
Mexico	79	11	0	None
Mexico	13	4	0	Rhinovirus/enterovirus
Mexico	45	10	4	None
Mexico	21	7	5	RSV
Mexico	18	8	0	hMPV

hMPV: human metapneumovirus; ILI: influenza-like illness; RSV: respiratory syncytial virus.

attended. ILI associated with influenza resulted in the highest proportion of children missing school or daycare (52%), although 20—40% of children infected with the other viruses also missed school or daycare. There was no difference between viruses in the proportion of children hospitalized. Clinical features were variable depending upon the viral infection associated with the ILI episode.

Study limitations have been described previously.[9] Only healthy children participated in the trial, limiting generalizability. In addition, our study did not include any children aged <6 months and only a limited number of children aged 6—11 months, so our findings are mainly relevant to older children. Fever was part of the ILI definition, and therefore we would have missed cases in children with no fever. To put this into perspective, in the Influenza Incidence Surveillance Project, 34% and 43% of cases among children aged 1—4 years and 5—17 years, respectively, met the ARTI definition which did not require fever, but did not meet the ILI definition which did require fever.[18] However, our definition of ILI was somewhat broader (except in children under 2 years of age) and US data may not be generalizable to the tropical and southern hemisphere countries in our study. The inclusion of only healthy children in the study and the exclusion of cases with no fever would have underestimated the burden. We also could not discriminate between rhinovirus and enterovirus by PCR, therefore the exact prevalence and incidence of each one could not be determined. Finally, our study included only a small number of pneumonia cases (n = 20), limiting the conclusions that can be drawn regarding the distribution of viral infection in these cases. The overall pneumonia detection rate in the clinical trial (0.9%) is higher than, but in line with, what has been reported in the US for hospitalized cases.[24] However, our sample collection rate among pneumonia cases was only 62.5% compared with 80.0% for ILI overall.

In conclusion, our active surveillance of healthy children as part of a vaccine efficacy trial provided evidence of the burden of respiratory illness associated with a range of viruses. A substantial burden of illness occurs in older children. Data on the epidemiology of respiratory viruses determined from active surveillance of healthy children are generally lacking, and are particularly sparse in the developing countries included in our study. A considerable amount of the burden would not be identified through hospital-based surveillance. These novel data fill an important gap in our knowledge of the epidemiology of viruses contributing to the substantial burden of respiratory disease in children, and may be useful in informing priorities for implementation of existing vaccine programs and development of new vaccines.

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Conflict of interest

Ping Li, Serge Durviaux, Gerco Haars, Sumita Roy-Ghanta, David W Vaughn and Sylvia Taylor are employed by the GSK group of companies. Ping Li, Gerco Haars, Sumita Roy-Ghanta, David W Vaughn and Sylvia Taylor own company stock options or restricted shares. Yang Feng was employed by the GSK group of companies at the time of the study and is currently working for GSK Vaccines as an independent consultant.

Terry Nolan reports a research contract from GSK to the Murdoch Children Research Institute (MCRI) for the conduct of the present study as well as research grants to MCRI from GSK for the conduct of clinical trials on the Meningococcal ACYW, H1N1 pandemic and birth dose pertussis vaccines, from Sanofi Pasteur for clinical trial on QIV vaccine, from Novartis for clinical trials of Men B and adjuvanted TIV vaccines.

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Lily Weckx declares research grants from GSK to Federal University of São Paulo for conduct of three clinical trials and received payment from GSK, Novartis, Pfizer, and Sanofi for board membership or lectures.

Rolando Ulloa-Gutierrez discloses having received honoraria from GSK for the original influenza A H1N1 clinical trial discussed here, as well as from GSK, Sanofi Pasteur, Pfizer/Wyeth and Merck as a speaker in the past.

Marco Aurelio P Safadi has received grants to support research projects and consultancy fees from Novartis, GSK, Pfizer and Sanofi Pasteur.

Fong Seng Lim discloses having received travel grants from GSK as well as a grant from GSK to his institution to perform clinical trials.

Marcela Hernandez-de Mezerville declares having received honoraria from GSK for the original influenza A/H1N1 clinical trial discussed here, as well as travel support from GSK, Sanofi Pasteur and Pfizer outside the submitted work in the past.

Idis Faingezicht received payment from GSK as principal investigator in a previous vaccine clinical trial and as co-investigator in the influenza A/H1N1 clinical trial.

Pio Lopez, Eduardo Lazcano-Ponce, Angkool Kerdpanich, Miguel Angel Rodriguez Weber, Abiel Mascareñas de Los Santos, Juan-Carlos Tinoco, and Aurelio Cruz-Valdez report having nothing to disclose.

Author contributions

All authors participated in the design, or implementation, or analysis and interpretation of the study results; as well as in the development of this manuscript. All authors had full access to the data and gave final approval before submission.

Terry Nolan, Charissa Borja-Tabora, Pio Lopez, Lily Weckx, Rolando Ulloa-Gutierrez, Eduardo Lazcano-Ponce, Angkool Kerdpanich, Miguel Angel Rodriguez Weber, Abiel Mascareñas de Los Santos, Marco Aurelio P Safadi, Aurelio Cruz-Valdez and Juan-Carlos Tinoco were coordinating investigators, and together with Sumita Roy-Ghanta, David W Vaughn and Ping Li were responsible for the conduct of the Flu Q-PAN H1N1-035 PRI (NCT01051661) trial. Fong Seng Lim, Marcela Hernandez-de Mezerville and Idis Faingezicht also contributed to study material and data collection.

Sylvia Taylor led the epidemiology team in collaboration with Gerco Haars.

Yang Feng was responsible for the statistical input; statistical expertise was also provided by Gerco Haars, Sumita Roy-Ghanta, Ping Li and Terry Nolan. Serge Durviaux led the laboratory analysis.

Terry Nolan, Charissa Borja-Tabora, Yang Feng, David W Vaughn and Sylvia Taylor were members of the core writing team. Terry Nolan and Sylvia Taylor contributed equally to this manuscript and the corresponding author was responsible for the submission of the publication.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jinf.2016.09.003.

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