CONCISE COMMUNICATION

Results of Molecular Detection of *Mycoplasma pneumoniae* among Patients with Acute Respiratory Infection and in Their Household Contacts Reveals Children as Human Reservoirs

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During a 30-month prospective study in The Netherlands, the distribution of *Mycoplasma pneumoniae* and respiratory viruses among 1172 patients with acute respiratory infection (ARI) who were treated in the outpatient general practitioner setting was studied. *M. pneumoniae,* as detected by polymerase chain reaction analysis, was present in 39 (3.3%) patients. The infection rate was similar in all age groups. Nose and throat samples collected from 79 household contacts of *M. pneumoniae*–positive index patients revealed *M. pneumoniae* in 12 (15%) cases. The frequency of *M. pneumoniae* among household contacts of index patients treated with appropriate antibiotics and untreated index patients was similar. Nine of the 12 *M. pneumoniae*–positive household contacts were <16 years old ($P = .02$), and 4 (44%) of them did not develop ARI. Apparently, children are a relevant reservoir for *M. pneumoniae.*

Mycoplasma pneumoniae, a common respiratory pathogen, usually causes mild upper respiratory tract infections, such as sore throat, pharyngitis, and tracheitis. In 5%–10% of patients, *M. pneumoniae* infection results in tracheobronchitis or pneumonia. An estimated 2%–4% of patients with *M. pneumoniae* infection require hospitalization [1]. Data on the incidence and transmission of *M. pneumoniae* are derived mainly from large-scale population studies that were performed in the 1960s and 1970s, which used classic methods, such as culture and serology, to diagnose *M. pneumoniae* [2, 3]. Modern techniques such as polymerase chain reaction (PCR) analysis of easily obtainable specimens, such as throat and nasal swabs, can be used to provide new insight into the epidemiology of *M. pneumoniae* infection in outpatient and community settings [1, 4, 5].

In The Netherlands, acute respiratory infections (ARIs), including those due to *M. pneumoniae,* in patients who were treated in an outpatient general practitioner (GP) setting are being studied within the framework for monitoring influenza virus outbreaks, as recommended by the World Health Organization [6].

We aimed to assess the following: (1) the frequency of *M. pneumoniae* infection among patients with ARI who were treated in the outpatient GP setting; (2) the distribution of patients with *M. pneumoniae* by patient age and by season, compared with patients with ARI due to respiratory viruses; (3) the frequency of *M. pneumoniae* infection, by age, among household contacts of *M. pneumoniae*–positive index patients; and (4) the influence of antibiotic treatment of *M. pneumoniae*–positive patients on transmission of *M. pneumoniae* in the household setting.

Patients, Materials, and Methods

Design of the surveillance. GPs from 45 practices, evenly distributed in The Netherlands, participated in a nationwide sentinel surveillance network coordinated by The Netherlands Institute of Primary Health Care (Utrecht, The Netherlands). This surveillance, covering ~1% of the population in The Netherlands (population of 15.7 million), was representative of the national population in terms of age, sex, and degree of urbanization [7]. GPs identified patients with ARI and register those patients who present with influenza-like illness (ILI). ILI is defined as a respiratory infection with acute onset, fever (rectal temperature, ≥38°C), and ≥1 of the following symptoms: coryza, sore throat, cough, frontal headache, retrosternal pain, or myalgia. ARI without ILI is defined as a respiratory infection with acute onset and ≥1 of the above-mentioned symptoms.

Patients and clinical samples. From 1 January 1997 through 30 June 1999, GPs were requested to collect a nose and throat sample weekly from 2 randomly selected patients with ARI. For each patient, the GP completed a questionnaire, including patient

Table 1. Distribution of *Mycoplasma pneumoniae* and various respiratory viruses in nose and throat samples obtained from 1172 patients with acute respiratory infection (ARI) who presented to their general practitioner, in 32 general practices in The Netherlands, 1 January 1997 to 1 July 1999.

Age category, years	AD of Dutch general population,[a] %	Patients with ARI sampled	Patients with a sample positive for									Patients with ≥1 RP
			M. pneumoniae[b]	Influenza A virus	Influenza B virus	Rhinovirus	RSV	Coronavirus	Enterovirus	PIV	Adenovirus	
0–4	6	114 (10)	3 (2.6)	22 (19.3)	4 (3.5)	17 (14.9)	23 (20.2)	4 (3.5)	3 (2.6)	2 (1.8)	3 (26)	61 (54)
5–15	12	140 (12)	8 (5.7)	38 (27.1)	20 (14.3)	11 (7.8)	3 (2.1)	2 (1.4)	3 (2.1)	0	1 (0.7)	74 (53)
16–25	13	170 (14)	5 (2.9)	33 (19.4)	17 (10.0)	24 (14.1)	3 (1.8)	3 (1.8)	3 (1.8)	1 (0.6)	0	84 (49)
26–40	25	348 (30)	15 (4.3)	44 (12.6)	40 (11.5)	64 (18.4)	6 (1.7)	6 (1.7)	7 (2.0)	2 (0.6)	0	173 (50)
41–60	26	291 (25)	6 (2.1)	51 (17.5)	20 (6.9)	34 (11.7)	11 (3.8)	6 (2.1)	6 (2.1)	2 (0.7)	2 (0.7)	129 (44)
>60	18	98 (9)	2 (2)	12 (12.2)	6 (6.1)	9 (9.2)	6 (6.1)	6 (6.1)	4 (4.1)	0	0	44 (45)
Unknown		11	0	4	2	1	0	0	1	0	0	8
Total	15.7 × 10^6	1172	39 (3.3)	204 (17.4)	109 (9.3)	160 (13.7)	52 (4.4)	27 (2.3)	27 (2.3)	7 (0.6)	6 (0.5)	573[c] (49)

NOTE. Data are no. (%) unless otherwise indicated. AD, age distribution; PIV, parainfluenza virus; RP, respiratory pathogen; RSV, respiratory syncytial virus.

[a] Percentages derived from figures per January 1999 [12].

[b] No significant difference in frequency of *M. pneumoniae* infection was observed between different age categories ($P > .05$, χ^2 test).

[c] Total number of patients with ≥1 RP is lower than the summarized totals of patients having specific RPs, since, in 56 (9.8%) patients, >1 RP was detected.

characteristics, time of illness onset, clinical signs and symptoms, and antibiotic treatment. A nose and a throat swab were placed together into 4 mL of Hanks's balanced salt solution containing gelatin, lactalbumin, yeast, and antibiotics. Specimens were sent to the Virology Department of the Laboratory of Infectious Diseases and Perinatal Screening (National Institute of Public Health and the Environment, Bilthoven, The Netherlands) by regular mail.

Detection of M. pneumoniae *and respiratory viruses.* When specimens arrived at the laboratory, transport medium was separated from the swabs, and a 400-μL aliquot was processed for *M. pneumoniae* by using PCR, as described elsewhere [5]. DNA was isolated by using a proteinase K lysis protocol, and *M. pneumoniae* DNA was detected by using nested PCR, with the P1 cytadhesin gene as the target. An amplification control was used to check for the presence of PCR inhibitors. In case of inhibition, the lysate was diluted 1:10 and was retested in the PCR. Reaction products were analyzed by 2% agarose gel electrophoresis.

Virus isolation and identification were performed, according to standard methods [8]. Five hundred microliters of the transport medium was inoculated into tertiary cynomolgus monkey kidney cells (tMKs) and human diploid lung fibroblasts in tubes and subsequently was incubated in roller drums at 33°C. A second aliquot of 500 μL was used to inoculate tMKs in flat-bottom tubes, which were centrifuged at 5000 g at room temperature for 75 min and then were incubated at 33°C. Influenza viruses were (sub)typed in hemagglutination-inhibition assays. PCR was performed for respiratory syncytial virus [9], rhinovirus and enterovirus [10], and coronaviruses OC43 and 229E [11].

Transmission of M. pneumoniae *among household contacts of the patients.* Household contacts of *M. pneumoniae*–positive patients were asked to participate in the transmission study. A nose and throat swab were collected by the GP within 4 weeks after sampling of the index patient. A questionnaire that included the characteristics of the sampled persons, the presence of current or recent symptoms and signs of ARI, and antibiotic usage was completed. Samples were processed for *M. pneumoniae* by using PCR.

Statistical analysis. The χ^2 test was used to compare the frequency of *M. pneumoniae* infection in the different age categories, in the winter (October through March) and the summer (April through September) periods (with Yates correction), and the frequency of *M. pneumoniae* among household contacts of index patients treated with appropriate antibiotics and of untreated index patients. The χ^2 test with Mantel-Haenszel trend analysis was used to compare the frequency of *M. pneumoniae* in the age categories of the household contacts.

Results

Distribution of M. pneumoniae *and respiratory viruses among patients with ARI.* During the 30-month study period, GPs in 32 (70%) of the 45 general practices participating in the surveillance collected nose and throat samples from 1172 ARI patients. The 1172 patients (772 [65%] patients with ILI and 400 [35%] patients with ARI without ILI) were a representative sample of the national population, with respect to geographic and sex distribution [12]. The youngest age category (0–4 years) was slightly overrepresented, and the oldest age category (>60 years) was slightly underrepresented (table 1). In total, 631 respiratory pathogens were detected in nose and throat samples from 573 (49%) patients. Mixed infections occurred in 56 patients with ARI, predominantly in the youngest age category (0–4 years).

M. pneumoniae DNA was present in samples from 39 (3.3%) of the 1172 patients (24 [3.1%] patients with ILI and 15 [3.7%] patients with ARI without ILI). Of the 39 *M. pneumoniae*–positive patients, 22 (56%) were men, and 17 (44%) were women. The percentage of *M. pneumoniae*–positive samples in the various age groups, with regard to the total number of samples in the various age groups, was similar ($P > .05$; table 1). Mixed infections with *M. pneumoniae* and rhinovirus were detected in 4 patients, *M. pneumoniae* and enterovirus in 2, and *M. pneumoniae* and respiratory syncytial virus in 1.

Chest radiography was performed in only 1 of the 39 *M. pneumoniae*–positive patients. The radiograph showed signs of pneumonia in this patient, a 36-year-old woman. She was the

only patient hospitalized because of *M. pneumoniae* infection. Symptoms and signs of *M. pneumoniae*–positive patients and of patients with a viral cause of ARI were similar.

The majority of the samples was obtained from patients presenting with ARI during the winter period (1012 [86%] of 1172 patients). *M. pneumoniae* infection occurred throughout the entire year. Although the frequency was higher during the summer months (9 [5.6%] of 160 patients) than during the winter period (30 [2.9%] of 1012 patients), this difference was not significant.

M. pneumoniae *among household contacts of index patients.* The 39 *M. pneumoniae*–positive index patients represented 39 households. In total, nose and throat samples from all 79 household contacts of 30 index patients were obtained. Sampling of household contacts in 4 households was not possible, and 3 patients and 2 GPs refused to participate in this part of the study. Samples were collected at a mean of 22 days (range, 14–30 days) after initial sampling of the index patient. *M. pneumoniae* was present in samples from 12 (15%) household contacts (9 children 0–15 years old and 3 persons >15 years old; *P* = .02; figure 1). Of the 12 *M. pneumoniae*–positive household contacts, 8 had ARI, and 3 of these 8 had consulted their GP because of ARI. Four of the *M. pneumoniae*–positive contacts, all young children (0–7 years old), were asymptomatic or had only a subclinical infection the 4 weeks prior to and the 4 weeks after sampling (figure 1).

Of the 23 household contacts of 8 patients treated with doxycyline or macrolide, 3 (13%) were *M. pneumoniae* positive. Of the 9 household contacts of 3 patients treated with β-lactam and the 47 household contacts of 19 untreated patients, 9 (19%) were *M. pneumoniae* positive.

Discussion

During this 30-month prospective study in The Netherlands, the distribution of *M. pneumoniae* and various respiratory viruses among 1172 patients with ARI was studied. *M. pneumoniae,* detected by PCR analysis of nose and throat samples, was present in 39 (3.3%) patients. For patients with ARI who presented to a GP, we calculated an incidence of *M. pneumoniae* of 587 per 100,000 persons per year, based on the frequency of *M. pneumoniae* among patients with ILI and patients with ARI without ILI found in our study and on the incidence of ILI and ARI among patients in The Netherlands [13]. In the community, the incidence of *M. pneumoniae* infection is expected to be much higher, since only 25% of the *M. pneumoniae*–positive household contacts of the index patients consulted their GP. In France, the incidence of *M. pneumoniae,* as diagnosed by PCR, among outpatients with ARI, was 190–1234 per 100,000 persons [4]. The high incidence of 1234 per 100,000 persons was estimated during an *M. pneumoniae* epidemic period in France, whereas the incidence of 587 per 100,000 persons in our study was found during an *M. pneumoniae*–endemic period in The Netherlands.
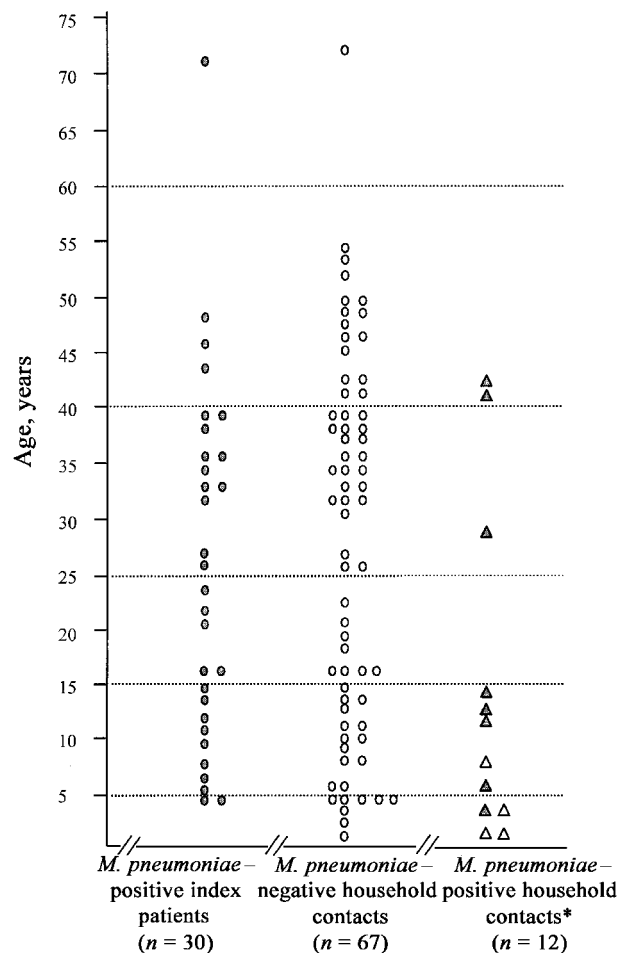

Figure 1. No. and age distribution of *Mycoplasma pneumoniae*–positive index patients participating in the transmission study (●) and their *M. pneumoniae*–negative household contacts (○) and *M. pneumoniae*–positive symptomatic (▲) and asymptomatic (△) household contacts. Age categories are indicated by the dotted lines. *More *M. pneumoniae*–positive household contacts were detected in the 2 youngest age categories (0–4 and 5–15 years) than in the other age categories (≥16 years; *P* = .02, Mantel-Haenszel χ² trend analysis).

ARI due to *M. pneumoniae* occurred throughout the year. The number of *M. pneumoniae*–positive patients was highest during winter months, but the proportion of *M. pneumoniae*–positive patients was highest during the summer months, because of the low frequency of respiratory virus infections (data not shown).

ARI due to *M. pneumoniae* occurred at all ages. The infection rate in the various age groups was similar, as was reported recently for a French outpatient population [4]. Thus far, in studies mainly of hospitalized patients with *M. pneumoniae* infection, school-age children (5–15 years old) and adults (30–45 years old) were identified as age groups with a higher incidence for *M. pneumoniae* infection [1, 14].

Among the 79 household contacts of 30 *M. pneumoniae* index

patients, significantly more *M. pneumoniae*–positive household contacts were in the age groups 0–4 and 5–15 years than in the other age groups. Since these children had either no signs and symptoms of ARI or ARI that was so mild that they did not visit their GP, children are a relevant reservoir for *M. pneumoniae,* playing an important role in transmission of the bacterium.

The frequency of *M. pneumoniae*–positive household contacts of index patients treated with doxycycline, macrolide, or β-lactam and of untreated index patients was similar ($P > .05$). Since doxycycline and macrolides are antibiotics by which *M. pneumoniae* infection is effectively treated [1], our findings suggest that *M. pneumoniae* transmission from effectively treated patients still occurs in the household setting. It may be that transmission can be prevented only by early treatment of the index patient, as has been shown for the prevention of the spread of *Bordetella pertussis* among household contacts [15].

In conclusion, we found a frequency of *M. pneumoniae* infection of 3.3% among patients presenting with ARI and of 15% among their household contacts, as detected by PCR. In the latter group, *M. pneumoniae* was mainly present in children in whom the infection was either mild or asymptomatic. Therefore, children may be an unrecognized reservoir for *M. pneumoniae.*

Acknowledgments

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