

# Viral Etiology of Influenza-Like Illnesses in Cameroon, January–December 2009

Richard Njouom,<sup>1</sup> Elsie Laban Yekwa,<sup>1</sup> Pierre Cappy,<sup>1</sup> Astrid Vabret,<sup>3</sup> Pascal Boisier,<sup>2</sup> and Dominique Rousset<sup>1</sup>

<sup>1</sup>Service de Virologie and <sup>2</sup>Service d'Epidémiologie et de Santé Publique, Centre Pasteur du Cameroun, Yaounde, Cameroon; and <sup>3</sup>Laboratoire de Virologie Humaine et Moléculaire, Centre Hospitalier Universitaire, Avenue Georges Clemenceau, Caen, France

**Background.** No information is available on the viral etiology of upper respiratory tract infections in Cameroon.

**Methods.** We prospectively enrolled outpatients with influenza-like illness (ILI) presenting at 14 sentinel clinics located across the country from January through December 2009. The specimens were tested using real-time and multiplex reverse-transcription polymerase chain reaction methods for the detection of 15 RNA respiratory viruses.

**Results.** We detected at least 1 respiratory virus in 365 of 561 specimens (65.1%). Overall, influenza virus was the most commonly detected virus (28.2% of specimens), followed by human rhinovirus (17.8%); parainfluenza virus (PIV) types 1–4 (7.5%); enterovirus (5.9%); respiratory syncytial virus (RSV; 5.7%); human coronavirus (HCoV) OC43, 229E, NL63, and HKU1 (5.3%); and human metapneumovirus (HMPV; 5.0%). RSV (26 of 31 specimens [83.9%]), PIV (30 of 39 [76.9%]), and HRV (64 of 99 [64.6%]) were most common among children <5 years of age. Coinfections were found in 53 of 365 positive specimens (14.5%), and most (71.7%) were in children <5 years of age. While influenza virus, enterovirus, RSV, and HMPV had a defined period of circulation, the other viruses were detected throughout the year.

**Conclusions.** We found that respiratory viruses play an important role in the etiology of ILI in Cameroon, particularly in children <5 years of age.

Acute respiratory infection (ARI) is the leading cause of morbidity and mortality worldwide. Approximately 2.2 million children die of ARI every year throughout the world, and about 40% of these deaths occur in Africa [1]. The etiology of influenza-like illness (ILI) has been well characterized in some parts of the world, particularly in temperate regions of the northern hemisphere [2–4]. Infections due to influenza virus, respiratory syncytial virus (RSV), parainfluenza virus (PIV) types 1–4, human metapneumovirus (HMPV), enterovirus, human rhinovirus (HRV), adenovirus, and human coronavirus (HCoV) account for a significant proportion of acute respiratory tract infections [3]. However, ARIs are generally subject to

presumptive treatment, and their causes are rarely sought. Clinical presentations of viral respiratory tract infections are similar, making it difficult to distinguish between etiologic agents. Nonetheless, differentiating between infections caused by these viruses may help policy makers in decisions related to vaccination and resource allocations [4].

In Africa, a limited number of studies have described the etiology of ILI due to viruses other than influenza virus [5–7]. In Cameroon, an influenza sentinel surveillance system for outpatients with ILI was instituted by the Centre Pasteur du Cameroun (CPC) in November 2007. Preliminary data from this surveillance network reported the circulation of seasonal influenza A virus subtypes H1N1 (A[H1N1]) and H3N2 (A[H3N2]) and influenza B virus during 2007–2008 [8]. Influenza viruses were detected throughout the year, with no marked seasonality. However, no studies have been conducted on the viral etiology of respiratory tract infections in Cameroon. We conducted an observational study nested within the existing influenza surveillance system in order to identify viral pathogens

Correspondence: Richard Njouom, PhD, Centre Pasteur du Cameroun, PO Box 1274, Yaounde, Cameroon (njouom@pasteur-yaounde.org; njouom@yahoo.com).

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associated with ILI and to determine the frequency of these pathogens among patients attending our ILI sentinel sites.

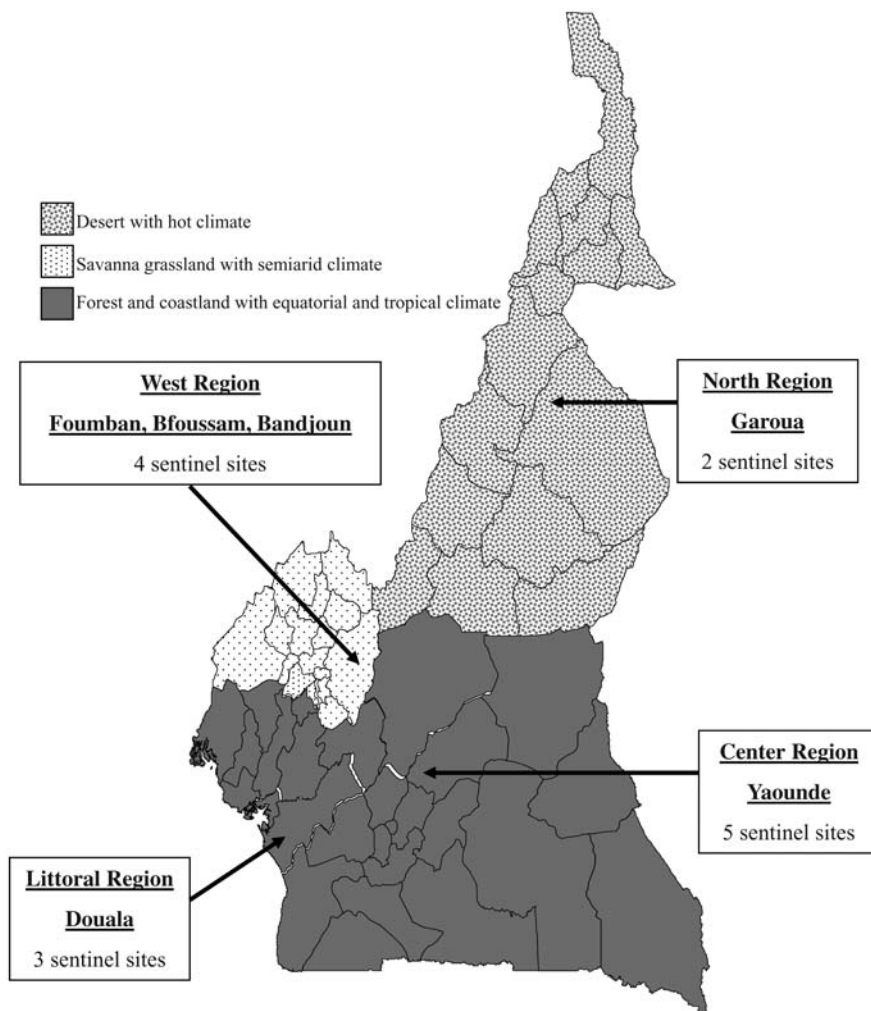
## MATERIALS AND METHODS

### Study Design and Settings

Cameroon is located in Central Africa, and it exhibits many of the major climates and ecosystems of the continent. Mountains with savanna grassland are located in the west, a desert is in the north, and rain forests and ocean coastline are the south and east, respectively. The climate varies from tropical along the coast to semiarid and hot in the north. In the western regions, the rainy season occurs between June and October. On the coastline, the rainy season begins in April and ends in November. The central and southern regions have an equatorial climate with moderate rainfall between March and June and intense precipitation between September and

November. In the northern part of the country, the rainy season occurs between May and September. The lowest temperatures (21°C) are recorded in the west, while the highest (32°C–40°C) are recorded in the north. In the south and along the coast, temperatures vary between 22°C and 29°C.

We conducted a prospective observational study from January through December 2009 in 14 ILI sentinel sites established for influenza surveillance in 2007, located in major urban centers in the following 4 regions of the country: Center (5 sites), Littoral (3 sites), North (2 sites), and West (4 sites) (Figure 1). The sentinel sites were private (7 sites) and public (7 sites) primary healthcare centers. At each sentinel site, trained nurses or physicians identified all ILI cases presenting at the clinics from Monday through Sunday. The surveillance staff consisted of facility staff who worked on the surveillance program on a voluntary basis. An ILI case was defined as an outpatient presenting with sudden onset of fever (temperature >38°C) and cough or sore throat, with the onset



**Figure 1.** Location of the sentinel sites for influenza-like illness detection and climatic and ecosystem characteristics in Cameroon.

of symptoms within the prior 5 days. All individuals with ILI were considered eligible for enrollment. Subjects were enrolled on a daily basis. Verbal informed consent was obtained from all identified cases who were  $\geq 18$  years of age. For case patients aged  $< 18$  years, proxy informed consent was obtained from parents or legal guardians. Patients who did not meet the case definition or did not provide verbal consent were excluded from the study.

A standardized questionnaire to record patients' demographic characteristics and medical history was used. The questions included information on date of enrollment and symptom onset, sex, age, clinical symptoms, and vaccination status for influenza. In very young children ( $< 3$  years of age), specific symptoms (eg, sore throat, headache, and myalgia) could not be properly ascertained.

The surveillance protocol was reviewed and approved by the National Research Ethics Committee and the Ministry of Health of Cameroon.

### Sample Collection and Laboratory Procedures

Throat and/or nasopharyngeal swab specimens were collected from all enrolled patients and then placed in 2-mL cryovials containing virus transport medium. If throat and nasopharyngeal swab specimens were collected from the same patient, both swabs were placed in the same cryovial. The specimens were kept refrigerated at 4°C at the sentinel site and then were transported twice per week to the CPC, where they were divided into 3 aliquots. One aliquot was used for molecular analysis, one was used for virus isolation, and one was stored at  $-80^{\circ}\text{C}$  for further analysis. Specimens were packaged using a triple packaging system at the sentinel sites and were transported refrigerated in cool boxes.

At the CPC, total RNA was extracted from 140  $\mu\text{L}$  of each sample, using the QIAamp viral RNA minikit (QIAGEN, Courtaboeuf, France) in accordance with the manufacturer's protocol. Purified RNA was frozen at  $-80^{\circ}\text{C}$  in aliquots.

We tested samples by real-time and multiplex reverse-transcription polymerase chain reaction (RT-PCR), using a modified version of a previously published protocol for RNA virus detection [9, 10]. The positive controls for each virus were made of RNA extracts of cell supernatants of cultivated viruses and were provided by the Virology Laboratory of the Caen University Hospital (France). Water was used as a negative control.

All samples were first tested using a 1-step real-time RT-PCR assay for the detection of influenza A and B viruses, according to the US Centers for Disease Control and Prevention (CDC) protocol. Subsequently, the same samples were tested using 4 multiplex RT-PCR methods, targeting 12 RNA respiratory viruses. HMPV and RSV were detected by multiplex RT-PCR method 1; PIV 1–4 by multiplex RT-PCR method 2; HRV, enterovirus, and influenza C virus by multiplex RT-PCR

method 3; and HCoV-OC43, -229E, -NL63, and -HKU1 by multiplex RT-PCR method 4. The products of multiplex RT-PCR methods 1–3 were subjected to heminested multiplex RT-PCR, as described previously [9]. However, multiplex method 1 was modified by using only primers for HMPV and RSV. Multiplex method 4 was developed for the simultaneous detection of the 4 HCoVs, using primers described by Vabret et al [10]. Multiplex RT-PCR and heminested PCR products were visualized under UV light after electrophoresis, using an ethidium bromide-stained 2% agarose gel.

We amplified each sample in the 4 different multiplex RT-PCR assays, each containing primers for 2 or 4 different viral targets. For each viral target, limit of detection (LOD) was assessed using 10-fold dilution series of the corresponding positive control. The LOD corresponded to the highest dilution of the positive control that gave a visualized band on the electrophoretic agarose gel. A sample was considered positive for a specific virus when its electrophoretic band was the same size as that of the corresponding sample of positive-control virus. We observed amplification only in the presence of RNA derived from the specific positive control virus. No amplification occurred in the negative controls. The LODs demonstrated a similar sensitivity between the different multiplex assays.

### Data Management and Statistical Analysis

Questionnaire information and laboratory results were recorded in a central database (MS Access) situated at the CPC. We analyzed the demographic and clinical characteristics of the study subjects and the positive cases, as well as the seasonal patterns of all respiratory viruses. The Student *t* test, the Mann-Whitney *U* test, and the Kruskal-Wallis rank test were used for continuous variables, and the Fisher exact test was used for categorical variables. We analyzed the clinical characteristics of infected patients, using a stepwise logistic regression model. Statistical significance was assessed at a *P* value of  $< .05$  for all parameters. Uncertainty was expressed as 95% confidence intervals (CIs). The statistical analysis was implemented using Stata, version 11.0 (StataCorp, College Station, TX).

## RESULTS

### Demographic and Clinical Characteristics of Patients With ILI

From January through December 2009, we obtained 561 samples from patients presenting with ILI (Table 1) at the 14 surveillance sites: 335 (59.7%) were from the Center region, 137 (24.4%) were from the West region, 58 (10.3%) were from the Littoral region, and 31 (5.6%) were from the North region. There were 281 males (50.1%), 273 females (48.7%), and 7 (1.2%) with no sex specified. The patients' age ranged between 1.2 months and 75 years (median, 6 years). A total of 44.6% of all enrolled patients with ILI were children  $< 5$  years of age.

**Table 1. Demographic and Clinical Characteristics of Outpatients With Influenza-Like Illness and 1 or >1 Respiratory Virus Detected, Cameroon, 2009**

Characteristic	ILI Detected (n = 561)	Respiratory Virus(es) Detected		
		1 (n = 312)	>1 (n = 53)	Total (n = 365)
<b>Sex</b>				
Male	281 (50.1)	149 (47.7)	34 (64.1)	183 (50.1)
Female	273 (48.7)	160 (51.3)	18 (34.0)	178 (48.8)
Missing	7 (1.2)	3 (1.0)	1 (1.9)	4 (1.1)
<b>Age</b>				
0–4 y	250 (44.6)	142 (45.5)	38 (71.7)	180 (49.3)
5–14 y	101 (18.0)	53 (17.0)	4 (7.5)	57 (15.6)
15–24 y	54 (9.6)	31 (9.9)	4 (7.5)	35 (9.6)
25–64 y	136 (24.2)	77 (24.7)	6 (11.3)	83 (22.7)
65+ y	5 (0.9)	1 (0.3)	0	1 (0.3)
Missing	15 (2.7)	8 (2.6)	1 (1.9)	9 (2.5)
<b>Symptom<sup>a</sup></b>				
Cough	511 (91.1)	292 (93.6)	50 (94.3)	342 (93.7)
Rhinorrhea	419 (74.7)	260 (83.3)	41 (77.4)	301 (82.5)
Fatigue	390 (69.5)	219 (70.2)	34 (64.2)	253 (69.3)
Headache	267 (47.6)	153 (49.0)	15 (28.3)	168 (46.0)
Sore throat	178 (31.7)	107 (34.3)	12 (22.6)	119 (32.6)
Arthralgia	123 (21.9)	69 (22.1)	4 (7.5)	73 (20.0)
Myalgia	114 (20.3)	63 (20.2)	4 (7.5)	67 (18.4)
Conjunctivitis	104 (18.5)	53 (17.0)	8 (15.1)	61 (16.7)
Wheezing	96 (17.1)	53 (17.0)	10 (18.9)	63 (17.3)
Vomiting	83 (14.8)	42 (13.5)	8 (15.1)	40 (11.0)
Diarrhea	52 (9.3)	22 (7.1)	7 (13.2)	29 (7.9)
Cutaneous rash	32 (5.7)	10 (3.2)	1 (1.9)	11 (3.0)
<b>Region</b>				
Center	335 (59.7)	204 (65.4)	30 (56.6)	234 (64.1)
West	137 (24.4)	66 (21.1)	18 (34.0)	84 (23.0)
Littoral	58 (10.3)	33 (10.6)	5 (9.4)	38 (10.4)
North	31 (5.6)	9 (2.9)	0	9 (2.5)

Data are no. (%) of patients.

<sup>a</sup> All patient had fever, because it was the inclusion criterion.

All patients presented with fever, because this was the inclusion criterion. Cough, rhinorrhea, and fatigue were present in >50% of cases (Table 1). Of the 561 samples tested, 365 (65.1%) were positive for at least 1 pathogen. Among the 365 positive samples, a single infection was detected in 312 (85.5%), while coinfections were found in 53 (14.5%). Single infections and coinfections occurred in all age groups, but the highest percentage of viral infections was observed in children <5 years old (180 of 365 [49.3%]). Of the 180 infections among children <5 years of age, 142 (78.8%) were single infections, and 38 (21.2%) were coinfections.

#### Virus Detection

Influenza virus and HRV were the most frequently identified viruses, with detection in 158 (28.2%) and 100 (17.8%) of

patients with ILI, respectively. Of the 158 influenza viruses detected, 154 (97.5%) were influenza A virus (4 were A[H1N1], 8 were 2009 pandemic influenza A virus subtype H1N1 [A{H1N1}pdm09], and 142 were A[H3N2]), 2 were influenza B virus, and 2 were influenza C virus. The detection rate of the other viruses was 7.5% for PIV types 1–4, 5.9% for enterovirus, 5.7% for RSV, 5.3% for HCoV, and 5.0% for HMPV (Table 2).

HRV was the most common virus detected in children <5 years of age (25.6%), followed by influenza A virus (18.4%), PIV (12.0%), and RSV (10.4%). In contrast, influenza A virus (35.3%) was the most common virus detected in the groups aged ≥5 years, followed by HRV (11.5%). The remaining viruses were detected in <10% of individuals in all age groups, with the exception of enterovirus, which was detected in 13.0% in the 15–24-year age group (Supplementary Table 1).

The detection rate was significantly higher among children <5 years old, compared with persons  $\geq 5$  years old, for RSV (26 of 250 [10.4%] vs 5 of 296 [1.7%];  $P < .001$ ), PIV (30 of 250 [12.0%] vs 9 of 296 [3.0%];  $P < .001$ ), and HRV (64 of 250 [25.6%] vs 35 of 296 [11.8%];  $P = .001$ ). Conversely, influenza viruses were more frequently detected in persons  $\geq 5$  years old, compared with children <5 years old (105 of 250 [42.0%] vs 48 of 296 [16.2%];  $P = .001$ ).

In the multivariate analysis adjusted by age, influenza viruses were more likely to be detected in patients with cough (odds ratio [OR], 3.2; 95% CI, 1.1–9.2), rhinorrhea (OR, 3.1; 95% CI, 1.6–6.2), and fatigue (OR, 2.2; 95% CI, 1.1–4.9), whereas RSV was more likely to be detected in patients with sore throat (OR, 6.5; 95% CI, 1.3–31.5). No clinical symptoms were specifically associated with the other surveyed viruses.

Among the positive patients with known age, the detection rate for coinfections was significantly higher ( $P = .001$ ) in the group aged <5 years (38 of 250 [15.2%]) than in the group aged  $\geq 5$  years (14 of 296 [4.7%]). No significant difference ( $P = .345$ ) was detected for single infections between the 2 age groups (142 of 250 [56.8%] vs 162 of 296 [54.7%]).

Age-adjusted analysis of clinical symptoms in patients with coinfections versus those with single infections found no statistical differences. Of the 53 coinfections, dual infections occurred in 49 cases, triple infections in 3 cases, and quadruple infections in 1 case. The viruses most frequently observed in coinfections were HRV (28 cases), influenza A virus (19 cases), enterovirus (13 cases), and HMPV (11 cases). HRV with HCoV or PIV or influenza A virus were the most common coinfections, constituting 9 (16.9%), 9 (16.9%), and 7 (13.2%) of all 53 coinfections, respectively.

### Seasonality

We observed virus circulation throughout the study period. [Supplementary Figure 1](#) shows the temporal patterns of virus detection. Influenza A virus was mostly detected from June through November, with 2 major peaks observed during June–July and October–November. Enterovirus was mostly detected from January through June. Higher HRV, PIV, and enterovirus activity coincided with a decrease in influenza A virus circulation. RSV was mostly detected from October through December, with a peak in October. HMPV was mostly detected in June and July. The period of higher activity of RSV and HMPV coincided with that of influenza A virus. HRV, PIV, and HCoV were detected throughout the study period, with no evident temporal patterns.

## DISCUSSION

This report contains the first description of the etiology of respiratory viruses associated with patients with ILI in Cameroon. We detected respiratory viruses in 65.1% of our samples;

influenza virus and HRV were the most common viruses detected. The high frequency of virus detection among patients with ILI in our study is consistent with that of studies conducted in other countries, including Madagascar (75.1%) [7], Senegal (58.5%) [6], Kenya (56%) [5], Shanghai (59.5%) [11], and Brazil (61.8%) [12]. In other studies in the developed and developing worlds, however, respiratory viruses were detected in <50% of cases [2, 4, 11, 13–16]. These differences could be due to true differences in overall burden, to differences in study populations, or to the use of different diagnostic techniques. Druce et al in Australia [14] included hospitalized patients in their study for 5 months over a 12-month period, and Puzelli et al in Italy [4] included hospitalized patients during the winter. Lina et al in France [2] used immunocapture enzyme-linked immunosorbent assay and immunostaining for the detection of respiratory viruses in their samples, and Laguna-Torres et al in Peru [13] used viral isolation. These techniques are known to be less sensitive than molecular techniques.

Like in many other studies [4, 6, 7, 13–15], influenza A virus was the most common viral pathogen detected in patients with ILI. In addition, PIV, RSV, HCoV, HMPV, and enterovirus were identified from patient's specimens and contributed collectively to 29.4% of all ILI cases in our study. The rate of detection of RSV in our study (5.7%) was lower than rates reported by other investigators. For example, in a study performed in Madagascar [7, 17], 21.2% of samples from patients with ILI were positive for RSV. The low proportion of RSV-positive samples in our study could be because we did not include hospitalized children, as RSV has been shown to be a common cause of lower respiratory tract infection in children admitted to the hospital [2, 14]. The detection rate for HCoV (5.3%), PIVs (7.5%), and HMPV (5.0%) was in agreement with data from other studies [16, 18–21].

The detection rate of influenza A virus was significantly higher among patients with ILI who were  $\geq 5$  years of age [13], while other viruses, such as HRV, PIV, and RSV, were prevalent among children aged <5 years. These findings are consistent with reports from other studies [2, 14, 18, 22–24]. A(H3N2) was predominant in 2009 in Cameroon, a finding that was similar in other Central and West Africa countries [25, 26] despite the introduction of A(H1N1)pdm09 in several countries worldwide in that year.

Recent data from tropical countries, including Senegal and Cambodia, demonstrate that the seasonality of influenza virus transmission corresponds mostly with the rainy season, and only a few cases can be detected during the dry season [27, 28]. This is consistent with our finding of a higher circulation of influenza virus during June–July and October–November 2009, corresponding to the rainy season in most of the areas where our sentinel sites are located. However, the seasonality of influenza virus in Cameroon in 2009 contrasts our earlier study that

showed that A(H1N1) was the prevalent influenza A virus subtype circulating in the country in 2008 and that it had no marked seasonality [8]. This highlights the need for multiple years of influenza surveillance to properly define the temporal patterns of influenza virus circulation, especially in tropical areas, where less demarcated seasonal patterns are observed.

HRV was the second most frequent viral agent detected, with positive results for 17% of patients with ILI, including all age groups. Our findings are in agreement with previous studies suggesting that HRV is responsible for a large proportion of acute respiratory tract infections in both adults and children, composing 10% to >40% of respiratory infections [12, 16, 29, 30]. HRV circulated year-round, a finding shown in other studies [7, 12, 15]. Increased HRV circulation coincided with a period of decreased activity for influenza virus, a situation also observed in Madagascar [7]. In our study, HRV was the most frequent virus detected in children <5 years of age (25.6%). This finding contrasts with other studies conducted in Senegal [6] and China [11], where the predominant virus detected in children <5 years of age was RSV. This difference could be due to different inclusion criteria for the study subjects. We included mostly children with upper respiratory tract infection; the studies conducted in Senegal and China also included children with lower respiratory tract infection. HRV has been reported to be the major cause of upper respiratory tract infections in children in Italy [29].

Our findings are subject to several limitations. First, we did not test for some respiratory DNA viruses (adenovirus and bocavirus), and we did not test for any respiratory bacteria. Adenovirus has been shown to be responsible for up to 13% of acute respiratory tract infections [18, 20]. As such, our results may underestimate the overall frequency of respiratory viral infections among ILI patients in Cameroon. Second, although our findings suggest considerable circulation of respiratory viruses in the country, we are limited in our ability to fully assess the burden of these viral agents: because our focus was on patients with ILI who presented at selected outpatient clinics, we lack data on the contribution of the investigated viruses to cases of pneumonia resulting in hospitalization. Third, the involvement of facility staff on a voluntary basis, with frequent shifts in personnel to other facilities, resulted in the poor performance of sentinel sites in some regions. This, coupled with the narrow interval of our study (1 year), hindered our ability to implement region-specific analysis to assess spatiotemporal variations in virus circulation in relation to climatic and geographic factors.

Despite these limitations, this study is the first to describe the etiology of RNA viral pathogens involved in upper respiratory tract infections in Cameroon. These viruses may also induce more severe disease, especially in young children, which should be investigated. We have also shown that the use of multiplex RT-PCR permits a rapid differential diagnosis of

ILI cases, which could potentially facilitate rapid detection of epidemics of respiratory virus infection and, therefore, rapid responses to outbreaks.

This study should be expanded over several years and in different settings (eg, hospitals) to ascertain the seasonal variation of ARI and individual viruses and to provide a better understanding of the epidemiology and spectrum of illness caused by respiratory viruses in Cameroon.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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