

REVIEW ARTICLES

Dual Respiratory Virus Infections

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We retrospectively reviewed eight prospective epidemiological studies conducted between 1991 and 1995 for dual respiratory virus infection (DRVI) to determine the frequency, associated comorbid conditions, clinical presentations, and morbidity related to DRVI among immunocompetent persons. Two viruses were identified as the cause of 67 (5.0%) of 1,341 acute respiratory virus infections. DRVI was detected in patients from <1 year to 79 years of age, in both sexes, and in many races. Forty-two percent of patients with DRVI were ≤ 4 years old. Fifty-eight percent of patients with DRVI had underlying chronic lung disease. DRVI was associated with upper respiratory tract illness; lower respiratory tract illness, including pneumonia; systemic influenza-like illnesses; and exacerbations of asthma or chronic obstructive pulmonary disease. All of the common acute respiratory viruses were identified; picornaviruses and influenza virus A were the most common. The rate of DRVI (11.6%) was highest in the epidemiological studies in which cell culture, serology, and polymerase chain reaction were used together. Patients with DRVI were hospitalized significantly more often than those with respiratory infection due to a single virus (46.3% vs. 21.7%; $P < .01$). The percentage of DRVIs increased proportionally with the number of diagnostic methods used.

Respiratory illness associated with multiple viral pathogens has been reported infrequently. The majority of the studies have been published in the pediatric literature, with most infections reported to be due to respiratory syncytial virus (RSV) and a second respiratory virus [1–8]. The frequency of dual respiratory virus infection (DRVI) varies widely in the literature, and the clinical relevance of DRVI is unresolved. Some authors have found that the morbidity associated with DRVI is higher than that associated with single respiratory virus infection (SRVI) [3], while other authors have not found that DRVIs are more severe than SRVIs [2, 5, 8–12]. We performed a retrospective review of prospective epidemiological studies of respiratory virus infection carried out by the Acute Viral Respiratory Disease Unit at Baylor College of Medicine between 1991 and 1995. We examined the incidence, demographics, and clinical presentations of DRVI. A review of the literature on DRVI was performed, and our results were compared with those previously reported.

Materials and Methods

Study design. We retrospectively reviewed the charts included in epidemiological studies of community-acquired respiratory virus infections conducted at our institution between 1991 and 1995. A DRVI was defined as an acute respiratory virus infection and any combination of culture(s), serological test(s), or PCR(s) positive for two different viruses. An SRVI was defined as an acute respiratory virus infection caused by a single virus detected by either culture, positive serology, or PCR. The charts of all patients with DRVI in these epidemiological studies were reviewed by one of us (A.L.D.), and data on demographics, comorbid conditions, date of onset of the acute respiratory illness, results of viral diagnostic tests, and clinical presentation were recorded. A computerized database was used to obtain the results of viral diagnostic tests performed for the other patients in these studies. Information on demographics and hospitalization status of those with SRVI was also abstracted.

Patient populations. A total of 4,336 patients were enrolled in eight different prospective epidemiological studies of acute respiratory viral disease conducted between 1991 and 1995. All studies were reviewed and approved by the Institutional Review Board of Baylor College of Medicine (Houston). The studies differed in their objectives, patient populations, time periods of study, and viral diagnostic tests used (table 1). Two studies were performed in outpatient clinics, and two included only hospitalized patients. Three of the studies focused on patients with underlying lung disease: in one study, patients

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Table 1. Data from epidemiological studies of acute respiratory viral disease reviewed for dual respiratory virus infections at Baylor College of Medicine, Houston, Texas.

Study no.	Population	No. of patients enrolled	Viral diagnostic tests performed
1	Outpatients (children and adults) with acute respiratory illnesses who were seen in community health clinics	1,560	Cell tissue cultures
2	Outpatients (children and adults) with acute respiratory illnesses who were treated at an HMO	1,226	Cell tissue cultures
3	Children and adults hospitalized with acute respiratory illnesses or CHF	902	Cell tissue cultures, serology
4	Children and adults from an HMO hospitalized with acute respiratory illnesses or CHF	171	Cell tissue cultures, serology
5	Patients with COPD and control patients ≥ 50 years old followed longitudinally for the development of acute respiratory illnesses	124	Cell tissue cultures, serology
6	Pairs of mothers and children (0–3 years old) followed longitudinally for the development of acute respiratory illnesses	195	Cell tissue cultures, serology
7	Young adults with asthma followed longitudinally for the development of acute respiratory illnesses	36	Cell tissue cultures, serology, PCR
8	Adults with acute asthma exacerbations presenting to county hospital emergency center	122	Cell tissue cultures, serology, PCR

NOTE. CHF = congestive heart failure; COPD = chronic obstructive pulmonary disease; HMO = health maintenance organization.

with chronic obstructive pulmonary disease (COPD) and control patients >50 years old were observed longitudinally for the development of acute respiratory illness; in another study, young adults with asthma were observed longitudinally for the development of acute respiratory disease; and in the third study, adults with exacerbations of acute asthma who presented to a county hospital emergency center (EC) were evaluated. In another smaller study, pairs of mothers and infants from birth to 3 years of age were observed for the development of acute respiratory disease. Cell culture alone was used for diagnosis in the two outpatient clinic studies. In all of the other studies, cell culture and serology were used, and in the two studies of asthmatics, PCR was used as well.

Viral diagnostic methods. Cell cultures for influenza virus types A and B, RSV, parainfluenzavirus types 1, 2, and 3, adenoviruses, and picornaviruses were performed by using the following cell lines: human embryonic lung fibroblast, human epidermoid laryngeal carcinoma, African green monkey kidney, and Madin-Darby canine kidney or primary rhesus monkey kidney. Standard detection and identification methods of viruses were used [13]. Cultures positive for herpes simplex virus (HSV) or cytomegalovirus (CMV) were excluded from the analysis.

Serology was performed by microneutralization tests for detection of influenza virus types A and B; RSV; and parainfluenzavirus types 1, 2, and 3 [14, 15]. An ELISA to detect antibody to coronavirus OC43 was performed as described previously [16]. Serology for influenza viruses A and B was also performed by hemagglutination inhibition by using previously described techniques [17]. For an acute respiratory illness to be consid-

ered an acute respiratory virus infection on the basis of serology of acute and convalescent sera, a significant increase in titers of antibody had to be detected within 6 weeks of the onset of the illness.

A significant rise in antibody titer was defined for influenza viruses A and B as follows: at least a sixfold increase by microneutralization assay or at least an eightfold increase by hemagglutination inhibition assay or at least a fourfold increase in antibody to the same antigen in two different assays. We excluded titer increases due to influenza vaccination by assuming that all simultaneous increases in antibody to influenza viruses A H1 and H3 antigens and influenza virus B antigen were due to vaccination. Furthermore, the charts of all patients with potential influenza virus infection, as determined by serology alone, were reviewed for influenza vaccination status. This information had been collected prospectively in all of the epidemiological studies reviewed. If there was documentation of influenza vaccination within 6 weeks of the acute respiratory illness, these patients were not considered to have influenza virus infection; however, they could be included as a case of SRVI or DRVI if they had infection(s) with any of the other respiratory viruses.

For parainfluenzaviruses and RSV, a significant rise in antibody titer was defined as at least a sixfold rise by microneutralization assay. Given the cross-reactivity of antibody to parainfluenzavirus types 1, 2, and 3, a positive serology for more than one type of parainfluenzavirus was not included as a DRVI; instead, it was considered a single parainfluenzavirus infection, and the type assigned was the prevalent type circulating in the community at that time.

Table 2. Demographics of patients with dual respiratory virus infections and single respiratory virus infections and other patients enrolled in epidemiological studies at Baylor College of Medicine, Houston, between 1991 and 1995.

Variable	DRVI (n = 67)	SRVI (n = 1,274)	All enrolled (n = 4,336)
Age (y)			
Range	<1–79	<1–84.6	<1–99
Mean \pm SD	25.0 \pm 26.6	23.5 \pm 23.5	27.4 \pm 23.6
Median	18.8	13.1	24
Sex			
Male	26 (39)	582 (46)	1845 (43)
Female	41 (61)	693 (54)	2491 (57)
Race			
White	18 (27)	516 (40)	1464 (34)
Black	26 (39)	280 (22)	1309 (30)
Hispanic	22 (33)	449 (35)	1482 (34)
Asian	1 (1)	20 (2)	59 (1)
Other or unknown	0	10 (1)	22 (1)

NOTE. Data are number (%) unless otherwise indicated. DRVI = dual respiratory virus infection; SRVI = single respiratory virus infection.

For coronavirus OC43, a significant rise in antibody titer was defined as either a single fourfold or greater rise or a ≥ 2.5 -fold rise that was reproducible upon repeated testing.

Reverse transcriptase-PCR was performed to detect coronaviruses and picornaviruses in two epidemiological studies and to detect influenza A in one of these studies. Viral nucleic acids were extracted from respiratory secretions, and previously described primers for amplification of coronavirus OC43 [18], influenza A [19], and picornavirus [20] were used. Complementary DNA synthesis and PCR amplification were performed by using a PTC-100 thermal cycler [20, 21]. Positive results were identified by slot blot or Southern blot hybridization with use of digoxigenin-labeled oligonucleotides [19, 22]. Pre- and post-PCR procedures were performed in separate rooms on different floors, and other standard precautions were used to prevent carryover contamination during the performance of reverse transcriptase-PCR assays [23]. For each study, only a single clinical specimen from the respiratory tract was used for the identification of virus by cell culture or PCR.

Statistical analysis. The demographics and hospitalization rates for patients with DRVI and SRVI were analyzed by use of the χ^2 test for discrete variables and the Mann-Whitney *U* test for continuous variables (non-normally distributed).

Results

Demographics. A total of 1,341 acute respiratory virus infections were identified in the eight studies, and 67 (5.0%) were associated with multiple respiratory viruses (66 infections were DRVIs, and one was a triple respiratory virus infection); 1,274 (95%) were SRVIs. The rate of DRVI varied from 1.8%–15.8% in the individual epidemiological studies. Table 2 shows

the ages, sexes, and races of all the patients enrolled in the studies. The patients with DRVI ranged in age from <1 year to 79 years (median age, 18.8 years). Ten patients (15%) were ≥ 65 years old, and 28 patients (42%) were ≤ 4 years old. Although there was no statistically significant difference between the median age of those with DRVI and those with SRVI, the percentage of patients with DRVI who were <1 year old was significantly greater than the percentage of patients with SRVI who were <1 year old (25% vs. 15%; *P* = .025). There was no significant difference in the percentage of those ≥ 65 years old with DRVI versus SRVI.

There were slightly more women than men with DRVI, but this difference reflects the sexes of the patients enrolled in these studies, and the difference was not significant. The racial distribution of the patients with DRVI differed significantly from that of patients with SRVI in that a greater percentage of DRVIs than SRVIs occurred in blacks, and fewer DRVIs than SRVIs occurred in whites (*P* = .004). Information regarding comorbid conditions was available for 53 of the 67 patients with DRVI. Thirty-one (58%) of these 53 had underlying chronic lung disease (COPD or asthma). Five patients had hypertension. Two patients had congestive heart failure, two had diabetes mellitus, and two had peptic ulcer disease.

Clinical presentations. Information was available regarding the clinical diagnosis of the acute respiratory illness for 60 of the 67 patients. Upper respiratory tract infection was the most common diagnosis (55% of patients), but 40% of the patients had lower respiratory tract infections. An exacerbation of underlying lung disease (COPD or asthma) was associated with DRVI in 42% of the patients. Eighteen percent of these patients presented with a systemic or influenza-like illness, and a small number (5%) had associated congestive heart failure.

Respiratory viruses causing DRVI. There were 20 different virus combinations isolated from patients with DRVI (table 3). Picornaviruses (33 patients), most of which were rhinoviruses, and influenza A (28) were the most common viruses identified in DRVIs, and the combinations of a picornavirus with influenza A (10 patients) or a picornavirus with coronavirus OC43 (10) were the most frequent combinations. The combinations of influenza B with parainfluenzavirus, RSV with adenovirus, and adenovirus with coronavirus OC43 did not occur.

Although all of the respiratory viruses were involved in both DRVIs and SRVIs, the frequencies of these viruses among cases of DRVI vs. SRVI differed (table 4). Picornavirus was the most common virus, followed by influenza A in both DRVIs and SRVIs. RSV was frequently involved in both DRVIs and SRVIs. Coronavirus OC43 was the third most common virus detected in patients with DRVI, but it was uncommon in patients with SRVI. Conversely, influenza B was the third most common virus detected in patients with SRVI, but it was the least frequently identified virus in patients with DRVI.

Table 3. Virus combinations detected in patients with dual respiratory virus infections.

Virus combination	No. of patients (% with DRVI)
Influenzavirus A, picornavirus	10 (14.9)
Picornavirus, coronavirus	10 (14.9)
Adenovirus, picornavirus	7 (10.4)
Influenzavirus A, RSV	6 (9.0)
RSV, parainfluenzavirus	4 (6.0)
RSV, picornavirus	4 (6.0)
RSV, coronavirus	4 (6.0)
Parainfluenzavirus, coronavirus	4 (6.0)
Influenzavirus A, adenovirus	3 (4.5)
Influenzavirus A, influenzavirus B	2 (3.0)
Influenzavirus A, parainfluenzavirus	2 (3.0)
Influenzavirus A, coronavirus	2 (3.0)
Influenzavirus B, RSV	2 (3.0)
Influenzavirus A (H1), influenzavirus A (H3)	1 (1.5)
Influenzavirus B, adenovirus	1 (1.5)
Influenzavirus B, picornavirus	1 (1.5)
Influenzavirus B, coronavirus	1 (1.5)
Parainfluenzavirus, adenovirus	1 (1.5)
Parainfluenzavirus, picornavirus	1 (1.5)
Influenzavirus A, RSV, coronavirus	1 (1.5)
Total	67 (100)

NOTE. DRVI = dual respiratory virus infection; RSV = respiratory syncytial virus.

Viral diagnostic methodology. Simultaneous cultures yielded two different respiratory viruses in 41.8% of the cases of DRVI. However, the results of serology and PCR added greatly to the diagnosis of DRVI. Thirty-one (46%) of the 67 DRVIs required serology for detection, and 13 (19.4%) of the DRVIs would not have been identified without the use of PCR. The rate of detection of DRVI increased with the number of viral diagnostic methods used (table 5). The studies in which cell culture was the only diagnostic method used had the lowest

Table 4. Frequency of respiratory virus involvement in cases of dual respiratory virus infection and in cases of single respiratory virus infection.

Virus identified	No. (%) of patients with DRVI (n = 67)	No. (%) of patients with SRVI (n = 1,274)
Picornaviruses	33 (49.3)	443 (34.7)
Influenzavirus A	28 (41.8)	276 (21.7)
Coronavirus	22 (32.8)	29 (2.3)
RSV	21 (31.3)	126 (9.9)
Adenovirus	12 (17.9)	113 (8.9)
Parainfluenzavirus	12 (17.9)	126 (9.9)
Influenzavirus B	7 (10.4)	161 (12.6)

NOTE. DRVI = dual respiratory virus infection; SRVI = single respiratory virus infection; RSV = respiratory syncytial virus.

rate of DRVI (1.9%), and the studies in which cell culture and serology were used had an intermediate rate of DRVI (8.1%). The rate of detection of DRVI was highest (11.6%) when cell culture, serology, and PCR were used. Since cell culture was the only viral diagnostic method that was performed in all of these epidemiologic studies, a separate analysis was performed to determine the rate of SRVI vs. DRVI based only on the results of cell culture. In this analysis, there were 1,200 infections, 28 (2.3%) of which were DRVIs.

The viral diagnostic method detecting the respiratory viruses differed for DRVI and SRVI. Cultures were positive for 90% of patients with SRVI vs. only 58.5% of patients with DRVI. Some additional SRVIs were detected with use of serology and PCR, but these diagnostic methods had a proportionally greater impact on the detection of DRVIs. PCR was very important in the diagnosis of coronavirus OC43 infection; >40% of all the coronavirus OC43 infections were detected by PCR alone. PCR was the only diagnostic method that detected 8.6% of the picornavirus infections. No additional influenzavirus A infections were detected by PCR; in all the PCR-positive cases, either a culture or serology was positive for influenzavirus A as well.

Morbidity. Thirty-one (46.3%) of the 67 patients with DRVI were hospitalized, whereas 277 (21.7%) of the 1,274 patients with SRVI were hospitalized ($P < .01$). Although the difference in hospitalization rates is significant, the different viral diagnostic tests performed for individual patients make interpretation of this finding difficult. To address this issue further, a subgroup analysis was performed to determine the rates of DRVI and SRVI based on the results of cell culture alone, since this was the single viral diagnostic technique performed for all patients. This analysis included 28 DRVIs and 1,172 SRVIs. Ten of 28 patients with DRVIs diagnosed by culture alone vs. 221 of 1,172 patients with SRVIs diagnosed by culture alone were hospitalized (36% vs. 19%; $P = .025$).

Overall, there was no significant difference in the age of hospitalized patients with DRVI vs. SRVI or in the percentage of those <1 year old or >65 years old. One asthmatic patient had two DRVIs and two SRVIs, and she was not hospitalized with any of these infections. Eight patients with DRVI also had an SRVI at another time. Four of these patients were not

Table 5. Increased frequency of detection of dual respiratory virus infection when additional diagnostic methods are used in addition to cell culture.

Viral diagnostic methods used	Total no. of respiratory infections	No. (%) of DRVIs detected
Cell culture	737	14 (1.9)
Cell culture and serology	483	39 (8.1)
Cell culture, serology, and PCR	121	14 (11.6)

NOTE. DRVI = dual respiratory virus infection.

hospitalized with either DRVI or SRVI, three were hospitalized with DRVI but not SRVI, and one was hospitalized with both DRVI and SRVI. Four patients each had one DRVI and two SRVIs; three were not hospitalized with any of these infections, and one was hospitalized with the DRVI but not with either SRVI. One patient had one DRVI and three SRVIs and was not hospitalized with any of these infections. No patient with both DRVI and SRVI was hospitalized with SRVI and not DRVI.

Discussion

Over the past 45 years, more than 430 cases of DRVI in immunocompetent hosts have been reported (table 6). The rate of DRVI in these reports varies widely. Many published studies of acute respiratory illness report no cases of DRVI [43–53]. In the published studies in which DRVIs have been reported, the rate of DRVI ranged from 1.33% to 100% in a single case report. In our study, the overall rate of DRVI was 5%, but the rate of DRVI in the individual epidemiological studies that we reviewed ranged from 1.8% to 15.8%. The wide range of DRVI rates in our studies and in the literature is due to multiple factors including differences in patient populations (e.g., differences in age and comorbid conditions), time of study (e.g., winter vs. summer or during epidemics of respiratory virus infections), and diagnostic methods employed. Both the literature and our analysis show that the number of DRVIs identified increases with the number of viral diagnostic methods used.

Most reports of DRVI have involved children, but there have been reports of DRVI in immunocompetent adults as well. Our study shows that a greater percentage of patients with DRVI than those with SRVI are <1 year old (25% vs. 15%). It is unclear from the literature and our study why younger patients are more often dually infected with respiratory viruses. The immature immune system of infants and lack of previous exposure to respiratory viruses could increase susceptibility to simultaneous infection with two or more respiratory viruses. Another possible but unproven explanation for the increased rate of DRVI among children is that there is something unique about RSV that facilitates infection with a second respiratory virus. It is also possible that prolonged shedding of respiratory viruses occurs more commonly in children. We cannot exclude the possibility that our apparent DRVIs were actually separate but closely timed infections.

Fifty-eight percent of our patients with DRVI had underlying lung disease (asthma or COPD). The importance of this observation is unclear because complete information regarding underlying lung disease for all enrolled patients in our studies was not available. This high rate may reflect the prevalence of underlying lung disease in our study populations rather than an increased susceptibility to DRVI in patients with underlying lung disease, since three of our studies specifically included patients with asthma or COPD. Most of the reports of DRVI

in the literature do not contain comments on comorbid conditions of the patients, but a few of the studies specifically included patients with asthma or COPD [31, 34]. The rates of DRVI in these reports were not higher than the overall rate of DRVI in all of the studies reviewed.

DRVIs have been reported in both inpatients and outpatients with diagnoses of upper respiratory tract illness, lower respiratory tract illness, systemic or flu-like illness, and exacerbation of asthma. Our review also found DRVI to be associated with these various clinical presentations; however, no predilection for DRVI in a specific clinical syndrome was apparent.

All of the usual respiratory viruses have been reported in cases of DRVI. In addition, many studies include HSV [3, 5, 8, 26, 28, 29, 31, 34, 41] and CMV [3–5, 8, 31, 41] in cases of DRVI. Measles virus [24], reovirus [24], and mumps virus [28] have also been reported in DRVI. Many of the studies in the literature do not specify which of the identified respiratory viruses were specifically involved in DRVI. In the studies that do specify the viruses involved in DRVI, RSV is generally the most common one, and influenzaviruses A, B, and C, parainfluenzaviruses, adenoviruses, and rhinoviruses also are frequently involved. In our patients with DRVI, picornaviruses and influenza virus A were the most common viruses, followed by coronavirus OC43.

Most of the published reports did not attempt to identify coronavirus as an etiologic agent, but Lina et al. [42] reported that 15 of 16 cases of DRVI in their study included a coronavirus. Coronavirus was detected in 32.8% of our patients with DRVI, while this virus was detected in only 2.3% of the patients with SRVI. The increased frequency of coronavirus in cases of DRVI may be an artifact of our viral detection methods rather than a true difference. Our detection methods included PCR for coronavirus, and >40% of our coronavirus infections were detected by PCR alone.

In most of the published studies of DRVI, more than one viral diagnostic technique was used to identify respiratory viruses. The rate of DRVI in the literature is dependent upon the number of viral diagnostic methodologies used. When only one diagnostic method was used, the overall rate of DRVI was 1.8%, while when two virus detection methods were used, the rate of DRVI was 9.9%, and when three methods were used, the rate was 8.4%. Likewise, our study shows that the rate of detection of DRVI increased with the number of viral diagnostic methods used. It seems that DRVIs occur more frequently than is currently appreciated, and as viral diagnostic ability improves, the number of DRVIs detected will increase.

In the published reports in which serology was used, a fourfold or greater increase in the antibody titer between acute and convalescent sera was accepted as the definition of infection. We used more stringent criteria for serological diagnosis, which affected the number of DRVIs detected. If a fourfold rise in antibody titer between acute and convalescent sera was considered diagnostic in our study as it is in the literature, then the

Table 6. Summary of data from studies of dual respiratory virus infection.

Reference	Date of study	No. of patients	No. (%) with DRVI	Age or type of patient	Viruses identified	Diagnostic method			Increased severity
						Culture	Serology	Immuno-fluorescence	
[9]	2/51	1	1 (100)	20 y	IA, IC	Yes	Yes	No	No
[24]	1/59–6/60	989	34 (11.4)	Children, adults	A, RSV, P, IA, IB, IC, measles, reovirus	Yes	Yes	No	NA
[10]	12/1/62–6/1/63	389	53 (26.9)	<13 y	A, P, R, I, RSV, untyped cytopathogenic agents	Yes	Yes	No	No
[25]	11/63–4/64; 11/64–2/65	142	2 (2.5)*	Children, adults (families)	RSV, R, A	Yes	Yes	No	NA
[1]	2/66–4/66	97	15 (28.3)	Children	NA	Yes	Yes	No	No
[26]	10/63–4/65	113	10 (13.2)	3 w–6 y	HSV, RSV, A, R, P, IC	Yes	Yes	No	NA
[27]	4/66–3/67	2,055	5 (1.3)	Children	NA	Yes	No	No	NA
[12]	1/1/67–6/30/68	746	69 (23.7)	<18 mo	NA	Yes	Yes	No	No
[28]	1/15/66–3/15/66	22	6 (35.3)	<6 y	IA, P, HSV, RSV, mumps, A	Yes	Yes	No	NA
[1]	11/1/65–4/30/67 (winter)	377	9 (4.5)	<1 y	RSV, I, R, E, A	Yes	Yes [†]	No	NA
[29]	7/1/67–6/30/68	427	6 (6.3)	Adults	IA, HSV, P	Yes	Yes	No	NA
[30]	1/68–3/69	477	10 (5.0)	<11 y	RSV, A, P, R, I	Yes	Yes	No	NA
[31]	1970	63	2 (9.1)	15–77 y	CMV, HSV, IA, A	No	Yes	No	NA
[32]	10/68–1/73	24	1 (7.7)	22 d–35 mo	IA, P	Yes	No	Yes	NA
[33]	11/15/77–3/15/78	71	1 (3.1)	18–96 y	RSV, IA	Yes	Yes	No	NA [‡]
[34]	1968–1975	150	10 (3.7)	Adults	HSV, A, R, C, P	Yes	Yes	No	NA
[35]	1978–1980 (during epidemics)	(1,030 illnesses) 164	2 (2.5)	1 mo–9 y	RSV, A	No	No	Yes	NA
[36]	1/80–4/80	46	17 (37.0)	NA	IA, IB, “other respiratory viruses”	Yes	Yes	No	NA
[2]	1981–1982 (winter)	20	2 (14.3)	2 w–5 mo	RSV, P	Yes	No	Yes	No
[37]	11/1/78–6/30/79	102	4 (5.3)	<5 y	P, R, RSV	Yes	Yes	Yes	NA
[38]	12/84–3/85	103	4 (7.1)	<1 y	RSV, I, R	Yes	No	Yes	NA
[39]	12/82–3/84	98	2 (5.3)	2 mo–15 y	RSV, IB, C	Yes	Yes	Yes	NA
[3]	1977–1986 (winters)	1,706	28 (4.4)	<3 y	RSV, A, IA, IB, CMV, P, HSV, E, R	Yes	No	Yes	NA
[4]	12/85–5/87 (winter/spring)	189	5 (2.6)	Children	RSV, A, CMV	Yes	No	Yes	Yes [§]
[5]	9/81–8/87	2,415	51 (7.7)	Children	RSV, CMV, R, A, I, P, HSV, E	Yes	No	Yes	No
[6]	7/84–11/87	31	1 (8.3)	<5 y	RSV, A	Yes	No	Yes	NA
[40]	5/87–4/88	204	4 (5.4)	<5 y	RSV, A, P	Yes	No	Yes	NA
[7]	1/87–12/87	738	24 (6.8)	<5 y	RSV, P	Yes	No	Yes	NA
[41]	1989	NA	9 (1.8)	NA	CMV, HSV, A	Yes	No	No	NA
[8]	5/80–10/84	1,246	29 (9.5)	0–3 y	RSV, C, CMV, IC, P, E, IA, IB, A, HSV	Yes	Yes	Yes	No
[42]	10/1/94–5/2/95	962	16 (4.6)	Children, adults	C, RSV, A, IA, IB, R	Yes	No	Yes	NA

NOTE. A = adenovirus; C = coronavirus, CMV = cytomegalovirus; DRVI = dual respiratory virus infection; E = enterovirus; HSV = herpes simplex virus; I = influenza virus; IA = influenza virus A; IB = influenza virus B; IC = influenza virus C; NA = data not available; P = parainfluenza virus; R = rhinovirus; RSV = respiratory syncytial virus.

* Diagnosed by culture alone; serology may have revealed more infections but does not distinguish between simultaneous and sequential infections.

[†] Serology for RSV only.

[‡] One patient with DRVI had pneumonia, as did six patients with single respiratory virus infections.

[§] There was an increase in the number of cases of respiratory failure among patients dually infected with RSV and adenovirus.

rate of DRVI would increase from 5.0% to 6.4%. Given the performance characteristics of the serological tests, we chose to use a higher threshold for serological diagnosis to exclude more potential false-positive increases in antibody titers.

It is not clear from the literature whether infection with two or more respiratory viruses causes more-severe clinical illness than infection with a single respiratory virus. Tristram et al. [4] reported an increased incidence of respiratory failure among patients dually infected with RSV and adenovirus, suggesting that DRVI may be more severe than SRVI. On the other hand, several reports state that the severity of DRVI is not greater than infection with either agent alone, as was stated in the first reported case of DRVI [9]. Similarly, by using duration of hospitalization as an indicator of severity, Portnoy et al. [10] did not find any significant difference in terms of severity between DRVI and SRVI.

Mufson et al. [12] found no association between DRVI and a specific disease syndrome. In reporting a simultaneous epidemic of RSV and parainfluenzavirus type 3 in a neonatal intensive care unit, Meissner et al. [2] stated, "The infants infected by two viruses simultaneously could not be distinguished clinically from infants infected by a single agent." In a case-control study of children infected with RSV alone vs. RSV and another respiratory virus, Subbarao et al. [5] found no difference between the two groups with use of a scoring system for severity of clinical illness. In a study comparing infants who had lower respiratory tract infections caused by RSV alone vs. RSV and a second viral agent, Ray et al. [8] found that infants <6 months old were more likely to have a second viral agent identified in addition to RSV than were older infants ($P = .08$), but there were no differences in type of lower respiratory tract illness (bronchitis, bronchiolitis, croup, or pneumonia) or duration of illness between the two groups.

When hospitalization is used as a marker for severity of disease, our findings suggest that DRVIs are associated with increased morbidity. We found a significantly increased rate of hospitalization among patients with DRVI than among those with SRVI (46.3% vs. 21.7%; $P < .01$). However, the number of viral diagnostic methods used differed in the different epidemiological studies, and the two outpatient clinic studies were the only ones in which cell culture was used alone for virus identification. Because the rate of DRVI increased as more viral diagnostic methods were used, the rate of DRVI in the outpatient studies may have been underestimated. Thus, the marked difference in the hospitalization rates for patients with DRVI vs. those with SRVI may be due to the decreased ability to identify DRVI in outpatients rather than a reflection of the increased morbidity associated with DRVI that resulted in hospitalization.

To address this further, we performed a separate analysis by using the results of cell culture alone and found that the hospitalization rate again was significantly higher for patients

with DRVI than for those with SRVI (36% vs. 19%; $P = .025$). The finding of a significantly higher hospitalization rate for patients with DRVI than for those with SRVI when identical viral diagnostic methods were used suggests that DRVIs are associated with increased morbidity. In addition, a small number of SRVIs were detected in asymptomatic patients, but all DRVIs were associated with illness. Whether DRVIs are associated with increased morbidity can only be determined by a prospective clinical study in which patients with DRVI, SRVI, and respiratory illnesses without a known etiology are followed longitudinally.

DRVIs occur in immunocompetent hosts of all ages, particularly infants. DRVIs are associated with all syndromes of respiratory illness and are found in both inpatients and outpatients. All of the common acute respiratory viruses are involved in DRVI. All viral diagnostic methods contribute to detection of DRVI, and the rate of DRVI increases as the number of diagnostic methods used increases. Wider application of PCR should increase the rate of detection of dual infections and allow clearer distinction between DRVI and SRVI. The percentage of patients with DRVI who are hospitalized is greater than that of patients with SRVI, suggesting possible increased morbidity associated with DRVI; however, only a prospective study with use of identical viral diagnostic methodologies for all patients could resolve this question. Determination of the clinical significance of DRVI should become more important as more DRVIs are detected by newer, more sensitive viral diagnostic techniques.

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