Journal of Medical Virology 82:866–872 (2010)

# Viral Etiology of Respiratory Infections in Children Under 5 Years Old Living in Tropical Rural Areas of Senegal: The EVIRA Project

**Mbayame Ndiaye Niang,[1]* Ousmane M. Diop,[1] Fatoumata Diene Sarr,[2] Deborah Goudiaby,[1] Hubert Malou-Sompy,[2] Kader Ndiaye,[1] Astrid Vabret,[3] and Laurence Baril[2]**

[1]*Unit of Medical Virology, Institut Pasteur of Dakar, Dakar, Senegal*
[2]*Unit of Infectious Disease Epidemiology, Institut Pasteur of Dakar, Dakar, Senegal*
[3]*Laboratory of Human and Molecular Virology, University-Hospital of Caen, Caen, France*

Acute respiratory infection is one of the leading causes of child morbidity, especially in developing countries. Viruses are recognized as the predominant causative agents of acute respiratory infections. In Senegal, few data concerning the causes of respiratory infections are available, and those known relate mainly to classical influenza infections. Clinical and virological surveillance of acute respiratory infections was carried out in a rural community in children less than 5 years old. A standardized questionnaire was used and a nasopharyngeal swab sample was collected from each patient. These samples were tested for the detection of 20 respiratory viruses by multiplex RT-PCR or by viral culture. A total of 82 acute respiratory episodes were included, and 48 (58.5%) were found to be positive, with a total of 55 viral detections; several samples were positive for two (n = 5) or 3 (n = 1) viruses. Ten different viruses were identified: influenza viruses A, B, and C (n = 25), human respiratory syncytial virus type A (n = 13), rhinoviruses (n = 8), human coronaviruses type 229E and NL63 (n = 6), parainfluenza viruses 3 and 4 (n = 2), and bocavirus (n = 1). These results provide evidence on the importance and the diversity of viruses as causative agents of acute respiratory infections in children living in a rural community in Senegal. The establishment of sentinel surveillance sites could help estimate the burden of acute respiratory infection in the pediatric population and should help prepare the health care systems to identify and respond to new viral respiratory emergencies. ***J. Med. Virol. 82:866–872, 2010.*** © 2010 Wiley-Liss, Inc.

**KEY WORDS:** respiratory viruses; acute respiratory infection; molecular diagnosis tools; sub-Saharan Africa; sentinel surveillance; emergency preparedness

## INTRODUCTION

Viruses are recognized as the predominant causative agents of acute respiratory infections in adults and in children. Acute respiratory infection is one of the leading causes of child morbidity and mortality throughout the world [Williams et al., 2002]. However, until recently acute viral respiratory infections were not considered to be a major public health concern. The 1997 H5N1 influenza outbreak in Hong Kong, the severe acute respiratory syndrome (SARS) epidemic in 2003 due to a coronavirus, and the current emergence of a novel swine-origin influenza A (H1N1) variant [Novel swine-Origin Influenza A (H1N1) Virus Investigation Team, 2009] emphasize the risk posed by acute viral respiratory infections in humans. These outbreaks emphasize the difficulties faced by unprepared public-health authorities and populations. The first two events of zoonotic origin were linked to farming practices, and were exacerbated by market trading in livestock; the current novel swine-origin influenza A (H1N1) variant epidemic is still under investigation. The risk of an

Grant sponsor: Institut Pasteur of Dakar; Grant sponsor: US Department of Health and Human Services (Project Name: "Support, Training and Capacity Building for Infectious Disease Surveillance Networks in Affected Countries in Southeast Asia, Africa and Other Regions of the World"); Grant sponsor: French Ministry of Health.

*Correspondence to: Mbayame Ndiaye Niang, Unit of Medical Virology, Institut Pasteur of Dakar, 36, avenue Pasteur, BP 220 Dakar, Senegal. E-mail: niang@pasteur.sn

Accepted 17 August 2009

DOI 10.1002/jmv.21665

Published online in Wiley InterScience (www.interscience.wiley.com)

influenza A virus pandemic, particularly due to an H5N1 virus or to the novel A (H1N1) variant, or of a further SARS-like epidemic has led public health authorities to implement or reinforce surveillance of acute respiratory infections in humans.

The three influenza pandemics that occurred during the last century were unpredictable, but children were often the primary vectors in the spread of the epidemic. Little is known about the respiratory viruses responsible for upper and lower acute respiratory infections circulating in the human population in tropical areas. Therefore, clinical and virological surveillance of acute respiratory infections is essential for public health, and children represent a key target population. In this context, it is important to develop and to implement diagnostic tools to identify other viruses involved in respiratory illnesses. Standardized procedures of collection, storage, and transportation of viral samples from the field to the laboratory should be implemented. Some respiratory viruses are diagnosed directly by a combination of isolation in cell culture and antigen detection, but often samples remain negative. Molecular biology techniques have been developed in recent years and multiplex RT-PCR methods are useful for the detection of viruses causing acute respiratory infections.

Lower acute respiratory infections are identified in hospital settings, health care centers, and outpatient consultations, but are documented very rarely. The identification of minor and mild respiratory illnesses requires sentinel surveillance in the community. In Senegal, almost 2 million inhabitants are children under the age of 5. Influenza-like illnesses are prevalent during the rainy season (July–October) in the urban areas of Dakar, and influenza viruses have been characterized in these infections [Dosseh et al., 2000]. However, other circulating viruses causing acute respiratory infections remain to be determined, particularly in rural areas. Identification would allow assessment of the magnitude of acute respiratory infections in these communities.

The aim of this study was to identify the respiratory viruses circulating in a rural community in Senegal. Because children are recognized in the first wave of the spread of viruses in the community, the study focused on acute respiratory infections occurring in children under the age of 5 and identification of the causative viruses. Episodes of upper and lower acute respiratory infections in two villages during the rainy season and during the two following months (from mid-June to December 2007) were surveyed.

## METHODS

### Study Area

The Dielmo and Ndiop villages are located in a rural area of the Sine Saloum region (280 km south of Dakar), near the Gambia border. The two villages are only 5 km apart from each other, but their ethnicities and ways of life are different, and there is little contact between the two populations. In 2007, 433 inhabitants in Dielmo and 612 in Ndiop were monitored for a multidisciplinary project on malaria [Noranate et al., 2007]. In July 2007, the population of children under the age of 5 comprised 67 and 76 individuals in Dielmo and Ndiop, respectively. The climate is tropical with a high relative humidity in the rainy season, from July to October, and is drier from November to June. The temperature varied between 30 and 40°C during the time of this study. There is holoendemic malaria transmission in Dielmo due to a small permanent stream (the Nema) and in Ndiop malaria transmission is highly seasonal and mesoendemic.

### Target Population

Upper and lower acute respiratory infections in children under the age of 5 in these two villages (the EVIRA project: "Étiologies Virales des Infections Respiratoires Aigues") were monitored. The study was approved by the National Health Research Council of the Senegalese Health Ministry and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from the children's parents or legal guardians before the EVIRA study.

Children were included if they had a temperature of >38°C and at least one respiratory symptom (rhinitis, pharyngitis, laryngitis, cough, rhonchi, crackles, or wheezing). A clinical examination (including pulmonary auscultation and otoscopy) was carried out and the results were reported using a standardized questionnaire. A nasopharyngeal swab sample to detect respiratory viruses was performed. The nasopharyngeal swab sample was collected in 2 ml of viral transport medium (Universal Transport Medium, COPAN Diagnostics, Inc., Murrieta, CA) and was stored at 4°C before shipment to the Unit of Medical Virology at the Institut Pasteur of Dakar on a weekly basis. Samples were shipped at a controlled temperature (4°C) and processed immediately on arrival at the laboratory. In addition, a systematic thick blood film to detect malaria was performed because the area is endemic for malaria. The thick blood film was read on site by a trained technician.

### Viral Isolation and Identification

Virological samples were examined by the Unit of Medical Virology at the Institut Pasteur of Dakar, a part of the WHO Global Influenza Surveillance Network since 1996. The nasopharyngeal swabs were removed from vials, and samples were processed and stored immediately at −80°C. For viral isolation, plates containing monolayers of Madin-Darby Canine Kidney (MDCK) cells, human lung laryngeal epidermoid carcinoma (HEp-2) or Vero cells lines were inoculated with 2 ml Dulbecco's minimum Essential's medium (DMEM reference M 2645, Sigma-Aldrich, St. Louis, MO). MDCK cells supplemented with 3 μg/ml trypsin (TPCK, reference 93630, Sigma-Aldrich) were used for the isolation of influenza viruses, as described previously [Dosseh et al., 2000]. The other two cell lines were used

to attempt to isolate other respiratory viruses (human respiratory syncytial virus (hRSV), parainfluenza viruses (PIV) and adenovirus).

Viruses were identified using the hemagglutination inhibition (HI) method and by direct immunofluorescence assays. Supernatants from influenza-positive specimens were typed by HI to determine the virus type (A or B), and subtyped for influenza virus type A (H1, H3) with specific antisera, as recommended by WHO standard protocols (WHO Manual on Animal influenza Diagnosis and Surveillance; WHO/CDS/CSR/NCS/2002.5 Rev.1). Slides were prepared from all inoculated samples, and were tested for hRSV, adenovirus, influenza, and parainfluenza viruses 1, 2, and 3 by direct immunofluorescence, according to the manufacturer's recommendations (Respiratory Viruses Panel I Viral Screening & Identification Kit, Chemicon Light Diagnostics, Temecula, CA).

### RNA and DNA Extraction

Viral RNA was extracted from 300 μl of each sample, using BioRobot M48 and the Mag Attract Viral RNA kit (Qiagen AB, Germantown, MD). The Mag Attract Viral DNA kit (Qiagen AB) to extract DNA from 200 μl of each sample was used.

### Multiplex RT-PCR

Four multiplex RT-PCR methods (m-RT/PCR), targeting 16 respiratory viruses, developed by the Laboratory of Human and Molecular Virology of the University Hospital of Caen (France), were used [Bellau Pujol et al., 2005; Freymuth et al., 2006; Vabret et al., 2008]. m-RT/PCR 1 detected influenza viruses A and B, human metapneumovirus (hMPV) and hRSV (types A and B). m-RT/PCR 2 detected parainfluenza virus types 1, 2, 3, and 4 (PIV 1–4). m-RT/PCR 3 detected rhinovirus, enterovirus and *influenza* C, and m-RT/PCR 6 detected the four human coronaviruses (HCoVs) except for SARS-CoV: HCoVs OC43, NL63, 229E, and HKU1. The products of m-RT/PCR 1, 2, and 3 were subjected to hemi-nested m-RT/PCR. An internal primer was designed for each virus and used together with the corresponding anti-sense primer used for m-RT/PCR. Multiplex RT-PCR and hemi-nested PCR products were visualized under UV light after electrophoresis through an ethidium bromide-stained 1% agarose gel. The m-RT/PCR 6 products were confirmed by hybridization (GEN-ETI-K DEIA Kit, Sorin Biomedica, Saluggia, Italy).

Influenza viruses were sub-typed for N1, H1, and H3 antigens [Wright et al., 1995] and for M, N2, H2, and H5 [Schweiger et al., 2000].

The presence of adenovirus and bocavirus genomes was sought by PCR [Hierholzer et al., 1993; Allander et al., 2005]. Lastly, the polyomaviruses KI and WU, isolated recently from respiratory samples, were amplified using primers as defined originally by Allander et al. [2007] and Gaynor et al. [2007].

Data were analyzed using STATA™ version 10.0 (College station, TX), statistical differences were considered significant if $P < 0.05$.

## RESULTS

Eighty-two acute respiratory episodes were evaluated in 67 children between July and December 2007: 54 children had one acute respiratory episode, 11 had two acute respiratory episodes, and 2 children had three acute respiratory episodes during the study period. Among these 67 children, the proportion of children was similar between male and female (36 (53.7%) male and 31 (46.3%) female; $P = 0.51$) and between the two villages (30 (44.8%) from Dielmo and 37 (55.2%) from Ndiop; $P = 0.41$). Consultations relating to acute respiratory infections represented between 15% and 25% of all consultations that occurred at the outpatient primary care setting during the study period.

The number of acute respiratory episodes was higher in children under 24 months of age (43 (52.4%) of the 82 acute respiratory episodes) compared to 39 in children between 24 and 60 months of age ($P = 0.03$). Among the children under 24 months of age, 8 (18.6%) were less than 6 months old. Ten (12.2%) acute respiratory episodes were associated with thick blood films positive for *Plasmodium falciparum*; 6 (7.3%) of these episodes occurred simultaneously with a clinical malaria episode, and these children were treated with artemisinin-based combination therapy. Thick blood films were negative in the other 72 respiratory episodes. In total, 74 (90.2%) episodes were classified as upper respiratory tract illness and 8 (9.8%), as lower respiratory tract illness. Antibiotic therapy with amoxicillin or cotrimoxazole was used in 24 (29.3%) episodes: 14 in children below 24 months of age and 10 in children between 24 and 60 months of age ($P = 0.07$). No children were admitted to the hospital for severe acute respiratory infection during the study period. No clinical signs of pharyngitis, laryngitis, crackles, or wheezing were listed in the questionnaire by the clinical team. Some of the children had underlying conditions, such as atopy (n = 7) and passive exposure to tobacco smoke (n = 20). No children were known to have immunosuppression and there were no premature newborns among the children with acute respiratory infections who were less than 24 months old.

Fifty-four of the 67 children had one acute respiratory episode: 35 viruses were identified in 31 (57.4%) episodes, and no viruses were detected in 23 (42.6%) episodes. Eleven children had two episodes of acute respiratory tract infections (total of 22 episodes): 17 viruses in 14 (63.6%) episodes were identified, and no viruses were detected in the other 8 (36.4%) episodes. Two children had three acute respiratory episodes each (total of six episodes): three viruses were identified in three (50.0%) episodes, and no viruses were detected in the remaining three episodes. In total, no virus was detected in 34 (41.5%) acute respiratory episodes. Table I details the characteristics of the total 82 episodes, in relation to the detected viruses.

TABLE I. Characteristics of the Children With Acute Respiratory Episodes (n = 82) in Relation to the Detected Viruses (n = 55*)

| Characteristics of children at the time of each acute respiratory episode (n = 82) | Episodes with influenza (n = 25) | Episodes with hRSV A (n = 13) | Episodes with rhinovirus (n = 8) | Episodes with HCoV (n = 6) | Episodes with PIV-3 and PIV-4 (n = 2) | Episodes with no viral detection (n = 34) |
|---|---|---|---|---|---|---|
| Children <6 months of age | 2 | 1 | 1 | 2 | 1 | 2 |
| Children between 6 and 23 months of age | 11 | 8 | 5 | 2 | 0 | 12 |
| Children ≥24 months of age | 12 | 4 | 2 | 2 | 1 | 20 |
| Male/female | 15/10 | 9/4 | 5/3 | 4/2 | 0/2 | 15/19 |
| Villages: Dielmo/Ndiop | 16 influenza A: 11/5<br>7 influenza B: 6/1<br>2 influenza C: 1/1 | 1/12 | 2/6 | 4/2 | 1/1 | 19/15 |
| Concomitant malaria episode | 1 | 2 | 1 | 0 | 0 | 6 |
| Anorexia | 11 | 7 | 4 | 2 | 1 | 6 |
| Upper respiratory tract symptoms | | | | | | |
| Rhinitis | 24 | 13 | 7 | 5 | 2 | 31 |
| Otitis | 0 | 1 | 0 | 0 | 0 | 0 |
| Lower respiratory tract symptoms | | | | | | |
| Cough | 19 | 12 | 8 | 5 | 1 | 28 |
| Rhonchi | 3 | 5 | 1 | 1 | 1 | 2 |
| Other symptoms | | | | | | |
| Conjunctivitis | 4 | 5 | 1 | 1 | 1 | 1 |
| Diarrhea | 4 | 6 | 4 | 2 | 2 | 3 |

One bocavirus was co-detected with influenza virus A + NL63 viruses.

Table II shows the 55 viruses detected and Figure 1 shows the viruses detected over 2-week periods. Influenza viruses (45.6%) were the most frequent, followed by hRSV (23.6%, all type A), rhinovirus (14.5%), and HCoVs 229E and NL63 (10.9%). The parainfluenza virus was detected only in two episodes (one PIV-3 and one PIV-4), and bocavirus was detected only in one episode. hMPV, HCoV-OC43, HCoV-HKU1, enterovirus, adenovirus, or polyomaviruses K1 and WU were not detected during the study period. All viruses were detected using genome detection tests except for influenza viruses where 11 samples were tested positive by MDCK cell culture; no virus was detected using the HEp-2 and vero cell lines. Among these 11 samples (8 influenza viruses A and 3 influenza viruses B), 2 influenza viruses A samples were negative by PCR.

TABLE II. Description of the 55 Viruses Detected During the Study Period

| Viruses | Number of positive detections | % of positive viral detection (n = 55) | % of episodes (n = 82) |
|---|---|---|---|
| Influenza viruses | 25 | 45.5 | 30.5 |
| A | 16 | 29.1 | 19.5 |
| B | 7 | 12.7 | 8.5 |
| C | 2 | 3.7 | 2.5 |
| hRSV | 13 | 23.6 | 15.9 |
| hRSV A | 13 | 23.6 | 15.9 |
| hRSV B | 0 | 0 | 0 |
| hMPV | 0 | 0 | 0 |
| PIV (1 PIV-3 and 1 PIV-4) | 2 | 3.5 | 2.5 |
| HCoV | 6 | 10.9 | 7.3 |
| HCoV-229E | 2 | 3.7 | 2.5 |
| HCoV-NL63 | 4 | 7.2 | 4.9 |
| HCoV-OC43 | 0 | 0 | 0 |
| HCoV-HKU1 | 0 | 0 | 0 |
| Rhinovirus | 8 | 14.6 | 9.7 |
| Enterovirus | 0 | 0 | 0 |
| Bocavirus | 1 | 1.8 | 1.2 |
| Adenovirus | 0 | 0 | 0 |
| Polyomaviruses KI, WU | 0 | 0 | 0 |
| Total | 55 | 100.0 | 67.1 |

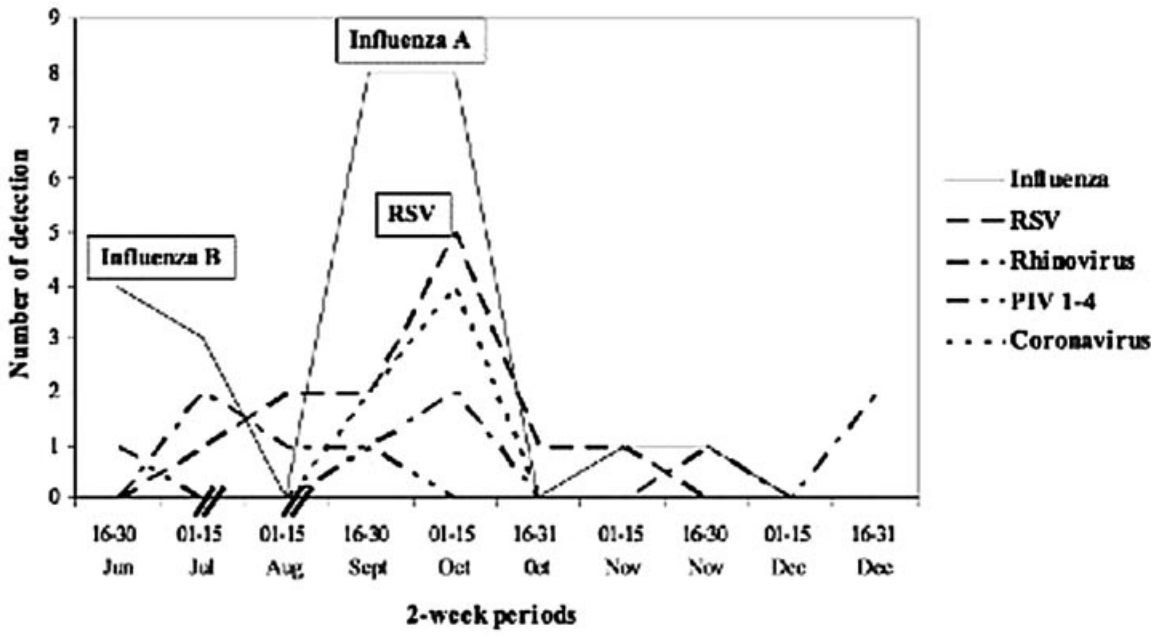


Fig. 1. Number of respiratory virus-positive specimens detected in each 2-week period of the study.

In total, influenza viruses were detected in 25 episodes (PCR or cell culture): 16 (64.0%) were due to influenza viruses A, 7 (28.0%) to influenza viruses B, and 2 (8.0%) to influenza viruses C. Among the 14 influenza viruses A which were positive by PCR, sub-typing by RT-PCR identified 13 H1N1 and 1 H3N2 viruses. Viruses were co-detected in six episodes: influenza viruses A + HCoV-NL63 (n = 2), hRSV + HCoV-229E (n = 2), hRSV and rhinovirus (n = 1), and influenza viruses A + HCoV-NL63 + bocavirus (n = 1). Table III details the results of viral detection in the 13 children who had more than one episode during the study period.

## DISCUSSION

The various respiratory viruses causing acute respiratory infections in a rural area of Senegal, a West African country, are described. The clinical team of a primary care center collected a total of 82 nasopharyngeal samples between mid-June and December 2007 from children under 5 years of age who presented signs of acute respiratory disease. At least ten different viruses circulating in the two test villages during the rainy season were detected using viral culture and PCR. Children with respiratory virus infections experienced respiratory symptoms consisting mostly of upper respiratory symptoms. As expected, influenza viruses and hRSV were detected most frequently. Rhinovirus [Peltola et al., 2008] and human coronaviruses were associated with respiratory symptoms in a quarter of the children. The frequency of PIV was remarkably low in this study; in the USA peaks of PIV are reported during the cold season [Fry et al., 2006]. In our community-based study, bocavirus was co-detected with influenza virus A and NL63 in only one episode, as expected from previous reports [Falsey et al., 2005; Longtin et al., 2008]. Bocavirus co-detected with influenza virus A and NL63 was not associated with a lower respiratory tract infection, as has been reported in rural Thailand [Fry et al., 2007]. Adenovirus or human metapneumovirus were not detected, possibly due to the small number of lower respiratory cases examined [Arnold et al., 2008] and the small number of acute respiratory infections in very young children in this study [Regamey et al., 2008].

The season of human influenza reported previously during the rainy season in Senegal is confirmed [Dosseh and Rogier, 1996; Dosseh et al., 2000]; episodes of influenza viruses B in late June and episodes of influenza viruses A between mid-September and mid-October were identified. The distribution of hRSV in sub-tropical countries throughout the year is still unknown; in Europe and the USA, symptomatic hRSV infections occur mainly in young children during the cold season [Meerhoff et al., 2006; CDC, 2007]. However, two simultaneous clusters of acute respiratory infections were observed: one consisted of influenza virus A and occurred in Dielmo, whereas the other cluster concerned hRSV in Ndiop between mid-September and mid-October. The two villages are close geographically to each other, but the biotope is very different and rural exchanges are rare. hRSV transmission in humans may occur through children or adults, including individuals without or with mild, symptoms [Falsey et al., 2005]. Among the 12 acute respiratory infection episodes associated with hRSV, episodes of clinical bronchiolitis were not reported (children were aged between 4 and 37 months). The absence of clinical bronchiolitis is possibly due to the role of breastfeeding in this population, where indeed women continue breastfeeding their children for up to 24 months (mixed

TABLE III. Results of Viral Detection in 11 Children With 2 Episodes of Acute Respiratory Infection and in 2 Children With 3 ARI Episodes During the Study Period

| 1st episode | Virus 1 | 2nd episode | Virus 2 | 3rd episode | Virus 3 | Village | Age (months) |
|---|---|---|---|---|---|---|---|
| 26 June | Influenza B | 21 November | ND* | — | — | Dielmo | 31 |
| 27 June | Influenza B | 26 September | Influenza A + NL63 + bocavirus | — | — | Dielmo | 42 |
| 29 June | PIV-3 | 6 October | Influenza A | — | — | Dielmo | 4 |
| 1 July | Rhino | 5 October | NL63 | — | — | Dielmo | 9 |
| 3 July | Influenza B | 12 August | ND* | 29 September | Influenza A + NL63 | Dielmo | 6 |
| 17 July | ND* | 8 October | Influenza A | — | — | Ndiop | 15 |
| 12 August | hRSV | 19 December | ND* | — | — | Dielmo | 22 |
| 28 September | Influenza A | 24 November | ND* | — | — | Dielmo | 49 |
| 28 September | ND* | 12 October | ND* | — | — | Ndiop | 3 |
| 4 October | hRSV | 23 November | ND* | — | — | Ndiop | 28 |
| 5 October | Influenza A + NL63 | 6 November | ND* | 30 December | ND | Dielmo | 24 |
| 5 October | Influenza A + 229E | 13 November | MIC | — | — | Dielmo | 18 |
| 5 October | Influenza A | 17 December | Rhinovirus | — | — | Dielmo | 23 |

*Not determined.

milk feeding). Also, the rural environment may protect children from severe forms of hRSV disease, whereas in urban areas, air pollution may act as a trigger in severe acute bronchiolitis cases [Diouf et al., 2003; Hertz-Picciotto et al., 2007; Karr et al., 2007; Segala et al., 2008].

Treatment with amoxicillin or cotrimoxazole was reported in more than a quarter of the children, and virus was detected in 14 (58.3%) episodes in those receiving antibiotic therapy. This shows that, even in tropical rural areas, the over-prescription of antibiotics may be frequent. The clinical symptoms associated with the prescription of antibiotics were mainly rhonchi with cough, suggesting bacterial bronchitis. Bacterial causes of acute respiratory infections were not documented in this study. However, an association between invasive pneumococcal disease and increased circulation of respiratory viruses has been reported in North America [Talbot et al., 2005].

In relation to the prescription of anti-malaria therapy, only laboratory-confirmed malaria episodes with clinical symptoms were treated with artemisinin-based combination therapy. Even during the rainy season, acute respiratory infections were frequent and only a few episodes were associated with clinical malaria and hence treated with artemisinin-based combination therapy. The malaria burden during the rainy season is probably over-estimated in sub-tropical countries. With the use of rapid detection tests for malaria, it should be possible to reduce the over-prescription of anti-malaria drugs in primary and secondary care settings.

In conclusion, the diversity of viruses responsible for respiratory tract infections in children under five living in a rural area of Senegal is described. Molecular diagnostic tests facilitated the identification of all viruses sought. The volunteers from the two villages participated originally in a malaria project, but the current study highlights the importance of examining other co-morbidity factors in rural settings in tropical areas. The implementation or reinforcement of influenza sentinel sites associated with reference laboratories in tropical areas is essential to ensure emergency preparedness of the health care systems.

## ACKNOWLEDGMENTS

We are indebted to the Dielmo and Ndiop children and their parents for their invaluable help and commitment in participating in this study. We thank the data management and field clinical staff who contributed to the study, in particular Joseph Faye, Abdoulaye Badiane and Julie Coutherut. We are also indebted to the scientific teams who have contributed to the multidisciplinary program on malaria in Dielmo and Ndiop over the last 15 years, in particular Adama Tall, Alioune Badara Ly, Cheikh Sokhna and Jean-François Trape. We thank François Freymuth for his suggestions concerning the clinical protocol. We also thank Katherine Kean and Richard Paul for revising the English of the manuscript. We acknowledge the Senegalese Ministry of Health, in particular the National Health Research Council for their help in implementing this study.

## REFERENCES

Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci USA 102:12891–12896.

Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MA, Dalianis T, Ramqvist T, Andersson B. 2007. Identification of a third human polyomavirus. J Virol 81:4130–4136.

Arnold JC, Singh KK, Spector SA, Sawyer MH. 2008. Undiagnosed respiratory viruses in children. Pediatrics 121:e631–e637.

Bellau-Pujol S, Vabret A, Legrand L, Dina J, Gouarin S, Petitjean-Lecherbonnier J, Pozzetto B, Ginevra C, Freymuth F. 2005. Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses. J Virol Methods 126:53–63.

Centers for Disease Control and Prevention. 2007. Brief report: Respiratory syncytial virus activity—United States, July 2006–November 2007. Morb Mortal Wkly Rep 56:1263–1265.

Diouf A, Garcon G, Thiaw C, Diop Y, Fall M, Ndiaye B, Siby T, Hannothiaux MH, Zerimech F, Ba D, Haguenoer JM, Shirali P. 2003. Environmental lead exposure and its relationship to traffic density among Senegalese children: A pilot study. Hum Exp Toxicol 22:559–564.

Dosseh A, Rogier C. 1996. Influenza survey in Senegal. Trans R Soc Trop Med Hyg 90:377.

Dosseh A, Ndiaye K, Spiegel A, Sagna M, Mathiot C. 2000. Epidemiological and virological influenza survey in Dakar, Senegal: 1996–1998. Am J Trop Med Hyg 62:639–643.

Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. 2005. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med 352:1749–1759.

Freymuth F, Vabret A, Cuvillon-Nimal D, Simon S, Dina J, Legrand L, Gouarin S, Petitjean J, Eckart P, Brouard J. 2006. Comparison of multiplex PCR assays and conventional techniques for the diagnostic of respiratory virus infections in children admitted to hospital with an acute respiratory illness. J Med Virol 78:1498–1504.

Fry AM, Curns AT, Harbour K, Hutwagner L, Holman RC, Anderson LJ. 2006. Seasonal trends of human parainfluenza viral infections: United States, 1990–2004. Clin Infect Dis 43:1016–1022.

Fry AM, Lu X, Chittaganpitch M, Peret T, Fischer J, Dowell SF, Anderson LJ, Erdman D, Olsen SJ. 2007. Human bocavirus: A novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. J Infect Dis 195:1038–1045.

Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, Brennan DC, Storch GA, Sloots TP, Wang D. 2007. Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoS Pathog 3:e64.

Hertz-Picciotto I, Baker RJ, Yap PS, Dostal M, Joad JP, Lipsett M, Greenfield T, Herr CE, Benes I, Shumway RH, Pinkerton KE, Sram R. 2007. Early childhood lower respiratory illness and air pollution. Environ Health Perspect 115:1510–1518.

Hierholzer JC, Halonen PE, Dahlen PO, Bingham PG, McDonough MM. 1993. Detection of adenovirus in clinical specimens by polymerase chain reaction and liquid-phase hybridization quantitated by time-resolved fluorometry. J Clin Microbiol 31:1886–1891.

Karr C, Lumley T, Schreuder A, Davis R, Larson T, Ritz B, Kaufman J. 2007. Effects of subchronic and chronic exposure to ambient air pollutants on infant bronchiolitis. Am J Epidemiol 165:553–560.

Longtin J, Bastien M, Gilca R, Leblanc E, de Serres G, Bergeron MG, Boivin G. 2008. Human bocavirus infections in hospitalized children and adults. Emerg Infect Dis 14:217–221.

Meerhoff TJ, Fleming D, Smith A, Mosnier A, van Gageldonk-Lafeber AB, Paget WJ. 2006. Surveillance recommendations based on an exploratory analysis of respiratory syncytial virus reports derived from the European Influenza Surveillance System. BMC Infect Dis 6:128.

Noranate N, Durand R, Tall A, Marrama L, Spiegel A, Sokhna C, Pradines B, Cojean S, Guillotte M, Bischoff E, Ekala MT, Bouchier C, Fandeur T, Ariey F, Patarapotikul J, Le Bras J, Trape JF, Rogier C, Mercereau-Puijalon O. 2007. Rapid dissemination of Plasmodium falciparum drug resistance despite strictly controlled antimalarial use. PLoS ONE 2:e139.

Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. 2009. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 361:2605–2615.

Peltola V, Waris M, Osterback R, Susi P, Ruuskanen O, Hyypia T. 2008. Rhinovirus transmission within families with children: Incidence of symptomatic and asymptomatic infections. J Infect Dis 197:382–389.

Regamey N, Kaiser L, Roiha HL, Deffernez C, Kuehni CE, Latzin P, Aebi C, Frey U. 2008. Viral etiology of acute respiratory infections with cough in infancy: A community-based birth cohort study. Pediatr Infect Dis J 27:100–105.

Schweiger B, Zadow I, Heckler R, Timm H, Pauli G. 2000. Application of a fluorogenic PCR assay for typing and subtyping of influenza viruses in respiratory samples. J Clin Microbiol 38:1552–1558.

Segala C, Poizeau D, Mesbah M, Willems S, Maidenberg M. 2008. Winter air pollution and infant bronchiolitis in Paris. Environ Res 106:96–100.

Talbot TR, Poehling KA, Hartert TV, Arbogast PG, Halasa NB, Edwards KM, Schaffner W, Craig AS, Griffin MR. 2005. Seasonality of invasive pneumococcal disease: Temporal relation to documented influenza and respiratory syncytial viral circulation. Am J Med 118:285–291.

Vabret A, Dina J, Gouarin S, Petitjean J, Tripey V, Brouard J, Freymuth F. 2008. Human (non-severe acute respiratory syndrome) coronavirus infections in hospitalised children in France. J Paediatr Child Health 44:176–181.

Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. 2002. Estimates of world-wide distribution of child deaths from acute respiratory infections. Lancet Infect Dis 2:25–32.

Wright KE, Wilson GA, Novosad D, Dimock C, Tan D, Weber JM. 1995. Typing and subtyping of influenza viruses in clinical samples by PCR. J Clin Microbiol 33:1180–1184.