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A prospective, community-based study on virologic assessment among elderly people with and without symptoms of acute respiratory infection

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Accepted 9 April 2003

Abstract

Background and Objective: Community-based elderly studies concerning microbiology of acute respiratory infections are scarce. Data on subclinical infections are even totally absent, although asymptomatic persons might act as a source of respiratory infections.

Methods: In a 1-year community-based study, we prospectively investigated the possible virologic cause of acute respiratory infections in 107 symptomatic case episodes and 91 symptom-free control periods. Participants, persons ≥ 60 years, reported daily the presence of respiratory symptoms in a diary. Virologic assessment was performed by polymerase chain reaction (PCR) and serology.

Results: In 58% of the case episodes a pathogen was demonstrated, the most common being rhinoviruses (32%), coronaviruses (17%), and influenzaviruses (7%). The odds ratio for demonstrating a virus in cases with symptoms vs. controls without symptoms was 30.0 (95% confidence interval 10.2–87.6). In 4% of the symptom-free control periods a virus was detected.

Conclusion: This study supports the importance of rhinovirus infections in community-dwelling elderly persons, whereas asymptomatic elderly persons can also harbor pathogens as detected by PCR, and thus might be a source of infection for their environment. © 2003 Elsevier Inc. All rights reserved.

Keywords: Virology; Etiology; Acute respiratory infections; Rhinovirus; Elderly; Community based

1. Introduction

Elderly people have an increased susceptibility for respiratory infections and related complications [1]. On average, community-dwelling elderly people suffer from 1.2–1.6 acute respiratory infections per year [2,3]. Medical consultation and hospitalization because of such an infection has been reported in 40 and 0.8% of community-dwelling elderly people, respectively, during the winters of 1992–1993 and 1993–1994 in England [2].

Viruses play a crucial role in acute upper respiratory tract infections, the most common being rhinoviruses, coronaviruses, influenzaviruses, and respiratory syncytial viruses [4]. However, laboratory diagnosis of acute respiratory infections in symptomatic elderly people so far focussed on institutionalized elderly persons [5–7], on patients reporting for medical consultation [8,9], and to a far less degree on

community-dwelling elderly persons [2]. Besides, no data are available on the presence of respiratory pathogens in asymptomatic elderly persons. Asymptomatic people with a subclinical infection might, however, transmit the pathogen to other persons and act as an unrecognized source of respiratory infections.

Therefore, in this prospective, community-based study, we investigated the presence of known respiratory viruses in elderly persons both with and without symptoms of an acute upper respiratory tract infection. Second, we compared the clinical characteristics of the persons suffering from an acute respiratory infection, during episodes with positive and negative virologic laboratory diagnosis.

2. Methods

2.1. Study population

Persons with and without symptoms of an acute respiratory infection, hereafter referred to as cases and controls,

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were recruited from October 1, 1998, until October 1, 1999, from an intervention trial investigating the effect of micronutrient supplementation on acute respiratory infections in community-dwelling elderly persons (≥ 60 years) [3]. During the 1-year study period, a diary was used daily by all participants for reporting symptoms that indicated an acute respiratory infection. Participants were requested to report the onset of symptoms of a possible infection to the study nurse. A subject was identified as case if (1) he/she had respiratory symptoms with a sudden onset; (2) rhinorrhoea/sneezing, sore throat/hoarseness, or dry cough were present for at least 2 days; and (3) the symptoms had a pattern that differed from any usual symptoms [10,11]. Apart from a check by telephone, the study nurse evaluated the symptoms of cases during home visits. From those cases that reported their symptoms within 3 days to the study nurse every other case, with a maximum of five cases per week, was selected for virologic assessment. Cases who reported their symptoms after 3 days to the study nurse were excluded for virologic assessment to overcome false negative test results. Each case episode, i.e., the period during which a case had respiratory symptoms, had to have been preceded by a 7-day symptom-free period. During the 1-year study period, 624 incident case episodes were reported by 346 cases. In total 107 (17%) case episodes—reported by 97 cases—were selected for virologic assessment.

For each case episode an asymptomatic control was selected as follows. Participant numbers, including all cases and controls, ranged from 1–652. If the participant number of the case was 325 or lower, a closest eligible control was selected on participant number by counting back on these numbers. If the participant number of the case was 326 or higher, a closest eligible control was selected by counting forward on these numbers. Controls were subjects without symptoms of a respiratory infection within a time window of 8 weeks before and 8 weeks after the symptomatic period of the index case. The study nurse checked the absence of symptoms at the time of recruitment of the control and the diary was checked for absence of symptoms in the previous eight weeks. In total, 99 controls were selected.

Cases and controls were matched on age (± 5 years) and sex, and they were not living in the same house or apartment, did not have Chronic Obstructive Pulmonary Disease, asthma, or cancer, and did not use severe immunosuppressive medication. Afterwards, we excluded 8 (8%) of the 99 originally enrolled controls because they developed symptoms after a median duration of 21 days (range 9–54). For six excluded controls serologic testing was negative, while for two it was missing. With PCR two times a rhinovirus and two times a coronavirus OC43 was detected in the eight excluded controls. Results presented are therefore based on the 107 case episodes and 91 control periods.

This study was approved by the Medical Ethics Committee of the Wageningen University, The Netherlands, and written informed consent was obtained from all participants prior to the study.

2.2. Data collection

All participants filled out a questionnaire concerning relevant subject characteristics at baseline. A diary was used daily for self-report of symptoms that indicated an acute respiratory infection. Apart from the symptoms that had to be present because of our case definition (rhinorrhoea/sneezing, sore throat/hoarseness, dry cough), also accompanying symptoms were recorded in the diary: (1) symptoms of a lower respiratory tract infection (sputum production, wheezing, pain on respiration), (2) systemic symptoms (fever—self-assessed by a supplied thermometer—malaise, headache, rigors, muscular pain, perspiration), (3) other symptoms (tearful eyes, pain in facial sinuses or ear), (4) restriction of activity (staying in bed, not being able to do daily activities, staying at home), (5) episode-related medication, including antibiotic use, (6) medical consultation, and (7) hospitalization [2].

If the study nurse judged during a home visit the case's symptoms as an acute respiratory infection, in both the case and the matched control an acute phase serum sample and one swab from the nose and one from the throat were taken within 3 days and a convalescent serum sample was taken within 2–4 weeks after onset of the first symptoms of the case. Samples in cases and controls were taken on the same day to exclude seasonal differences.

2.3. Microbiologic diagnosis

PCR or serology was used to diagnose infection with the eight most common respiratory viruses and *Mycoplasma pneumoniae* (*M. pneumoniae*). PCR was performed for those viruses for which either no or only aspecific serology was available and for which validated PCR tests were available in our lab. Infections with rhinovirus, enterovirus, coronavirus OC43 and 229E, and respiratory syncytial virus were diagnosed by PCR. Serology was performed for those viruses for which either no PCR was available, or the nucleic acid extraction method had to be changed for DNA isolation (in the case of *M. pneumoniae*). Infections with influenza virus A and B, parainfluenzavirus 1, 2, and 3, adenovirus and *M. pneumoniae* were diagnosed by serology.

2.3.1. Polymerase chain reaction

Swabs from the nose and from the throat, hereafter referred to as “nose/throat samples,” were placed together in 4-mL Hanks' balanced salt solution containing gelatin, lactalbumin, yeast, and antibiotics. Upon receipt of the nose/throat samples at the laboratory, the swabs were twirled in the transport medium and removed. An aliquot of 200 μ L of the sample was used for nucleic acid extraction by using the High Pure RNA isolation kit (Boehringer, Mannheim, Germany). Five microliters of the eluted RNA preparation was used in a 25 μ L single-tube RT-PCR followed by a nested-PCR using primer pairs as described previously for rhino-/enterovirus [12]. Another 5 μ L of extracted RNA was used in a single 25 μ L single-tube RT-PCR followed by a

nested-PCR using primer pairs as described previously for respiratory syncytial virus (RSV) and coronavirus OC43 and 229E [13,14] in a multiplex format.

In the RNA isolation procedure and PCR-method for RSV detection, sensitivity for RSV A was about one virus particle and for RSV B about 70 virus particles. The virus particle count was determined by quantitative EM (Advanced Biotechnologies Incorporated, Columbia, MD).

Positive controls from culture were used in each PCR test for the respective viruses. To prevent carryover contamination within the laboratory, preparation of the patient samples and PCR mixtures was performed in safety hoods in separate dedicated positive pressure laboratories. To check for carryover contamination of samples and for amplicon contamination during the procedure, negative controls, consisting of transport medium, were included after every fifth patient sample. Subjects with a positive PCR result were considered to be infected by a known virus, which was interpreted as a laboratory-confirmed infection.

2.3.2. Serology

Paired sera from all cases and controls were analyzed for IgG antibodies against influenza virus A and B, adenovirus, and *M. pneumoniae*. For para-influenza virus 1, 2, and 3, IgA antibodies, combining the three antigens in one assay, were detected. Analyses were performed using commercially available ELISA (Serion Immunodiagnostica GmbH, Würzburg, Germany), and quantitative results, expressed in units/milliliter, were calculated using a lot-specific standard curve and calculation table as supplied in the test kit. Results were interpreted as negative, indeterminate, or positive according to the manufacturer instructions. In the case of indeterminate results for the parainfluenza IgA assay on paired sera, detection of total antibodies against separate parainfluenza 1, 2, and 3 antigens was repeated in a complement fixation assay (CFA), using commercially available parainfluenza 1, 2, and 3 antigens (Virion, Ruschlikon, Switzerland). In ELISAs, a change from negative to positive result and in the CFA a fourfold rise in antibody titer between the paired sera was interpreted to be a laboratory-confirmed respiratory infection.

2.4. Statistical methods

Data analysis concerning virologic (including *M. pneumoniae*) assessment was performed with the 107 case episodes and the 91 control periods. Differences in the distributions for continuous data, i.e., age, self-perceived health, and illness duration were compared with Independent Sample Student's *t*-test. Illness duration was not normally distributed, and was log transformed to obtain normality.

A chi-square test or a Fisher's Exact Test was used to test the correlation between discrete variables, i.e., sex, influenza vaccination, smoking habits, allergy, sharing an apartment, presence of micro-organisms, symptoms of a lower respiratory tract infection, systemic and other symptoms, restriction

of activity, fever, medical consultation, hospitalization, episode-related medication, and episode-related antibiotic use. A Fisher's Exact Test was used to calculate the odds ratio for demonstrating a virus in cases with symptoms of acute respiratory infection vs. controls without symptoms of such an infection.

Alpha was taken as 0.05 in all analyses.

3. Results

The matching procedure on sex and age resulted in well-balanced groups of cases and controls with respect to these and other relevant variables (Table 1). Micronutrient supplementation related to the intervention trial was also similar between cases and controls [3].

The 97 symptomatic cases had 107 case episodes of respiratory infection, during which virologic (including *M. pneumoniae*) tests were performed. In 62 (58%) of these case episodes at least one micro-organism was demonstrated, whereas in two of these 62 two different micro-organisms were demonstrated. In 45 (42%) case episodes none of the applied tests was positive. Of 10 cases, two case episodes were included. For seven out of the mentioned 10 cases, test results were different, i.e., different pathogens, or negative in one and positive virology in the other episode. In two cases, both episodes had negative virology. Only in one case, in both episodes rhinovirus was detected.

The most common viruses demonstrated were rhinoviruses (32%) and coronaviruses (17%) followed by influenza viruses (7%), enteroviruses (2%), parainfluenza viruses (2%) and *M. pneumoniae* (1%). Respiratory syncytial virus and adenovirus were not detected.

Three of the seven cases diagnosed with an influenza virus infection had been vaccinated against influenza. None of the titer rises on which the influenza virus infection was diagnosed, was related to vaccination, as 2 to 4 months passed between vaccination and the diagnosis of an influenza virus infection.

Table 1

Characteristics of persons with (cases) and without (controls) symptoms of an acute respiratory infection in who virological (including *M. pneumoniae*) tests were performed

	Cases (<i>n</i> = 97)	Controls (<i>n</i> = 91)
Age (years), mean (SD)	72.2 (6.8)	72.2 (5.6)
Men	44 (45%)	47 (52%)
Self-perceived health (range 1–10), mean (SD)	7.5 (1.2)	7.5 (1.2)
Influenza vaccination in 1998	73 (75%)	73 (80%)
Current smoker	50 (52%)	45 (49%)
Former smoker	7 (7%)	3 (3%)
Allergy ^a	12 (12%)	11 (12%)
Sharing an apartment	61 (63%)	64 (70%)

Data are *n* (%) unless otherwise indicated.

^a Allergy against house-dust mite and feces, pollen grains, domestic pets or moulds.

Presence of rhinovirus infections was almost five times higher compared to influenzavirus infections in this community-dwelling elderly population (Table 2).

In 4 out of 91 control periods (4%) a virus was demonstrated, i.e., two times a rhinovirus and two times a coronavirus. Two out of these four controls with positive virology never showed symptoms of a respiratory infection during the 1-year study period. The two remaining controls with positive virology did not have any symptoms at least 3.5 and 4 months before and 8 and 4 months after sample collection, respectively.

Overall, the odds ratio for demonstrating a virus (or *M. pneumoniae*) in cases with symptoms vs. controls without symptoms of acute respiratory infection was 30.0 (95% confidence interval 10.2–87.6).

Despite small numbers ($n = 5$) significantly more influenzavirus A infections were identified during symptomatic periods in winter (October–March) compared to summer ($P = .02$). Enteroviruses, parainfluenzaviruses and *M. pneumoniae* were only detected in summer (April–September).

Clinical characteristics of the persons suffering from an acute respiratory infection, during episodes with positive and negative virologic laboratory diagnosis, are described in Table 3. Influenzavirus infection was associated with significantly longer illness duration and more systemic symptoms than the other infections with positive and negative virology. Restriction of activity, presence of fever, medical consultation, and antibiotic use were also more frequently reported during influenzavirus infections, although not significantly different from the other infections with positive and negative virology.

4. Discussion

This study shows that subclinical respiratory infections occur in a minor part (4%) of asymptomatic elderly persons. Besides, we showed the importance of rhinovirus infections in community-dwelling elderly people because of its high frequency.

Table 2

Viruses (including *M. pneumoniae*) demonstrated in symptomatic case episodes of acute respiratory infection and symptom-free control periods of community-dwelling elderly persons, in The Netherlands from October 1, 1998, until October 1, 1999

	Case episodes ($n = 107$)	Control periods ($n = 91$)
Negative microbiology	45 (42%) ^a	87 (96%)
Rhinoviruses ^b	34 (32%)	2 (2%)
Coronavirus (OC43 + 229E) ^b	18 (17%)	2 (2%)
Influenzavirus A	5 (5%)	0
Influenzavirus B ^b	2 (2%)	0
Enterovirus	2 (2%)	0
Parainfluenzavirus (1, 2, +3)	2 (2%)	0
<i>Mycoplasma pneumoniae</i>	1 (1%)	0
Respiratory syncytial virus	0	0
Adenovirus	0	0

^a Significantly different with symptom-free controls, $P < 0.0001$.

^b During two case episodes two viruses were demonstrated: one case episode with rhinovirus + coronavirus OC43 and one with coronavirus OC43 + influenzavirus B.

To our knowledge, this is the first study to investigate several common respiratory pathogens in community-dwelling elderly persons both with and without symptoms of an acute respiratory infection. So far, only two studies reported on microbiologic evidence of respiratory infection in community-dwelling healthy subjects with and without symptoms of such an infection. One study focussed on detection of rhinoviruses and enteroviruses by PCR in children and adults [15]. In 12 and 4% of the asymptomatic children and adults, respectively, virologic assessment was positive. Although Johnston et al. [15] tested only for rhinoviruses and enteroviruses, the frequency of subclinical respiratory infections in those healthy adults is similar to what we observed in our older population. Preliminary results of a Dutch study being performed in persons consulting their general practitioner for signs and symptoms of an acute respiratory infection, showed a positive virologic assessment in 19% of the controls [16]. This percentage is higher than observed in our study. However, that study population existed of participants from all age categories, including babies and children. As showed by Johnston et al. [15] the percentage of asymptomatic persons with positive virologic assessment is clearly higher in children, which might explain the discrepancy.

Common viral pathogens demonstrated during symptomatic periods in children and adults [4,17], in institutionalized elderly patients [7,11], in patients with medical consultation [8], and in community-dwelling elderly persons [2] are rhinoviruses, coronaviruses, influenzavirus A and B, and RSV, which is in line with our results. The frequency of the most common viruses varies between the different subpopulations. Corresponding to one previously performed community-based elderly study, we also showed that rhinovirus infections are highly prevalent, and can cause a great overall disease burden in this population [2]. Corresponding to the results of Nicholson et al. in community-dwelling elderly persons [2], but in contrast to studies in more frail elderly persons as those living institutionalized and to studies with a general practitioner-based setting [7,11,18], we also observed that influenzavirus infections and RSV infections seem to occur less frequent in free-living elderly people. A severe morbidity is caused by viruses such as influenzavirus and RSV [19], which corresponds to our results on influenzavirus infections. This might explain the higher frequency of RSV and influenzavirus infections demonstrated in studies with general practitioner-based or institutionalized setting [19]. In total, 4 out of 7 patients with influenzavirus infection were not vaccinated against influenza. This might indicate the need for preventive vaccination in elderly persons.

In agreement with other studies in institutionalized [7,11] and in community-dwelling elderly subjects [2], we found that infections with parainfluenzavirus, enterovirus, adenovirus, and *M. pneumoniae* are of minor importance in causing acute respiratory infections in elderly persons.

Table 3

Relation between virology and clinical characteristics in community-dwelling elderly persons during symptomatic episodes of acute respiratory infection

	Rhinoviruses (n = 34)	Coronavirus OC43 + 229E (n = 18)	Influenzavirus A and B (n = 7)	Negative virology (n = 45)	Case episodes—total (n = 107)
Episode duration (days), median (min, max)	9 (2, 41)	7 (2, 34)	12 (9, 32) ^f	8 (2, 29)	9 (2, 41)
Symptoms of lower respiratory tract infection ^a	20 (58.8%)	9 (50.0%)	5 (71.4%)	21 (46.7%)	57 (53.3%)
Systemic symptoms ^b	19 (55.9%)	12 (66.7%)	7 (100.0%) ^f	30 (66.7%)	68 (63.6%)
Other symptoms ^c	15 (44.1%)	6 (33.3%)	3 (42.9%)	14 (31.1%)	39 (36.4%)
Restriction of activity ^d	11 (32.4%)	6 (33.3%)	6 (85.7%)	14 (31.1%)	39 (36.4%)
Fever	3 (8.8%) ^f	5 (27.8%)	5 (71.4%) ^g	10 (22.2%)	25 (23.4%)
Medical consultation ^e	3 (8.8%)	2 (12.5%)	4 (57.1%)	4 (9.1%)	14 (13.5%)
Hospitalization ^e	0	0	0	0	0
Episode-related medication ^e	12 (35.3%)	6 (37.5%)	3 (42.9%)	22 (50.0%)	45 (43.3%)
Episode-related antibiotic use ^e	2 (5.9%)	0 (0.0%)	2 (33.3%)	2 (4.6%)	7 (6.8%)

Data are number of case episodes (%) unless otherwise indicated.

^a Lower respiratory tract symptoms: sputum production, wheezing, and pain on respiration.

^b Systemic symptoms: malaise, headache, rigors, muscular pain, and perspiration.

^c Other symptoms: tearful eyes, pain in facial sinuses, pain in ear.

^d Restriction of activity: staying in bed, staying at home, not able to do daily activities.

^e Data of three case episodes are missing.

^f $P < 0.05$ compared with cases episodes-total.

^g $P < 0.01$ compared with cases episodes-total.

We obtained a microbiologic diagnosis in 58% of the case episodes. The diagnostic deficit of 42% is relatively low, as in most studies a micro-organism was demonstrated in at maximum 50% of the case episodes [2,7,8]. Other, partly new or unknown viruses, bacteria, and atypical micro-organisms other than *M. pneumoniae* may be responsible for some of the clinical and possible additional subclinical infections with negative microbiology. Bacterial, atypical and viral micro-organisms in adult patients consulting for respiratory infection have been shown in 12, 20, and 50% of the patients, respectively [20]. Also, *Chlamydia* species are reported to cause acute respiratory infections in community-dwelling elderly persons [2], although the proportion of bacterial infections is reported to be rare in adult patients with common cold [4]. Besides, *Chlamydia* infections occurred in 1% only of the community-dwelling elderly people, and were mainly analyzed in patients with COPD and asthma, while we excluded those patients [2,21]. However, we cannot exclude that part of the diagnostic deficit in our study might be explained by such bacterial and atypical micro-organisms.

Little is known about the time period after infection during which PCR-based tests are positive [12,14,22]. This issue is especially crucial in interpreting PCR positive results in nose/throat samples obtained from subjects both with and without symptoms of a respiratory infection. Andeweg et al. [12] demonstrated that rhinoviruses were no longer detected by PCR in patients who had recovered from disease. In our study, all cases and controls were followed day to day by using a self-reporting diary system. It was therefore possible to include only controls not having any symptoms 2 months before and 2 months after sampling. Thus, it is very unlikely that the controls, in which a virus was detected, were in the postinfectious or incubation period of a symptomatic infection. Moreover, in nose/throat samples of four of the

eight excluded controls a respiratory virus was detected, and those controls apparently were in the incubation period.

Detection of rhinovirus, enterovirus, RSV, and coronavirus infections by the PCR method has been used before and is widely accepted [12–14]. Although PCR-based tests are highly sensitive and specific, false positives due to contamination of negative samples with PCR product in the laboratory might have occurred [23]. However, given the strict conditions under which PCR was performed [24], this is very unlikely. Negative controls included after each fifth test sample were PCR-negative in all samples, indicating that contamination was effectively prevented.

Underreporting could have occurred if cases were admitted to the hospital when having an acute respiratory infection. Because none of the cases reported having been admitted to a hospital because of acute respiratory infection or its complications, underreporting because of hospitalization is no issue in this study.

The subjects who participated in this study were recruited from an intervention trial studying the effect of micronutrient supplementation on acute respiratory infections. Random selection of participants of this double-blind intervention trial resulted in a similar distribution of supplementation between cases and controls. There was no significant correlation between positive microbiologic testing and (type of) supplementation [3]. Therefore, confounding by the supplementation is likely to be negligible in this study.

In conclusion, rhinovirus infections cause substantial morbidity among community-dwelling elderly persons because of its high prevalence in this population. Also, although definitely more respiratory micro-organisms were demonstrated among persons with symptoms of an acute respiratory infection, asymptomatic elderly persons can also harbor respiratory pathogens, and thus might be a source of infection for their environment.

Acknowledgments

This work was funded by grant 28-2639 from The Netherlands Organization for Health Research and Development (Zon-MW), The Hague. We are grateful to Francis van Rooij, Division of Human Nutrition and Epidemiology, Wageningen University, The Netherlands for her assistance in data and specimen collection. We want to thank Berry Wilbrink, Henk Boswijk, Hans van der Nat, and Henk Hoof, Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, National Institute of Public Health and the Environment, The Netherlands, for their technical help.

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