

Incidence of Respiratory Viruses Among Travelers With a Febrile Syndrome Returning From Tropical and Subtropical Areas

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Fifty million people are estimated to travel from industrial countries to the tropics annually. In spite of exhaustive studies and widely different diagnosis among returned patients, some cases of febrile illnesses remain without an etiological diagnosis, suggesting that these cases could be due to viral respiratory tract infections. From August 2005 to October 2006, 118 febrile patients without a specific diagnosis in their first visit at the Center for International Health of the Hospital Clínic of Barcelona were included. In all of them, in order to study respiratory viruses, a nasopharyngeal swab was collected. Clinical and radiological features and epidemiological data, as well as other samples for microbiologic studies, were also collected during consultation. Based on the physician's judgment at the time of consultation, patients were classified into four groups: respiratory symptoms (62%), febrile syndrome with nonspecific symptoms (24%), digestive symptoms (10%), and patients presenting both respiratory and digestive symptoms (4%). A pathogen microorganism was detected in 61 patients (52%). Respiratory viruses were detected in 44 out of 118 (37%) travelers included in the study, representing 56% of the patients with respiratory symptoms. The most frequently viruses detected were influenza virus (38%), rhinovirus (23%), adenovirus (9%), and respiratory syncytial virus (9%). Respiratory viruses have been shown to play an important role in imported fever. In light of the fact that international tourism is an increasing phenomenon, new strategies to prevent the spread of respiratory viruses should be considered, specially for influenza when a vaccine is available. **J. Med. Virol.** 80:711–715, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: respiratory tract infection; virological diagnosis; febrile illness

INTRODUCTION

Fifty million people are estimated to travel from industrial countries to the tropics annually [Ryan et al., 2002; Hill, 2006], with up to 50% of travelers visiting developing countries presenting a travel-related health problem [Spira, 2003]. Furthermore, 3–11% of travelers to tropical areas report febrile illnesses on their return [Bruni and Steffen, 1997; Hill, 2000; Steffen et al., 2003; Antinori et al., 2004]. Nowadays, epidemiological investigations of health risks in travelers have focused on infections perceived as specific for this population, such as malaria, gastroenteritis, hepatitis, and other travel-related vaccine-preventable diseases [Humar and Keystone, 1996; Magill, 1998; Parola et al., 2006]. In spite of exhaustive studies and widely different diagnosis of febrile illnesses, some cases remain without an etiological diagnosis. It is thought that a percentage of these cases could be due to viral respiratory tract infections. In Spain, most people travel in August and September where tropical areas experience local variations in temperature, humidity and rainfall. These conditions are optimal for the circulation of respiratory viruses.

The present evaluated the role of respiratory viruses in febrile patients after being in the tropics.

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MATERIALS AND METHODS

Patients

From August 2005 to October 2006, 118 febrile patients older than 14 years old were prospectively studied at the Center of International Health of the Hospital Clínic of Barcelona. Patients with febrile symptoms 10 days before or after their return from a tropical or subtropical area without a specific diagnosis on their first visit were included in the study. Fever was defined by a documented axillary temperature of 37.5°C or higher or by the combination of febrile sensation, chills and sweats within 3 days prior to consultation.

Before the collection of a nasopharyngeal swab to study respiratory viruses, all patients were asked to participate in this study. Only patients in whom a nasopharyngeal swab was available and was collected within the 10 days posttravel were included. Specific serologies for dengue and herpes virus were performed when clinical suspicion was present. Blood cultures were done when temperature reached 38°C or clinical signs of bacteremia showed. Stool cultures were done to patients who presented diarrhea. *Leptospira* spp. serology (Microagglutination Test—MAT) was performed in one case. Patients with a diagnosis of malaria were excluded.

Clinical and radiological features, epidemiological data and a sample collection were performed by physicians from the Center for International Health. Clinical data were collected during consultation using case-record forms and included: age, sex, country visited, days of stay, travel history, antimalarial chemoprophylaxis and up-to-date vaccinations, time interval between the onset of fever and the day of consultation, time interval between the date of return and the day of consultation and a questionnaire referring to respiratory symptoms. Processing of samples and laboratory diagnosis were performed in the Department of Microbiology of the Hospital Clínic of Barcelona.

The Ethics Committee of the Hospital Clínic approved the study.

Microbiological Methods

Nasopharyngeal samples (1 nasal plus 1 oropharyngeal swab) were collected from all patients as described previously [Angeles Marcos et al., 2006]. Within the first 24 hr, nasopharyngeal swabs were processed for antigen detection by indirect immunofluorescence assay (IFA). Samples were stained with fluorescein-conjugated antibody to influenza virus A, influenza virus B, human parainfluenza virus 1–3, adenovirus and respiratory syncytial virus (Respiratory Panel 1, Viral Screening and Identification Kit; Light Diagnostics, Chemicon, Temecula, CA). The presence of viral antigen in respiratory cells was indicated by the appearance of characteristic intracellular apple-green fluorescence in ≥ 1 cell. Upon sample collection, an aliquot of each fresh specimen was collected to be used for RT-PCR analysis. Nucleic acids from any DNA/RNA viruses present in the

nasopharyngeal swab were extracted from 200 μ l of specimen using *NucliSense easyMAG* (BioMérieux, Boxtel, The Netherlands) according to the manufacturer's instructions. The lysis buffer included 500 molecules of the cloned amplified product used as internal control in each reaction tube and then excluded false-negative results due to nonspecific inhibitors or extraction failure. Two independent multiplex nested RT-PCR assays were used to detect influenza virus A, influenza virus B, influenza virus C, respiratory syncytial virus, respiratory syncytial virus B and adenovirus [Coiras et al., 2003], and, human parainfluenza virus 1–4A and 4B, human coronavirus 229E, human coronavirus OC43 and the generic detection of enterovirus and rhinovirus [Coiras et al., 2004]. All positive results were confirmed in two sequential assays. A RT-PCR previously standardized by the Spanish National Center of Microbiology (Instituto de Salud Carlos III) that amplifies H1, H3, and H5 subtypes viruses, was performed in the influenza A positive samples. Retrospectively, a RT-PCR for human metapneumovirus was performed using the fusion (F) and polymerase (L) genes as targets. A negative (viral transport medium containing no nucleic acid) and positive control obtained from our viral isolates was included in each assay in order to control extraction and amplification handling.

Only influenza A H1 or H3 positives and H5 negative samples were inoculated into a MDCK (Madin Darby Canine Kidney) cell line (Vircell, Granada, Spain) for virus isolation.

A viral etiology was considered when a respiratory virus was detected at least in PCR, IFA or in both.

RESULTS

Epidemiology

From August 2005 to October 2006, 118 travelers presenting fever after returning from tropical or subtropical areas were included, with 61 being men (52%) and 57 women (48%) with an average age of 37 years. During the study period the highest number of traveler consultation occurred during the months of July through October, with a peak in August 2006. Geographical regions to which the patients had traveled recently or had visited during the time of probable acquisition included Asia (53%), Africa (36%), and Latin America (11%). All patients had received pre-travel advice. Depending on the travel destination, 84 travelers (71%) were vaccinated for: hepatitis A, hepatitis B, typhoid fever, diphtheria-tetanus, polio or yellow fever and 59 travelers (50%) received antimalarial prophylaxis. The average of time spent in trip was 23 days.

Clinical Features

The average time from the onset of fever and the hospital consultation was of 4.4 days, occurring in most cases 2.3 days after their arrival. In general, the clinical symptoms had a duration of 5 days.

TABLE I. Frequency of Presenting Symptoms in Febrile Returned Travelers

Symptom	No. (%) of patients
Fever	118 (100)
Cough	63 (53)
Headache	57 (48)
Rhinorrhea	53 (45)
Arthromyalgia	46 (40)
Sore throat	40 (34)
Asthenia	33 (28)
Diarrhea	24 (20)
Vomiting	17 (14)
Rash	10 (8)

In all patients, fever was associated to other symptoms, which are summarized in Table I. Based on the physician's judgment at the time of consultation, patients were classified into four groups: respiratory symptoms (62%, n = 73), febrile syndrome with non-specific symptoms (24%, n = 28), digestive symptoms (10%, n = 12), and patients presenting both respiratory and digestive symptoms (4%, n = 5).

Hospitalization was required for 10 patients (8%), 4 of whom had a febrile syndrome with nonspecific symptoms, 2 a respiratory tract infection, 2 had digestive complications, and 2 remained without an etiological diagnosis.

Diagnosis of Imported Fever

A pathogen microorganism was detected in 61 patients (52%). Nasopharyngeal swabs were the only samples collected in 60 patients. Only 18 of the non-diagnosed patients had microbiological samples other than nasopharyngeal swabs. According to the clinical symptom classification, respiratory tract infections and febrile syndrome with nonspecific symptoms were the main groups that remained with an unknown etiological diagnosis, 45% and 61% respectively.

The microorganisms detected according to the different clinical groups are described in Table II. Respiratory viruses were detected in 44 out of 118 (37%) travelers

included in the study, representing 56% of the patients with respiratory symptoms. Respiratory viruses were only detected in patients presenting respiratory symptoms.

Forty-five respiratory viruses were detected (Fig. 1) influenza A and influenza B (n = 18, 38%), being the most frequently detected followed by rhinovirus (n = 11, 23%), adenovirus (n = 4, 9%), and respiratory syncytial virus A (n = 4, 9%). Among the influenza A subtypes found, 8 were A/H3 and 4 A/H1. No A/H5 subtype was detected. Considering all the patients included, influenza viruses accounted for 15% of the infections. In two cases an enterovirus was identified; one patient presenting respiratory and digestive symptoms and the second only with digestive symptoms.

Two of the patients with influenza A infection had been pre-travel influenza vaccinated. One traveled to Vietnam (A/H1) and the second returned from South Africa (A/H3). Moreover, influenza viruses were involved in 2 out of the 10 patients requiring hospitalization (Table II), being the sole pathogen detected in one of them.

According to the travel destination, respiratory viral infection was diagnosed in 6/13 of patients having traveled to Latin America (46%), 16/43 to Africa (37%) and 23/62 returning from Asia (37%) (Table III).

DISCUSSION

Fever is relatively common after tropical travel, and in light of the fact that international tourism is an increasing phenomenon, it will be observed more frequently. Although many reports have evaluated the etiology of febrile illnesses among travelers [Doherty et al., 1995; O'Brien et al., 2001; Antinori et al., 2004], no previous studies have focused specifically on respiratory viruses as a cause of a febrile disease.

In the present study, 118 travelers with fever without a specific diagnosis in their first visit were included, representing 8.5% of the patients that attended the Center for International Health of the Hospital Clinic of Barcelona during the study period. A pathogen microorganism was

TABLE II. Distribution of Microorganisms According to the Traveler's Clinical Symptoms

Respiratory symptoms (n = 73)	Digestive symptoms (n = 12)	Resp + digestive symptoms (n = 5)	Febrile syndrome with nonspecific symptoms (n = 28)
Influenza virus A	10 <i>C. jejuni</i>	2 Rhinovirus + EAEC	1 Dengue virus
Influenza virus A + dengue ^a	1 <i>S. choleraesuis</i> ^a	1 CoV229E + <i>C. jejuni</i>	1 Herpes virus 6 ^a
Influenza virus A + <i>K. pneumoniae</i>	1 <i>Salmonella</i> C2 ^a	1 Enterovirus	1 Epstein-Barr virus ^a
Influenza virus B ^a	5 <i>Giardia</i>	1 Rhinovirus	1 <i>K. pneumoniae</i> ^a
Influenza virus B + parainfluenza virus 3	1 <i>Shigella sonnei</i>	1 Parainfluenza virus 4	1 <i>Leptospira canicola</i> ^a
Respiratory syncytial virus A	4 Enterovirus		
Parainfluenza virus 2	1		
Parainfluenza virus 3	1		
Parainfluenza virus 4	2		
Rhinovirus	9		
Adenovirus	4		
Total (n)	39	7	10

^aCases requiring hospitalization. EAEC, *enteroaggregative Escherichia coli*.

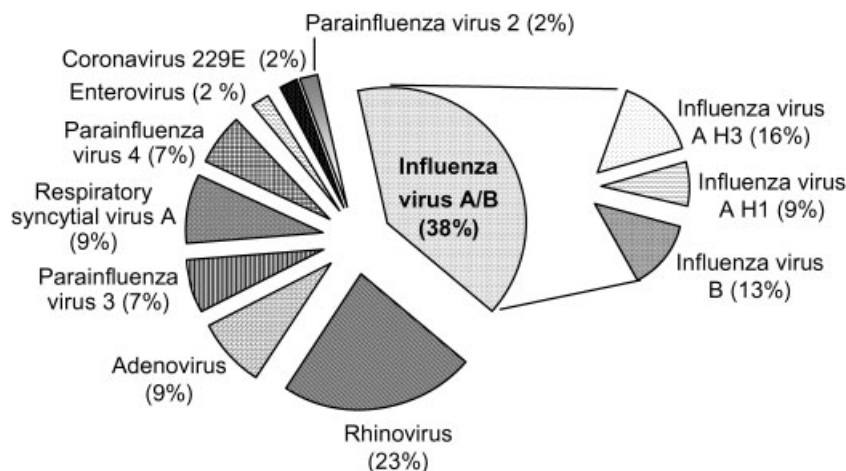


Fig. 1. Distribution of respiratory viruses detected.

detected in 52% of the travelers involved in the study. Recent reports have shown that respiratory tract infections are common in travelers [O'Brien et al., 2001; Parola et al., 2006]. In our study, 66% of the travelers included had a respiratory infection, with more than half being caused by a respiratory virus. It is well known that the incidence of some respiratory viruses, mainly respiratory syncytial virus and influenza virus, show seasonal trends and vary with the prevailing environmental conditions which are specially variable in the tropics [Shek and Lee, 2003]. In general, the presence of influenza virus infection has been observed with increased rainfall in several reports performed in Singapore [Chew et al., 1998], Senegal [Dosseh et al., 2000] and in Northeast Brazil [de Arruda et al., 1991].

The higher incidence of viruses as etiologic agents of respiratory infection in our study could be explained by the large profile of viruses studied and the use of two different detection techniques. We must take into account that due to the lack of another microbiological sample besides the nasopharyngeal swab in some patients, it may lead us to overlook other pathogens. In a recent study carried out in Switzerland [Mutsch et al., 2005] among 1,450 travelers returning from tropical and subtropical countries, seroconversion for influenza virus infection was found in 27 (12.8%) out of 211 travelers

that provided paired serum samples. However, whether influenza virus infection occurred just before home departure, en route, abroad, or shortly upon return cannot be ruled out. We obtained similar results concerning influenza virus. However, using molecular methods we were able to extend the range of respiratory viruses detected and consider that these respiratory viral infections took place abroad.

It should be highlighted that the study showed that respiratory viruses were only involved in travelers presenting respiratory symptoms, suggesting that respiratory virus screening would only be useful in patients with a respiratory infection. Influenza viruses were the most frequently detected, followed by rhinovirus, adenovirus, and respiratory syncytial virus.

Human metapneumovirus was not detected among travelers included in the study, is quite rare in healthy adults whereas among children [Manoha et al., 2007], the elderly [van den Hoogen, 2007] and immunocompromised patients [Kim et al., 2007] is more common.

Influenza A/H3 was the most commonly detected subtype, followed by influenza A/H1 and, less frequently, influenza B virus, as expected according to the epidemiological data of the 2005–2006 influenza season.

The study shows that influenza may be the most common vaccine-preventable disease in travelers

TABLE III. Distribution of Respiratory Viruses According to Travel Destinations

	Asia (n = 62 patients)	Africa (n = 43 patients)	Latin America (n = 13 patients)	Total (n = 118 patients)
Influenza virus A	8	2	2	12
Influenza virus B	4	0	2	6
Respiratory syncytial virus A	1	2	1	4
Parainfluenza virus 2	1	0	0	1
Parainfluenza virus 3	1	0	1	2
Parainfluenza virus 4	1	2	0	3
Rhinovirus	4	6	1	11
Enterovirus	0	2	0	1
Coronavirus 229E	0	1	0	1
Adenovirus	3	1	0	4
Total patients (no. viruses)	23 (23)	16 (16)	6 (7)	44 (45)

returning from tropical areas that present fever without a specific diagnosis, although, the complexity of this issue needs further discussion. Two pre-travel-vaccinated travelers returned from Vietnam and South Africa with influenza A/H1 and A/H3 infection, respectively. The trip to Vietnam took place in August and although this is not the season of influenza activity, previous reports have shown that influenza virus circulates at low levels all year-round in the tropics [Hampson, 1999; Harper et al., 2005].

Respiratory viruses have shown to play an important role in imported fever of travelers returning from tropical and subtropical areas as well as emergent pathogens. The results obtained in the present report raise the need for further investigations in virological and epidemiological surveillance of respiratory viruses among travelers. Furthermore, strategies to prevent the spread of respiratory viruses may be discussed, specially for influenza viruses when a vaccine is available.

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REFERENCES

- Angeles Marcos M, Camps M, Pumarola T, Antonio Martinez J, Martinez E, Mensa J, Garcia E, Penarroja G, Dambrava P, Casas I, Jimenez de Anta MT, Torres A. 2006. The role of viruses in the aetiology of community-acquired pneumonia in adults. *Antivir Ther* 11:351–359.
- Antinori S, Galimberti L, Gianelli E, Calattini S, Piazza M, Morelli P, Moroni M, Galli M, Corbellino M. 2004. Prospective observational study of fever in hospitalized returning travelers and migrants from tropical areas, 1997–2001. *J Travel Med* 11:135–142.
- Bruni M, Steffen R. 1997. Impact of travel-related health impairments. *J Travel Med* 4:61–64.
- Chew FT, Doraisingham S, Ling AE, Kumarasinghe G, Lee BW. 1998. Seasonal trends of viral respiratory tract infections in the tropics. *Epidemiol Infect* 121:121–128.
- Coiras MT, Perez-Brena P, Garcia ML, Casas I. 2003. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay. *J Med Virol* 69:132–144.
- Coiras MT, Aguilar JC, Garcia ML, Casas I, Perez-Brena P. 2004. Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. *J Med Virol* 72:484–495.
- de Arruda E, Hayden FG, McAuliffe JF, de Sousa MA, Mota SB, McAuliffe MI, Geist FC, Carvalho EP, Fernandes MC, Guerrant RL, et al. 1991. Acute respiratory viral infections in ambulatory children of urban northeast Brazil. *J Infect Dis* 164:252–258.
- Doherty JF, Grant AD, Bryceson AD. 1995. Fever as the presenting complaint of travellers returning from the tropics. *QJM* 88:277–281.
- 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.
- Hampson AW. 1999. Epidemiological data on influenza in Asian countries. *Vaccine* 17:S19–S23.
- Harper SA, Fukuda K, Uyeki TM, Cox NJ, Bridges CB. 2005. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 54:1–40.
- Hill DR. 2000. Health problems in a large cohort of Americans traveling to developing countries. *J Travel Med* 7:259–266.
- Hill DR. 2006. The burden of illness in international travelers. *N Engl J Med* 354:115–117.
- Humar A, Keystone J. 1996. Evaluating fever in travellers returning from tropical countries. *BMJ* 312:953–956.
- Kim YJ, Boeckh M, Englund JA. 2007. Community respiratory virus infections in immunocompromised patients: Hematopoietic stem cell and solid organ transplant recipients, and individuals with human immunodeficiency virus infection. *Semin Respir Crit Care Med* 28:222–242.
- Magill AJ. 1998. Fever in the returned traveler. *Infect Dis Clin North Am* 12:445–469.
- Manoha C, Espinosa S, Aho SL, Huet F, Pothier P. 2007. Epidemiological and clinical features of hMPV, RSV and RVs infections in young children. *J Clin Virol* 38:221–226.
- Mutsch M, Tavernini M, Marx A, Gregory V, Lin YP, Hay AJ, Tschopp A, Steffen R. 2005. Influenza virus infection in travelers to tropical and subtropical countries. *Clin Infect Dis* 40:1282–1287.
- O'Brien D, Tobin S, Brown GV, Torresi J. 2001. Fever in returned travelers: Review of hospital admissions for a 3-year period. *Clin Infect Dis* 33:603–609.
- Parola P, Soula G, Gazin P, Foucault C, Delmont J, Brouqui P. 2006. Fever in travelers returning from tropical areas: Prospective observational study of 613 cases hospitalised in Marseilles, France, 1999–2003. *Travel Med Infect Dis* 4:61–70.
- Ryan ET, Wilson ME, Kain KC. 2002. Illness after international travel. *N Engl J Med* 347:505–516.
- Shek LP, Lee BW. 2003. Epidemiology and seasonality of respiratory tract virus infections in the tropics. *Paediatr Respir Rev* 4:105–111.
- Spira AM. 2003. Assessment of travellers who return home ill. *Lancet* 361:1459–1469.
- Steffen R, Banos A, deBernardis C. 2003. Vaccination priorities. *Int J Antimicrob Agents* 21:175–180.
- van den Hoogen BG. 2007. Respiratory tract infection due to human metapneumovirus among elderly patients. *Clin Infect Dis* 44:1159–1160.