TABLE 1. Microbiological findings for 200 patients with signs and symptoms of the common cold

Organism or infection	No. (%) of patients positive by:				Total no. of patients (%)
	Virus antigen detection	Virus culture	Rhinovirus PCR	Serology	
Rhinovirus		80 (40)	103 (51.5)		105 (52.5)
Influenza A virus	6 (3)	3 (1.5)		9 (4.5)	10 (5)
Influenza B virus	2 (1)	1 (0.5)		1 (0.5)	2 (1)
Adenovirus	1 (0.5)			1 (0.5)	2 (1)
Parainfluenza virus type 1	1 (0.5)	1 (0.5)			1 (0.5)
Parainfluenza virus type 2				2 (1)	2 (1)
Parainfluenza virus type 3	3 (1.5)	3 (1.5)			3 (1.5)
Parainfluenza virus type 1 or 3				5 (2.5)	1 (0.5)
RSV	4 (2)	3 (1.5)		3 (1.5)	4 (2)
Enterovirus				1 (0.5)	1 (0.5)
Coronavirus OC43				7 (3.5)	7 (3.5)
Coronavirus 229E				10 (5)	10 (5)
C. pneumoniae				4 (2)	4 (2)
M. pneumoniae				1 (0.5)	1 (0.5)
S. pneumoniae				1 (0.5)	1 (0.5)
H. influenzae				1 (0.5)	1 (0.5)
M. catarrhalis					0
Double viral infection					10 (5)
Viral and bacterial infections					6 (3)
Total	17 (8.5)	91 (45.5)	103 (51.5)	46 (23)	139 (69.5)

Rhinovirus reverse transcription-PCR. Nucleic acids were isolated from the nasopharyngeal samples by using proteinase K-sodium dodecyl sulfate treatment followed by phenol extraction and ethanol precipitation. For detection of rhinoviruses, two reverse transcription-PCR assays were used (16). The first one utilizes primers from the conserved 5′ noncoding region and the VP2 capsid protein coding region of the viral genome (3), while the other test uses two primers from the 5′ noncoding region (10, 15).

Bacterial culture and antibody assays. For detection of beta-hemolytic streptococci, swabs dipped into nasopharyngeal mucus were inoculated onto blood agar plates and incubated for 24 h in an atmosphere of 5% carbon dioxide at 37°C. Those plates showing uncertain growth were incubated another 24 h.

Immunoglobulin G (IgG) antibodies to pneumococcal pneumolysin and C-polysaccharide were measured by EIA as described earlier, and a twofold-or-higher rise in antibody levels between paired sera was considered diagnostic (18). Antibodies to nontypeable *Haemophilus influenzae* and *Moraxella catarrhalis* were measured by EIA using whole bacterial cell antigen (a mixture of 10 different strains), and a threefold-or-higher antibody rise between paired sera was considered diagnostic for acute infection (6). IgG and IgM antibodies to chlamydial species were measured by a microimmunofluorescence method by using elementary bodies of *Chlamydia pneumoniae* Kajaani 7 and *Chlamydia trachomatis* 1.2 as antigens as described elsewhere (7), except that the sera were incubated overnight. The presence of IgM antibodies and/or a fourfold-or-greater change in antibody levels between paired sera was considered diagnostic for acute chlamydial infection. These antibody assays have been successfully used in the etiological diagnosis of pneumonia in children both in industrialized and developing countries (28, 29).

Mycoplasma IgM antibodies from the second serum samples taken on day 21 were measured with a commercial kit (Platelia; Sanofi Diagnostics Pasteur S.A., Marnes la Coquette, France) routinely used in our virology laboratory.

RESULTS

Virological findings. Evidence for virus infection was found in 138 of the 200 patients (69%). Rhinovirus was found in 105 patients (52.5%) by virus culture or PCR. The percentage of rhinoviruses was the same in both males and females. Overall, results by virus culture and PCR had a high level of agreement: 78% of the PCR-positive samples were also culture positive (80 of 103 patients). Coronaviruses were the second most common group of causative agents and were detected in 17 patients by serology. A total of 12 patients had influenza A or B virus infection (Table 1).

In addition to the 80 patients culture-positive for rhinoviruses, 11 nasopharyngeal-aspirate samples were positive by virus culture. Three cases of influenza A were detected by serology only, whereas the other seven patients were positive by antigen detection, culture, or serology. For the other respiratory viruses, five patients remained negative by culture or antigen detection but paired serum antibodies showed a rise. In addition, coronaviruses were detected by serology only. Taken together, virus culture was positive for the respiratory viruses in 91 cases (45.5%), virus antigen detection gave positive results in 17 cases (8.5%), and serology gave a diagnosis for 39 patients (19.5%) (Table 1).

Evidence of a double viral infection was found in 10 patients. Of these patients, three had both rhinovirus and coronavirus OC43, two had rhinovirus and influenza A virus, two had rhinovirus and parainfluenza virus type 2, one had rhinovirus and adenovirus, one had rhinovirus and influenza B virus, and one had rhinovirus and enterovirus infections.

Figure 1a shows the number of virus infections detected monthly and the total number of recruited patients. Figure 1b presents numbers of rhino- and coronavirus infections detected in the study population. An outbreak of rhinoviruses occurred in the fall, when 92% (33 of 36) of the patients recruited into the study had rhinovirus infections. Figure 1c shows the epidemiology of other respiratory viruses in the community during the study period. Influenza A and B virus infections peaked in March, followed by parainfluenza virus type 3 infections in April and RSV infections in May. Adenoviruses were endemic.

Bacteriological findings. Serological assays suggested bacterial infections in seven patients. Of these, four patients had a rise in IgG antibodies against *C. pneumoniae*; three of these patients were also rhinovirus positive, and one patient was positive for both rhino- and coronaviruses. One patient had a rise in antibodies against *Streptococcus pneumoniae*, one patient had a rise in antibodies against both *H. influenzae* and coronavirus, and one patient had serological evidence of both mycoplasma and coronavirus infections. None of the patients had beta-hemolytic group A *Streptococcus* in their nasopharynges.

For monitoring complications of the common cold, patients were examined clinically on days 1, 7, and 21 and according to