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Laboratory Diagnosis and Surveillance of Human Respiratory Viruses by PCR in Victoria, Australia, 2002–2003

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Respiratory viruses were identified by the polymerase chain reaction (PCR) in more than 4,200 specimens collected during 2002 and 2003 in Victoria, Australia from patients admitted to hospitals or participating in an influenza surveillance program. Influenza viruses and picornaviruses were important causes of morbidity in both years. Additional testing of picornavirus-positive samples suggested that rhinoviruses but not enteroviruses were more likely to be associated with respiratory symptoms, irrespective of the season in which they circulated. Detection of influenza viruses was strongly associated with the clinical symptoms of cough, fever, and fatigue; but each of the other respiratory viruses occasionally caused these symptoms or was responsible for symptoms severe enough to require hospitalization. Human coronaviruses HCoV-OC43 and HCoV-229E circulated at low levels throughout the study period with peak activity in winter, but overall did not circulate as widely as has often been reported for these agents. Evidence for the human metapneumovirus (hMPV) was only sought in the second year of the study and revealed low-level circulation of this virus, mainly in the cooler months among the very young and adult populations. The detection rate of all viruses declined with increasing age of the patient, particularly in hospital patients. Infection with more than one respiratory virus occurred in a small number of patients; picornaviruses were most commonly implicated in these dual infections. ***J. Med. Virol. 75:122–129, 2005.*** © 2005 Wiley-Liss, Inc.

KEY WORDS: nucleic acid detection; viruses causing respiratory symptoms; hospitalized patients; influenza surveillance

INTRODUCTION

Viruses are known to be common causes of respiratory illness throughout the world, with seasonal variation that is more marked in temperate rather than tropical climates. These viruses cause illnesses that can range from a brief upper respiratory tract infection to severe systemic illness resulting in death. The risk of infection is often related to age. Influenza, for example, is known to have its highest attack rates amongst the very young and the very old [McIntyre et al., 2002]. Respiratory syncytial virus (RSV) affects mainly the young, although less typical infections amongst adults are recognized [Hall, 2001]. The risk of infection also depends on pre-existing medical conditions and immunocompetence.

Historically, laboratory diagnosis of respiratory infection has been slow because of its reliance on virus isolation or serological techniques. The use of immunofluorescence-based assays has improved turnaround times, but a nasopharyngeal aspirate is required for optimal results and the method suffers from a limited number of viruses being detectable with the reagents available. The availability of new classes of drugs active against both influenza A and B viruses [Cooper et al., 2003], preclinical and clinical trials of drugs targeted at respiratory viruses other than influenza [Hayden et al., 2003; Cianci et al., 2004; Uckun et al., 2004], and the potential for new vaccines [Power et al., 2001] has encouraged further a shorter diagnostic turnaround time for the detection of respiratory viruses. An increasing number of diagnostic laboratories use

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polymerase chain reaction (PCR)-based assays to rapidly identify a wide range of viruses in specimens collected from a variety of respiratory sites [Billaud et al., 2003; Echavarria et al., 2003; Vuorinen et al., 2003].

The results of a 2-year retrospective study of respiratory viruses in more than 4,200 specimens obtained from individuals living in Victoria, Australia are presented. The seasonality of the viruses detected, their occurrence by age group, their presentation in hospitalized patients compared to those participating in an influenza surveillance program, and the occurrence of dual infections were investigated.

PATIENTS AND METHODS

Patients and Specimens

Specimens were received from individuals included in a Victoria-wide influenza surveillance program operating between May and September each year [Watts et al., 2003] or from patients who had been admitted to Victorian hospitals with a respiratory illness (or acquired their infection nosocomially during their admission) (Table I). Clinical material sent for laboratory testing from hospitalized patients included nose swabs, throat swabs, nasopharyngeal aspirates (NPAs), endotracheal aspirates, bronchoalveolar lavages (BALs), bronchial washings, lung biopsies, sputum samples, and pleural fluids. Nose and throat swabs (NTS) pooled into viral transport medium were received from surveillance patients. They were only taken from individuals with symptoms of 3 days or less of fever, cough, and fatigue. When multiple samples from a single patient were sent for testing, only one result was included in the analysis. A total of 333 additional respiratory specimens, including 20 from asymptomatic laboratory staff was used to validate the PCR assays for influenza A virus (H1 and H3 subtypes), influenza B virus, RSV, parainfluenza viruses (at least one of types 1–3), picornaviruses (a mixture of enteroviruses and rhinoviruses), and adenoviruses (each of different serotype) (Table III).

Development and Validation of PCR Assays

PCR assays were developed and validated on clinical material (NTS, NPAs, and BALs) in parallel with the existing techniques of virus isolation [Lewis and Kennett, 1976] and/or immunofluorescence (performed using a Bartels Viral Respiratory Screening and Identification Kit (Trinity Biotech, County Wicklow, Ireland)). The process included the design and evaluation of primers; optimization of nucleic acid extraction conditions; establishment of optimum PCR amplification conditions; evaluation of applicable specimen types; determination of assay sensitivity compared to conventional assays; specificity testing using clinical material likely to be negative (including asymptomatic staff volunteers); or material previously shown to be positive for respiratory viruses by conventional assays or by sequencing of an amplified product where no other confirmatory method was available. Because of the lack of immunofluorescence and routine isolation methods to confirm PCR positivity for human metapneumovirus (hMPV), human coronavirus type OC43 (HCoV-OC43), and human coronavirus type 229E (HCoV-229E), sequencing of the PCR-amplified products of these viruses was carried out with the same second round primers used to derive the product. Sequences obtained were confirmed by comparison with those in the National Center for Biotechnology Information (Genebank) database.

Nucleic Acid Extraction and Reverse Transcription

Prior to testing, each specimen was spiked with a low copy number of bovine diarrheal disease virus (BVDV) to act as an internal control for the nucleic acid extraction, reverse transcription, and PCR amplification steps. The approach of using a non-human virus as an internal inhibition control has been reported previously [Druce et al., 2002]. Viral nucleic acid was extracted using one of two commercially available methods. The preferred method used a Magnapure LC automated extraction robot with a Total Nucleic Acid Isolation Kit (Roche Diagnostics, Mannheim, Germany). This involved the extraction of viral nucleic acid from 200 μl of the clinical material in viral transport medium, with elution into a final volume of 50 μl of the supplied elution buffer. Specimens containing inhibitors of the PCR as shown by failure of the BVDV internal control to amplify were re-extracted through a column (Highpure Viral Nucleic Acid Extraction Column; Roche Diagnostics) and re-tested. Ten microliters of RNA was linearized for 10 min at 65°C, added to 12 μl of reverse transcription master mix consisting of random hexamers (Roche Diagnostics), dNTPs (Amersham Biosciences, Buckinhamshire, UK), and AMV-RT enzyme and buffers (Promega, Madison, WI) and incubated for 30 min at 42°C followed

TABLE I. Source, Gender, and Virus Detection Rates in 4,254 Patients From Whom Specimens Were Received for Respiratory Virus Polymerase Chain Reaction (PCR) in 2002 and 2003

	2002			2003		
	Surveillance	Hospital	Total	Surveillance	Hospital	Total
No. of patients	602	1,414	2,016	557	1,681	2,238
No. (%) of males	276 (45.7)	806 (57.0)	1,082 (53.7)	262 (46.9)	1,041 (62.0)	1,303 (58.2)
No. (%) of females	326 (54.3)	608 (43.0)	934 (46.3)	295 (53.1)	640 (38.0)	935 (41.8)
No. (%) of viruses detected	324 (53.8)	427 (30.2)	751 (37.3)	275 (49.4)	557 (33.1)	832 (37.2)

by 10 min at 100°C. The cDNA product was stored at 4°C until tested. Adenovirus DNA extracted by either of the above methods survived this process (results not shown).

PCRs for Detection of Respiratory Viruses

A panel of nested PCR assays capable of detecting 11 respiratory viruses was developed using primers reported by others or designed in-house. Tube 1 included influenza A virus (H1 and H3 subtypes) [Zhang and Evans, 1991], influenza B virus [Zhang and Evans, 1991], adenoviruses [Allard et al., 1992], and the BVDV internal control [Cleland et al., 1999]. Tube 2 included RSV [Osiowy, 1998] and picornaviruses (with primers specific for all enteroviruses and rhinoviruses) [Ireland et al., 1993]. Tube 3 included parainfluenza virus types 1, 2, and 3. Tube 4 included HCoV-229E and HCoV-OC43, and tube 5 included hMPV only. When required, a separate PCR assay was used to distinguish enteroviruses from rhinoviruses when the picornavirus component of tube 2 was positive [Zoll et al., 1992; Steininger et al., 2001]. A negative control of nuclease-free water was included in every assay and a virus positive control consisting of low copy number cDNA (or DNA for adenovirus) included in the relevant PCR targeting that virus. The primers used for first and second round amplifications, and their gene/product targets, are listed in Table II. For first round amplification, 3 μl of cDNA template was added to 40 μl of a mastermix containing 500 nM first round primers (100 nM for the picornavirus primers), 1.8 mM $MgCl_2$, 2 μM dNTPs and 0.3 U of Taq polymerase (Qiagen, Hilden, Germany). The first round PCR conditions consisted of one cycle of 94°C for 5 min followed by 35 cycles of 94°C (30 sec), 55°C (30 sec), 72°C (60 sec), then 72°C for 5 min. The conditions for tube 2 were reduced to 20, 20, and 30 sec, respectively, for cycles 2–36. For second round amplification, 2 μl of the first round product was transferred to fresh mastermix containing second round primers. The second round PCR amplification conditions were the same as for round 1 except the 35 cycle component for tube 2 was reduced to 25 cycles and the annealing temperature reduced to 50°C. Separation of amplified material and molecular weight markers (Roche Diagnostics) was performed by electrophoresis for 30 min at 80 mA on a 2% agarose gel prestained with ethidium bromide. Gels were photographed using a Gel Doc 2000 (Biorad, Hercules, CA).

RESULTS

A total of 4,254 patient specimens were examined in 2002 and 2003 (Table I). In both years, more specimens were received from hospitalized patients than from

TABLE II. First and Second Round Primers (5′–3′), and Their Gene Product Targets, Used for the Respiratory Viruses Identified in the Study

Virus [target]	First round primers	Second round primers
Flu A, H1N1 [HA]	CAGATGCAGACACAATATGT	CTTAGTCCTGTAACCATCCT
	AAACCGGCAATGGCTCCAAA	ATAGGCTACCATGCGAACAA
Flu A, H3N2 [HA]	CAGATTGAAGTGACTAATGC	AGCAAAGCTTTCAGCAACTG
	GTTTCTCTGGTACATTCCGC	GCTTCCATTTGGAGTGATGC
Flu B [HA]	GTGACTGGTGTGATACCACT	CATTTTGCAAATCTCAAAGG
	TGTTTTCACCCATATTGGGC	TGGAGGCAATCTGCTTCACC
Parainfluenza [L protein]	GTWCAAGGAGAYAATCARGC	GCATCAGACCCTTATTCATG
	GRTCYGGAGTTTCWARWCC	GTTGTATCAAGCATCCCGC
		CAGCCGATCCATACTCATTG
		CTTGTGGTGTCAAAAAATCC
		GCTGTTACTACAAGAGTACC
		GTTGCCAGATTTGAGGATGC
Respiratory syncytial virus (RSV) [nucleoprotein]	TGGGAGARGTRGCTCCAGAATACAGGC	ACYAAATTAGCAGCAGGR
	ARCATYACTTGCCCTGMACCATAGGC	CTCTKGTWGAWGATTGTGC
Picornavirus [5′UTR]	CGGACACCCAAAGTAG	GCATTCAGGGGCCGGAG
	GCACTTCTGTTTCCCC	GCACTTCTGTTTCCCC
Enterovirus [5′UTR]	STCACCGGATGGCCAATCC	ATTGTCACCATAAGCAGCCA
	GGCCCCTGAATGCGGCTAAT	GGCCCCTGAATGCGGCTAAT
Rhinovirus [5′UTR]	CCCCTGAATGYGGCTAACCT	GAATGYGGCTAACCTTAAMCC
	CGGACACCCAAAGTAGTYGGT	CAAAGTAGTYGGTCCCRTCC
Adenovirus [hexon protein]	GCCGCAGTGGTCTTACATGCACATC	GCCACCGAGACGTACTTCAGCCT
	CAGCACGCCGCGGATGTCAAAG	TTGTACGAGTACGCGGTATCCTCGCGGTC
		CMGASACSTACTTCAGYMTG
		GTASGYRKTRTCYTCSCGGTC
Corona 229E [nucleocapsid]	GGTACTCCTAAGCCTTCTCG	GGTACTCCTAAGCCTTCTCG
	GACTATCAAACAGCATAGCAGC	ACAACACCTGCACTTCCAAA
Corona OC43 [nucleocapsid]	AGGAAGGTCTGCTCCTAATTC	CTGGCAATAGAACCCCTACC
	GCAAGAATGGGGAACTGTGG	TATTGGGGCTCCTCTTCTGG
Human metapneumovirus (hMPV) [pol]	GAACGTGCTACACTGACAACAC	GAGAGATCCTCAAGCTGTTGGCTC
	GTCAGCAATGATTCCGACCTC	CTGTAGTGTATAGCACTATCGCTG
Bovine diarrheal disease virus (BVDV) [5′UTR-pro]	CGAAGGCCGAAAAGAGGCTAGC	CATGCCCTTAGTAGGACTAGCA
	GGCCCYGGYTTCAGGTAGA	TTACCCGACCTGCAGTCACCTC

K = G + T, M = A + C, R = A + G, S = G + C, W = A + T, Y = C = T.

those participating in the influenza surveillance program conducted during May–September. There were more specimens received from females (53.6%) than males (46.4%) participating in the surveillance program. Conversely, 59.7% of specimens from hospitals were from male patients. The overall respiratory virus detection rate was higher in specimens received from surveillance patients than hospital patients in both years (54% and 49% in 2002 and 2003, respectively, for surveillance patients compared to 30% and 33%, respectively, for hospital patients).

Experiments carried out during the development and validation process showed that it was not possible to incorporate all the required primers into a single tube assay without significant loss of sensitivity for some viruses (results not shown). Overall, the PCR assays developed were able to detect at least 0.1 tissue culture infectious dose (50%) using the methods described (results not shown). The sensitivity of the influenza A virus and influenza B virus PCR components was 86% and 87%, respectively, compared to the combined conventional assays of immunofluorescence and virus isolation (Table III). However, additional testing using matrix specific primers (not shown) confirmed that the influenza virus PCR positive/conventional test negative samples were true positives. The true sensitivity for influenza A was therefore 89.5% (95% CI, 66.9–98.7) and specificity 100% (95% CI, 98.9–100). For influenza B the sensitivity was 90.0% (95% CI, 67.1–98.8) and specificity 100% (95% CI, 98.9–100). A total of 44 specimens were positive for picornaviruses by PCR but negative by virus isolation, the only conventional test available. Positive results for picornaviruses were confirmed using enterovirus- and rhinovirus-specific primers, giving an assay sensitivity for picornaviruses of 100% (95% CI, 92.5–100) and specificity 100% (95% CI, 98.7–100). Although based on small numbers in some instances, the sensitivity of the PCR assays for each of RSV, parainfluenza viruses, and adenoviruses was 100% (Table III). The 20 specimens collected from asymptomatic volunteer staff were negative in each of the PCR assays.

The temporal circulation of respiratory viruses during 2002 and 2003 is shown in Figure 1. All viruses for which a PCR was available were detected during this time. Overall, respiratory viruses were detected in 1,583 (37%) of 4,254 specimens received for testing. The virus detection rates for 2002 and 2003 during the surveillance period (May–September) and the official winter months of June–August are shown in Table IV. A greater proportion of viruses were detected in surveillance patients in both years during these overlapping times. The average winter detection rate of 40% in hospitalized patients decreased to 22% in the summer of 2002/3.

Influenza A virus was the most common virus detected (Fig. 1). Discrete winter peaks occurred in both years, with activity for this virus being greater in 2003 than 2002. Identification of influenza A virus was rare outside the peak incidence times of May–September. Collectively the winter influenza epidemics of 2002 and 2003 were similar, but in 2002 this was due to both influenza A and influenza B virus activity, whereas in 2003 influenza B virus activity was very low.

Picornaviruses were the second most common virus detected after influenza A virus (Fig. 1) and were detected in every month of the year, although they were most common in the cooler months. Although the screening PCR did not discriminate between rhinoviruses and enteroviruses, a subgroup of 36 picornavirus-positive specimens, 10 collected during August 2003 (winter) and 26 collected during December 2002 and January–February 2003 (summer), were rhinovirus positive when retested using a genus-specific PCR.

RSV circulated in discrete winter peaks in both years, with little activity outside these times (Fig. 1). The circulation of coronaviruses was similar to RSV, although the peak incidence in both years was approximately one month later for the coronaviruses (compare Fig. 1b and c). The most commonly detected coronavirus was HCoV-OC43, with sporadic appearance of HCoV-229E mainly in autumn and spring. Parainfluenza viruses were detected in low numbers throughout both years, with a tendency for increased detection

TABLE III. Clinical Validation of the Respiratory Multiplex PCR on a Specimen Panel Previously Characterized by Results of Virus Isolation and/or Immunofluorescence Testing

	Positive by IF/isolation		Negative by IF/isolation		PCR	
Virus[a]	PCR positive	PCR negative	PCR positive	PCR negative	Sensitivity (95% CI)	Specificity (95% CI)
Influenza A	12	2	5[b]	314	85.7 (57.2–98.2)[b]	98.4 (96.4–99.5)[b]
Influenza B	13	2	5[b]	313	86.7 (59.5–98.3)[b]	98.4 (96.4–99.5)[b]
RSV	30	0	13	290	100 (88.4–100)	95.7 (92.8–97.7)
Picornavirus	3	0	44[b]	286	100 (29.2–100)[b]	86.7 (82.5–90.1)[b]
Adenovirus	1	0	5	327	100 (2.5–100)	98.5 (96.5–99.5)
Parainfluenza	4	0	4	325	100 (39.8–100)	98.7 (96.9–99.7)

[a]Validation of the HCoV-OC43, HCoV-229E, and hMPV assays was by sequencing of the amplified product as described in the "Patients and Methods."

[b]When these are included as true positives (see "Results"), the sensitivity for influenza A was 89.5% (95% CI, 66.9–98.7) and specificity 100% (95% CI, 98.9–100); sensitivity for influenza B was 90.0% (95% CI, 67.1–98.8) and specificity 100% (95% CI, 98.9–100); and sensitivity for picornaviruses was 100% (95% CI, 92.5–100) and specificity 100% (95% CI, 98.7–100).

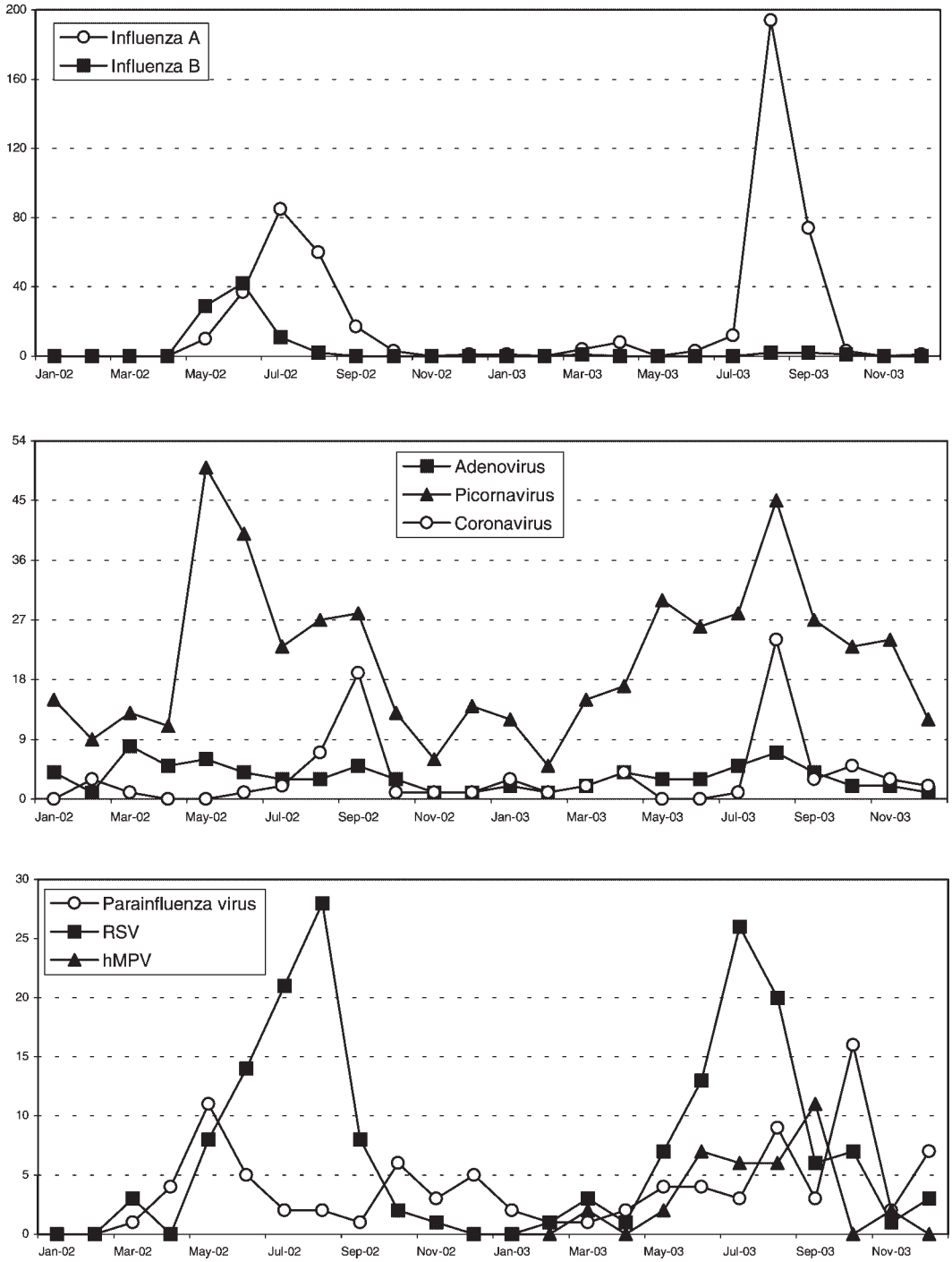

Fig. 1. Temporal distribution of respiratory viruses detected by polymerase chain reaction (PCR) in Victoria, Australia in 2002 and 2003. Results shown include combined figures for hospitalized patients and those participating in the influenza surveillance program. The vertical axis shows the number of detections for each virus.

in October and November. Adenoviruses were detected in low numbers throughout the year. In 2003, when testing was available, hMPV detection was highest in the cooler months of June–September.

The proportions of each of the respiratory viruses detected according to age-groups and whether the specimens were tested as part of the influenza surveillance program or for hospital patients is shown in Table V. Irrespective of the source of the specimens, individuals aged 10 years and under were more likely to have a respiratory virus detected. In adults, the rate of detection decreased with increasing age and this was more

TABLE IV. Respiratory Virus Detection Rates During Periods of Influenza Surveillance (May–September), Winter (June–August), Summer (December–February), and Totals for 2002 and 2003, According to Whether Specimens Were Tested as Part of the Influenza Surveillance Program or Were From Hospitalized Patients

	2002 hospital		2003 hospital		2002 surveillance		2003 surveillance	
	Tested	PCR+ (%)	Tested	PCR+ (%)	Tested	PCR+ (%)	Tested	PCR+ (%)
May–Sept	745	287 (39)	905	345 (38)	602	324 (54)	557	275 (49)
June–Aug	456	183 (40)	559	232 (42)	424	236 (56)	415	212 (51)
Dec–Feb	235	51 (22)	n/a	n/a	n/a	n/a	n/a	n/a
Year	1,414	427 (30)	1,681	557 (33)				

pronounced in the hospitalized patients. The most common viruses detected in hospital patients, irrespective of age, were influenza A and picornaviruses. In hospitalized children under 6 years of age, and hospitalized adults, the picornavirus detection rate was higher than that for influenza. In surveillance patients, influenza A and B viruses together accounted for at least 60% of the viruses detected in all age groups.

Sixty three patients (4%) were infected with 2 viruses. The most common viruses implicated in these dual infections were picornaviruses and adenoviruses (Table VI). Picornaviruses were present with another virus in 37 patients (representing 59% of the cases of dual infection) while adenoviruses were present in 21. These two viruses were detected together in 12 specimens. There was insufficient clinical information supplied to assess the influence of dual infections on the degree of morbidity compared to patients infected with a single virus.

DISCUSSION

This study reports virological testing of specimens from hospitalized patients and community-based patients who were part of Victoria's annual winter influenza surveillance program. The overall detection rate was influenced by the source of specimen referral, with a higher proportion of specimens from surveillance than hospitalized patients yielding a recognized respiratory virus.

The introduction of nucleic acid detection techniques into the diagnostic laboratory has decreased the time to diagnosis of many viral infections and also enabled the detection of one or more pathogens in a single specimen using individually directed PCRs or multiplexed assays [Billaud et al., 2003; Echavarria et al., 2003; Vuorinen et al., 2003]. These assays have also increased the proportion of specimens in which an aetiologic agent can be detected, as evidenced by the recent discoveries of hMPV [Van den Hoogen et al., 2001] and coronavirus HCoV-NL63 [van der Hoek et al., 2004], agents which do not replicate reliably in cell lines used in many diagnostic laboratories.

As expected in our study, influenza viruses were the most common agents identified, although viruses other than influenza sometimes caused an influenza-like illness. Picornaviruses were an important cause of morbidity in both hospital and surveillance patients. Although the picornavirus PCR detected both enteroviruses and rhinoviruses, all the picornavirus-positive specimens that were analysed further, including those detected during summer, were identified as rhinoviruses. Although some PCR-based assays have

TABLE V. Source (Hospital or Influenza Surveillance (Flu Surv)) and Overall Percentage of Respiratory Viruses Detected According to Age of the Patients From Whom Specimens (Number Tested) Were Received

Age group (years)	0–4		5–9		10–19		20–39		40–59		≥60	
Source	Hospital	Flu Surv	Hospital	Flu Surv	Hospital	Flu Surv	Hospital	Flu Surv	Hospital	Flu Surv	Hospital	Flu Surv
Number tested	987	64	44	55	111	205	598	436	662	292	693	107
Influenza A	10.4	48.8	23.8	52.8	48.4	55	27.2	62.1	15.3	50.4	32.3	53.8
Influenza B	0	9.3	14.3	25	0	22.9	3.1	7.5	2.9	10.6	1.6	7.7
RSV	17.9	7	9.5	5.6	3.2	3.1	7.4	3.3	16.8	4.9	15.3	5.8
Parainfluenza	8.6	0	0	0	0	3.1	4.9	1.9	13.1	3.3	8.9	1.9
Adenovirus	9.8	0	9.5	0	12.9	0.8	4.3	2.3	5.1	0	3.2	0
Picornavirus	46	7	23.8	11.1	22.6	14.5	41.4	19.6	36.5	22.8	33.9	23.1
Coronavirus	4.1	27.9	19	5.6	12.9	0	10.5	2.3	6.6	5.7	0.8	3.8
hMPV	3.1	0	0	0	0	0.8	1.2	0.9	3.6	2.4	4	3.8
All viruses	51.6	67.2	47.7	65.5	27.9	63.9	27.1	49.1	20.7	42.1	17.9	48.6

The combined detection rate for each of the viruses from both hospital and influenza surveillance patients is shown in the last row.

TABLE VI. Occurrence of Dual Respiratory Viruses in Patients From all Sources

	Number of patients concurrently infected with the coinciding viruses							
	Adenovirus	Flu A	Flu B	Picornavirus	RSV	Paraflu	HCoV-OC43	hMPV
Adenovirus	—	—	—	12	1	3	4	1
Influenza A	—	—	—	7	6	1	4	—
Influenza B	—	—	—	4	—	—	—	—
Picornavirus	—	—	—	—	4	7	3	4
RSV	—	—	—	—	1	—	—	—
Parainfluenza	—	—	—	—	—	1	—	1
HCoV-OC43	—	—	—	—	—	—	—	—
hMPV	—	—	—	—	—	—	—	—

indicated a low asymptomatic carriage rate for picornaviruses [Johnston et al., 1993], it is likely that the rhinoviruses detected by the current assays are the causative agents of the illness. Further studies are needed to analyze the severity of clinical illness associated with dual infections involving rhinoviruses. However, the ability of rhinoviruses to produce symptoms sufficiently severe to be mistaken for influenza infection or sometimes requiring hospitalization is noteworthy.

Of the viruses associated regularly with lower respiratory tract infection, RSV was the most common. Although RSV was detected most commonly in hospitalized children as expected, it was also detected regularly in adults. RSV has been shown previously to contribute to community and hospital cases of influenza-like illnesses [Zambon et al., 2001]. The role of the newly identified hMPV was investigated only in 2003. This virus was associated mainly with hospitalized cases, suggesting that it may be clinically distinguishable from influenza infection in the community setting. It was less common in older children and teenagers than in infants, young children and adults. A similar epidemiological pattern has been reported in other parts of the world [Falsey et al., 2003], but more detailed studies on the epidemiology of this virus are warranted. Coronaviruses, which have been reported to cause up to 35% of all cases of the common cold [McIntosh, 1997], and are associated with hospitalizations among older adults [Falsey et al., 2002], accounted for a few cases of influenza-like illness, particularly in younger children, but otherwise were present in only 6% of surveillance patients and 12% of hospitalized patients. The role of HCoV-OC43 in causing discrete outbreaks in elderly residents of aged-care facilities has been described [Birch et al., in press], and further studies on the impact of the newly recognized HCoV-NL63 are needed.

Because the diagnostic testing algorithm involved multiple targets, it was anticipated that some samples would contain more than one virus. Most of the viruses were identified on a prolonged background of adenovirus and picornavirus activity throughout the 2 year period. It is not surprising therefore that these viruses were most commonly detected as part of dual infections. Rhinoviruses have been previously shown to occur commonly with RSV in cases of acute bronchiolitis in infancy and to increase the risk for severe disease [Papadopoulos et al., 2002]. The availability of PCR technology now provides the opportunity for prospective studies on the epidemiology and clinical impact of dual infection with respiratory viruses.

Because of the lack of clinical information supplied at the time of specimen receipt, we were unable to link many of the virological findings with clinical symptoms or outcome for hospitalized patients. However, the influenza surveillance program shows that other viruses, especially rhinoviruses, are often associated with symptoms of cough, fever, and fatigue. We found relatively low levels of coronaviruses in both years, in contrast to previous reports. This study contributes to the demonstration of the importance of PCR in the investigation of the epidemiology and laboratory diagnosis of respiratory viral infections. PCR assays provide an opportunity to identify new viruses and detect those which do not replicate reliably in cultured cells. They also provide a rapid and sensitive means of diagnosis which may impact on the treatment and duration of hospitalization in many cases.

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