JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1997, p. 2864–2868 Vol. 35, No. 11
0095-1137/97/$04.00+0

Frequency and Natural History of Rhinovirus Infections in Adults during Autumn

EURICO ARRUDA,[1–3] ANNE PITKÄRANTA,[1,4] THEODORE J. WITEK, JR.,[5] CAROLINE A. DOYLE,[5] AND FREDERICK G. HAYDEN[1,2]*

Departments of Internal Medicine[1] and Pathology,[2] University of Virginia School of Medicine, Charlottesville, Virginia 22908; Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut 06877[5]; University of Sao Paulo School of Medicine, Ribeirão Preto, Sao Paulo 14049-900, Brazil[3]; and University Hospital of Helsinki, Helsinki, Finland[4]

Received 29 April 1997/Returned for modification 25 July 1997/Accepted 4 August 1997

Human rhinovirus (HRV) accounts for a significant portion of common-cold illness, with the peak incidence being in the early fall. Three hundred forty-six adults who had self-diagnosed colds of 48 h or less were enrolled in a study during September and October 1994 to determine the frequency and clinical course of HRV infections. Nasal wash specimens for viral culture and reverse transcription-PCR (RT-PCR) for HRV RNA and human coronavirus OC43 and 229E RNA detection were collected on enrollment, and participants recorded their symptoms twice daily for 14 days. Middle ear pressure (MEP) was measured with a digital tympanometer on days 1 and 7. Picornaviruses (224 HRV and 7 enterovirus isolates) were detected by culture in 67% (231 of 346) of the subjects. Among 114 samples negative by culture, HRV was detected by RT-PCR in 52 (46%) for an overall picornavirus infection rate of 82% (283 of 346 subjects). Among the remaining 62 negative samples, human coronavirus RNA was detected by RT-PCR in 5 patients, so that 288 (83%) of patients had documented viral infection. The first symptom noticed most often was sore throat (40%) in HRV culture- or PCR-positive patients and stuffy nose in HRV-negative patients (27%). No differences in symptom scores over time or in the presence of individual symptoms were noted between groups. The median duration of the cold episodes was 11 days in HRV culture-positive patients, 9.5 days in HRV RT-PCR-positive patients, and 11.5 days in HRV-negative patients. On enrollment, abnormal MEPs (≤ -100 or $\geq +100$ mm of H_2O) were found for 21% of HRV culture-positive patients, 14% of HRV RT-PCR-positive patients, and 10% of HRV-negative patients. No important differences in the clinical course of HRV culture-positive, HRV culture-negative and RT-PCR-positive, or HRV-negative colds were found. These results represent the highest frequency of virologically confirmed natural colds to date and document the importance of rhinoviruses as the cause of colds during fall months.

Human rhinoviruses (HRVs) are the most frequently recognized cause of acute upper respiratory infections among diverse populations, including working adults in the United States (9) and children in an impoverished tropical slum (1). The annual incidence of rhinovirus infection has been estimated to be approximately one per person in temperate areas (11). For reasons not entirely understood, the peak of incidence of colds caused by HRV in temperate regions of the northern hemisphere occurs in the autumn months, when about 40 to 60% of colds have been found to be associated with cultures positive for HRV (10, 14, 15).

The proportion of colds that are caused by HRV has been determined primarily on the basis of the recovery of HRV in cell cultures. Many studies have used cell types which may not be broadly susceptible to a range of HRV serotypes. The use of combinations of HRV-sensitive cell lines and optimal culture techniques enhances HRV recovery from the nasal washings of adults with colds (3). The type of specimen (e.g., nasal swab, throat swab, or nasal washing) may also affect isolation rates (3). Assays by reverse transcription-PCR (RT-PCR) with primers derived from conserved sequences within the 5′ nontranslated region have been found to be sensitive for HRV detection in prospective clinical studies of wheezing children (4, 18) as well as in studies with retrospectively selected upper respiratory tract samples obtained from adults with experimentally (2) and naturally (12, 17) acquired colds. Consequently, the present study used both virus isolation and RT-PCR for HRV RNA to determine the frequency and clinical course in adults with colds acquired naturally during the autumn seasonal peak. Our objectives were to determine the incremental diagnostic yield that a sensitive RT-PCR-based assay might provide compared to that provided by cell culture alone, reassess the role of HRV as a cause of fall colds in adults, and determine whether there are clinical differences between HRV culture-positive and culture-negative, PCR-positive colds caused by HRV.

MATERIALS AND METHODS

Subjects and monitoring of illness. As described previously (3), adults with colds during the months of September and October of 1994 were enrolled in the study if they had at least two of the following symptoms for less than 48 h: rhinorrhea, nasal stuffiness, sore throat, and cough. Symptomatic subjects were recruited by advertisement and were compensated for their participation. Written informed consent was obtained from all participants. Only one illness was assessed for each person. The study was approved by the Human Investigation Committee of the University of Virginia, and written informed consent was obtained from all participants.

The duration of illness prior to sampling was determined retrospectively at enrollment by a detailed interview with a study nurse. This included determination of the initial symptom, the time between the initial symptom and the moment that the subject knew that he or she had a cold, and the most bothersome symptom. They were also instructed to record in a diary record the severities of their symptoms (headache, malaise, muscle aches, chills, fever, sneezing, stuffy nose, runny nose, sore throat, cough, ear ache and pressure, and disturbance of sleep) on a scale ranging from zero (absence of the symptom) to 4 (very severe) twice daily (morning and evening) for 14 days. In addition, they were asked to rate the overall cold severity and interference with daily activity twice

* Corresponding author. Mailing address: University of Virginia Health Sciences Center, Box 473, Charlottesville, VA 22908. Phone: (804) 924-5059. Fax: (804) 924-9065.

TABLE 1. Demographic data and symptoms for adults with self-diagnosed colds

Characteristic	HRV negative (n = 62)	HRV PCR positive (n = 52)	HRV culture positive (n = 231)	Total[e] (n = 345)
Gender (no. [%] of subjects)[a]				
Male	25 (40)	21 (40)	96 (42)	142 (42)
Female	37 (60)	31 (60)	132 (58)	200 (58)
Median age (yr [range])[b]	20.5 (18–57)	21 (18–63)	20 (17–56)	20 (17–63)
Smokers (no. [%] of subjects)[c]	4 (6)	1 (2)	16 (7)	21 (6)
No. (%) of subjects with the following times before self-diagnosis:				
Within 4 h	21 (34)	19 (37)	89 (39)	129 (37)
4–8 h	13 (21)	12 (23)	69 (30)	94 (27)
8–16 h	16 (26)	10 (19)	31 (13)	57 (17)
1 day	12 (19)	10 (19)	34 (15)	56 (16)
>1 day	0	1 (2)	6 (3)	7 (2)
Symptomatic therapy used (no. [%] of subjects)				
NSAIDS[d] or analgesics	8 (13)	12 (23)	55 (24)	75 (22)
Decongestant	0	0	2 (1)	2 (1)
Antihistamine	0	2 (4)	7 (3)	9 (3)
Antitussives	1 (2)	0	1 (0.5)	2 (1)

[a] Gender was not reported for three subjects.
[b] Age not reported for one subject who was HRV culture positive.
[c] Smoking history not reported for one subject who was HRV PCR positive and one subject who was HRV culture positive.
[d] NSAIDS, nonsteroidal anti-inflammatory drugs.
[e] One sample negative by cell culture was not available for testing by RT-PCR.

daily. Middle ear pressures (MEPs) were determined with a digital tympanometer by previously described techniques (8). The MEP was considered abnormal if measurements were equal to or less than −100 mm of H_2O or equal to or greater than +100 mm of H_2O.

Specimens and virus detection. A total of 346 nasal washes and a concurrent subset of 100 nasal swabs were cultured in monolayers of human embryonic fibroblast cells and several strains of HeLa cells as described previously (3). Rhinoviruses and enteroviruses were distinguished by acid susceptibility. A total of 114 samples from which neither HRV nor enteroviruses were isolated and a randomly selected subset of 35 samples positive for HRV by cell culture were tested for HRV RNA by RT-PCR.

RT-PCR for HRV and HCV. Total RNA was extracted from 100 μl of sample diluted in an equal volume of phosphate-buffered saline (PBS) by matrix affinity chromatography (QIAamp blood kit; QIAGEN, Chatsworth, Calif.). The RT-PCR protocols were carried out as described previously (2, 23). Rhinovirus type 39 (American Type Culture Collection, Rockville, Md.), human coronavirus (HCV) 229E (kindly provided by Kathryn V. Holmes, University of Colorado), and HCV OC43 (American Type Culture Collection) were used as positive controls, and both nasal washings from healthy persons and phosphate-buffered saline were used as negative controls in each reaction series.

Nonincorporated primers and deoxynucleoside triphosphates were removed from the PCR products on a SELECT-B spin column (5Prime-3 Prime, Boulder, Colo.), and the product was frozen at −20°C until it was used for hybridization. Oligonucleotide probes were labelled at the 3′ end with digoxigenin dUTP by using 3′ nucleotide transferase, following the protocol of the manufacturer (Boehringer Mannheim, Indianapolis, Ind.). The locations of these primer sequences within the picornavirus and coronavirus sequences have been published previously (2, 16, 20, 21).

Hybridization was conducted in a volume of 50 μl in 1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% Sarkosyl, 0.02% sodium dodecyl sulfate, 1% blocking reagent (Boehringer Mannheim), 20 mM EDTA, 1 μg of yeast tRNA per μl, and 1 μg of salmon testis DNA per μl. The mixture was heated at 95°C for 10 min, quenched on an ice-ethanol bath, incubated at 45°C overnight, transferred to streptavidin-coated wells (NEN DuPont, Boston, Mass.), and incubated for 2 h at 37°C. The wells were washed with 1× SSC for 5 min, twice at room temperature and twice at 45°C, and then they were blocked with 5% fetal bovine serum in PBS–0.1% Tween 20 for 1 h at 37°C, followed by one wash with PBS–0.1% Tween 20. Coronavirus RT-PCR products were detected by a previously published microplate hybridization procedure which was simplified by using published methods (5, 23, 25).

Data analysis. The determination of the duration of the cold was done by examining three of the items in the daily diary: sleep disturbance, severity of the cold, and interference with daily activity. The day when the ratings for these three symptoms returned to zero defined the end of the cold, and these ratings had to be maintained for three consecutive ratings. For those subjects who still did not have three consecutive ratings of zero by the end of day 14, a duration of 15 was recorded. Differences in proportions between the three groups of interest were analyzed statistically by the Mantel-Haenszel chi-square test and by Fisher's exact test. Differences in continuous variables (symptom score, times to resolution) were analyzed by the Kruskal-Wallis test.

RESULTS

Patients. A total of 346 persons with fresh colds were enrolled in the study over 2 months, September and October 1994 (Table 1). Overall, the enrolled population was young (median age, 20 years; age range, 17 to 63 years), nonsmoking (93%), largely Caucasian (81%), and predominantly female (58%). More than 80% of the patients reported that the interval from symptom onset to perception of cold onset was 16 h or less. The three different diagnostic groups were generally comparable with respect to age, gender, smoking behavior, and the interval between the first symptoms and self-diagnosed colds (Table 1).

Only a few bacterial complications were observed. One patient in the HRV-negative group and two patients in the HRV-positive group received antibiotic treatment. The overall frequency of using symptomatic self-treatments (nonsteroidal anti-inflammatory agents, analgesics, antitussives, decongestants, and antihistamines) did not differ between HRV PCR-positive (35%) and culture-positive (31%) patients but was lowest among HRV-negative (16%) patients (Table 1). The most common reason for symptomatic therapy was headache. One HRV culture-negative subject whose specimens were not tested by PCR was not included in the three groups.

Etiologic diagnosis. A picornavirus was isolated in cell culture from 231 (224 HRV and 7 enterovirus isolates) of 346 (67%) subjects. Of the 115 samples negative for picornavirus by cell culture, 114 were available for testing by RT-PCR. Picornavirus RNA was detected by RT-PCR in 52 (46%) of the 114 cell culture-negative nasal wash specimens and in all 35 randomly selected cell culture positive nasal wash specimens.

TABLE 2. Clinical features and duration of illness in adults with common colds

Clinical feature	HRV negative	HRV PCR positive	HRV culture positive	Total
First symptom (no. [%] of subjects)[a]				
Sore throat	14 (25)	22 (42)	88 (39)	124 (37)
Stuffy nose	15 (27)	7 (13)	39 (17)	61 (18)
Runny nose	7 (13)	9 (17)	39 (17)	55 (17)
Sneezing	2 (4)	3 (6)	18 (8)	23 (7)
Most bothersome symptoms (no. [%] of subjects)[b]				
Runny nose	11 (18)	11 (22)	81 (36)	103 (31)
Stuffy nose	15 (25)	13 (26)	45 (20)	73 (22)
Sore throat	10 (16)	11 (22)	43 (19)	64 (19)
Malaise	9 (15)	4 (8)	22 (10)	35 (10)
Median duration (days) of symptoms[c]				
Cold episode	11.5	9.5	11	11
Sleep disturbance	6	4	4	5
Interference with daily activities	8	6	7	7

[a] If subjects recorded more than one first symptom, they were excluded. A statistically significant difference ($P = 0.025$) was found for the distribution of first symptoms across the groups by the Mantel-Haenszel chi-square test. Data are for a total of 332 subjects in the HRV-negative ($n = 56$), HRV PCR-positive ($n = 52$), and HRV culture-positive ($n = 224$) groups.

[b] If subjects recorded more than one symptom as most bothersome at enrollment, they were excluded. No statistically significant difference across the groups was found for the distribution of the most bothersome symptoms. Data are for a total of 335 subjects in the HRV-negative ($n = 61$), HRV PCR-positive ($n = 50$), and HRV culture-positive ($n = 224$) groups.

[c] Three subjects did not have diary data and were excluded. No significant ($P < 0.05$) differences in the duration of cold episodes, sleeping disturbance, or interference with daily activities were found between the groups by Kruskal-Wallis testing. Data are for a total of 342 subjects in the HRV-negative ($n = 60$), HRV PCR-positive ($n = 52$), and HRV culture-positive ($n = 230$) groups.

Thus, a picornavirus was detected by cell culture and/or RT-PCR from a total of 283 (82%) of 346 patients during the 2-month study period. This indicates that the use of RT-PCR afforded an incremental 15% increase in the detection of picornavirus in nasal washes compared to the level of detection by cell culture alone.

Sixty-two nasal wash specimens negative for picornavirus by cell culture and RT-PCR were tested for HCV by RT-PCR. Five (8%) of the 62 specimens were positive for HCV, for an overall rate of detection of HCV of 1.4%. Two samples were positive for HCV 229E and three samples were positive for HCV OC43. Overall, a virus was detected in 288 (83%) of 346 patients.

Illness course. The first symptom noticed most frequently was sore throat in HRV culture-positive patients (39%) and in HRV PCR-positive patients (42%), while stuffy nose was the first symptom noticed in HRV-negative patients (25%) (Table 2). The single most bothersome symptom at enrollment was runny nose in HRV culture-positive patients (36%) and stuffy nose in HRV PCR-positive patients (26%) and HRV-negative patients (25%) (Table 2). However, there were no major differences in the resolution of specific cold symptoms between those with and those without positively identified HRV infections (Fig. 1). The median duration of illness was 11 days in HRV culture-positive patients, 9.5 days in HRV PCR-positive patients, and 11.5 days in HRV-negative patients (Table 2). The median duration of sleep disturbance was 4 days in both HRV culture-positive and PCR-positive patients and 6 days in HRV-negative patients. Similarly, the median duration of interference with activity did not differ between HRV culture-positive, HRV PCR-positive, and HRV-negative patients (Table 2). The total respiratory symptom score (mean ± standard deviation) did not differ significantly between HRV PCR-positive (46 ± 29) and culture-positive (48 ± 24) or HRV-negative (52 ± 26) patients. Similarly, the total symptom scores over 14 days did not differ among the HRV culture-positive (96 ± 53), HRV RT-PCR-positive (97 ± 66), or HRV-negative (109 ± 61) patients.

MEPs. A higher proportion of HRV culture-positive patients tended to have MEP abnormalities on the first day of illness compared with the number of HRV PCR-positive and HRV-negative patients with such abnormalities (Table 2). On day 7, most of the HRV PCR-positive patients had MEPs in the normal range, while 14% of HRV-negative patients had abnormal MEPs (Table 3). The proportions of persons having abnormal MEPs on both day 1 and day 7 were 12 of 229 (5%) HRV culture-positive patients, 3 of 59 (5%) of HRV-negative patients, and 0 of 52 HRV PCR-positive patients.

DISCUSSION

Using a combination of virus isolation and RT-PCR for picornavirus RNA, we found that HRV caused 80% of colds among adults during the seasonal autumn peak. A picornavirus was detected by RT-PCR in nearly one-half of nasal washings negative by viral isolation, even when a highly sensitive combination of cell lines was used (3). Prior studies by virus isolation methods with comparable populations of adults presenting with self-diagnosed colds found HRV by culture in 57% of 228 patients (14) and 54% of 201 patients (15). Other studies have found that RT-PCR increased the frequency of picornavirus detection by up to fivefold compared to the frequency of detection by culture (22). Another important cause of upper respiratory tract infections, HCV, was detected by RT-PCR in 8% of the samples negative for HRV, or 1.4% of the patients with colds studied. This low proportion of HCV was not unexpected, given that HCV typically causes colds in the winter and spring months. Other respiratory viruses associated with colds during the fall (e.g., parainfluenzavirus, adenovirus, and respiratory syncytial virus) were not sought in this study, but our results indicate that an etiology can be established in almost all autumnal common colds in adults by a combination of virus isolation and nucleic acid amplification techniques.

The RT-PCR assay that we used is picornavirus specific (2) and does not distinguish between rhinoviruses and enteroviruses. Although certain enteroviruses can cause colds, it is

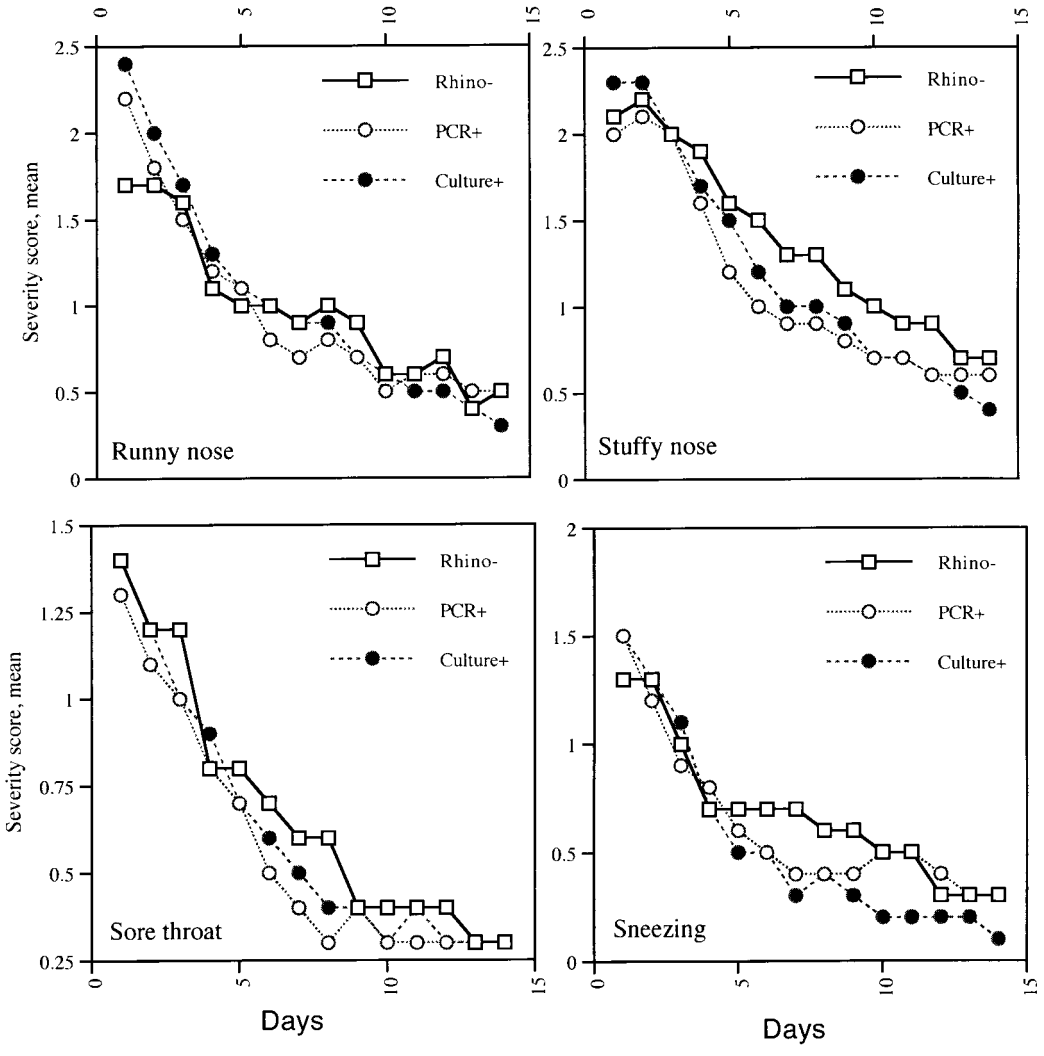

FIG. 1. Mean severity of runny or stuffy nose, sore throat, and sneezing in adults with self-diagnosed common colds positive for HRV by cell culture (filled circles) or RT-PCR (empty circles) or negative for HRV (squares). Symptom severity was highest upon presentation and declined over the study period. Symptoms were scored twice daily on a scale ranging from zero (absence of the symptom) to 4 (very severe).

reasonable to assume that HRVs constituted the vast majority of picornaviruses detected in association with colds in the autumn (10). Our culture results are in keeping with this observation, since we isolated only seven (3%) enteroviruses. In addition to its higher sensitivity for HRV detection compared to that of cell culture alone, this RT-PCR assay is suitable for the analysis of large numbers of samples in a relatively short period of time and is applicable in large epidemiological studies.

We wanted to determine whether the clinical course of HRV culture-positive colds might differ from that of culture-negative, RT-PCR-positive ones, since RT-PCR can detect small quantities of viral RNA. However, we did not find significant

TABLE 3. MEPs in adults with common colds

Group	No. (%) of patients							
	Day 1				Day 7			
	Total studied	Underpressure	Overpressure	Either[a]	Total studied	Underpressure	Overpressure	Either
HRV culture positive	230	46 (20)	4 (2)	49 (21)	229	18 (8)	1 (<1)	19 (8)
HRV RT-PCR positive	52	6 (12)	1 (2)	7 (14)	52	1 (2)	0	1 (2)
HRV Negative	62	6 (10)	1 (2)	6 (10)	59	8 (14)	0	8 (14)
Total	344	58 (17)	6 (2)	62 (18)	320	27 (8)	1 (<1)	28 (8)

[a] Subjects may have had underpressure in one ear and overpressure in the other. They are only counted once in this column. No statistically significant differences in the frequency of MEP abnormalities were observed between groups on days 1 ($P = 0.07$) or 7 ($P = 0.07$) by Mantel-Haenszel chi square test.

differences in the duration or severity of illness. Runny nose seemed to be more frequently bothersome among HRV culture-positive patients than among PCR-positive patients at enrollment, but the severity of this symptom and its resolution did not differ overtime between the groups. Sore throat was the first symptom in 40% of patients with HRV-positive colds, while sore throat was the first symptom in only 25% of the HRV-negative patients. This differs from the results of an earlier study (9), which found that sore throat was more frequent at presentation in patients with HRV-negative respiratory illnesses. Sore throat has been shown to be the early symptom of experimental and natural colds caused by rhinovirus (13, 24). In adults with upper respiratory illnesses identified by prospective symptom recording, the occurrence of rhinorrhea, nasal obstruction, hoarseness, and cough was more frequent in persons with HRV-positive illness than in those with HRV culture-negative illness (9). However, our study, which enrolled persons with self-diagnosed colds, found that the patterns of symptoms in each group overlapped considerably, so that HRV-positive colds could not be distinguished from HRV-negative ones.

Substantial evidence suggests that rhinovirus-induced MEP abnormalities play a role in the development of otitis media (8, 19). The frequency of significant MEP abnormalities observed in this study was similar to that observed in previous studies (6–8). Despite these functional changes, the risk of developing clinically apparent otitis media was very low in our patient population. Only 0.7% of the HRV-positive patients and 1.6% of the HRV-negative ones received antibiotics for complications presumed to be caused by bacteria, and no episodes of otitis media were documented. This low frequency and the relatively low level of use of therapies for symptoms may reflect the fact that the colds in our study population represented a broad range of severity. In addition, the subjects were discouraged by the study nurses from using unnecessary medications. Despite this range of severity, we observed that the illnesses were associated with considerable functional impact.

To our knowledge, these results represent the highest frequency of natural colds with an HRV etiology that has been detected to date and indicate that HRVs cause a larger proportion of colds in adults than was previously estimated on the basis of virus isolation alone. Furthermore, these results indicate that colds caused by HRVs are associated with considerable morbidity and functional impairment including sleep disturbance. No specific intervention is available for the prevention or treatment of HRV infections, but these findings support the need for such modalities.

ACKNOWLEDGMENTS

We are grateful to Paula K. Joyner for technical support.

This work was supported in part by a gift from Boehringer Ingelheim Pharmaceuticals, Inc.

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