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Viral Respiratory Infections in Hospitalized and Community Control Children in Alaska

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Respiratory syncytial virus (RSV) in Alaska Native children from the Yukon Kuskokwim (YK) Delta is associated with a hospitalization rate five times higher than that reported for the general US child population. The role of other viral respiratory pathogens has not been studied in this population. YK Delta children <3 years of age hospitalized with respiratory infections and same aged community control children were prospectively enrolled between October 2005 and September 2007. Polymerase chain reaction detection of viruses was performed on nasopharyngeal samples. Characteristics of hospitalized and asymptomatic control children were analyzed. From October 2005 to September 2007, 440 hospitalized and 425 control children were analyzed. Respiratory viruses were detected in 90% (395) of hospitalized children: 194 (44%) rhinovirus, 131 (30%) adenovirus, 102 (23%) RSV, 77 (18%) para influenza viruses (PIV), 66 (15%) human metapneumovirus (hMPV), 23 (5%) influenza, and 25 (6%) coronavirus. Fifty-two percent (221) of control children had a virus detected, most commonly rhinovirus (33%), and adenovirus (16%). RSV, PIV, hMPV, and influenza were significantly more common in hospitalized cases than control children, but rhinovirus, adenovirus, and coronavirus were not. RSV and hMPV were associated with higher severity of illness. In this study, RSV remains the most important virus associated with respiratory hospitalization, although hMPV and PIV were also common. RSV and hMPV were associated with more severe illness. Rhinovirus and adenovirus were detected in two-thirds of hospitalized children, but their frequent detection in control children made their role in respiratory hospitalization uncertain. ***J. Med. Virol. 82:1282–1290, 2010.***

KEY WORDS: respiratory syncytial virus; RSV; respiratory infections; respiratory viruses; Alaska native; viral etiologies

INTRODUCTION

Alaska Native infants have been reported to experience one of the highest hospitalization rates for lower respiratory tract infection (LRTI) and respiratory syncytial virus (RSV) among US children [Holman et al., 2004; Peck et al., 2005]. LRTIs in Alaska Native

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children are associated with environmental factors such as household crowding and lack of running water and result in significant morbidity and chronic respiratory sequelae, including bronchiectasis [Singleton et al., 2000; Hennessy et al., 2008]. For Alaska Native children in the Yukon Kuskowkim (YK) Delta, the LRTI hospitalization rate was 284/1,000 infants/year during 1994–1997 [Karron et al., 1999; Singleton et al., 2006], while the RSV-associated hospitalization rate was 178/1,000 infants/year, a rate five times higher than that reported for the general US infant population. High RSV hospitalization rates and prolonged seasonality led to specific palivizumab recommendations for this population in the 2009 Redbook RSV chapter [Pickering et al., 2009]. Despite a decrease in RSV hospitalizations from 178 to 104/1,000 infants/year between 1997 and 2004, possibly due to increased availability of running water, decreased household smoking, and palivizumab use [Singleton et al., 2003, 2006], there was little change in the overall infant LRTI hospitalization rate during this period, and three-fourths of LRTI hospitalized cases pathogen had not detected [Singleton et al., 2006].

The purpose of the current study was to characterize the etiology of LRTI hospitalizations in YK Delta children by testing for respiratory viruses (RSV), human metapneumovirus (hMPV), influenza A and B, parainfluenza (PIV) 1–3, rhinovirus, coronavirus, and adenovirus in order to inform policies and interventions. Viruses were detected using polymerase chain reaction (PCR) testing of nasopharyngeal samples from children hospitalized with LRTI. Findings for these cases were compared with those from same-aged asymptomatic community control children.

MATERIALS AND METHODS

Population

Alaska's YK Delta region is home to approximately 25,000 primarily Yup'ik Eskimo people (85%) who live in 52 villages and the regional town of Bethel. Annually, there are approximately 600 live births. The region is roadless and villages are reached by airplane, boat, or snow machine. Most homes are small and 40% of homes are without running water [Bulkow et al., 2002; Hennessy et al., 2008].

The YK Delta Regional Hospital (YKDRH), a 50-bed primary care facility, is the only hospital in the region. Children requiring intensive care are transferred to the Alaska Native Medical Center (ANMC) or other hospitals in Anchorage.

This study was approved by the Alaska Area and the Centers for Disease Control and Prevention (CDC) Institutional Review Boards, and the Boards of Directors for Alaska Native Tribal Health Consortium, YK Health Corporation, and Southcentral Foundation Tribal Health Organizations.

Study Design—Hospitalized Children

From October 4, 2005 through September 30, 2007, we recruited YK Delta children <3 years of age who were hospitalized with LRTI at YKDRH or ANMC. After obtaining parental consent, study personnel asked questions about the child's recent respiratory symptoms, medical history, and household features. We reviewed individual medical records for underlying medical conditions, respiratory hospitalizations during the year before hospitalization and outpatient respiratory visits during the month before hospitalization. Positive bacterial cultures from normal sterile sites in study children were reported. Disease severity was assessed using a severity index for RSV (1 point each for length of stay >5 days, SaO_2 <87, PCO_2 >45, pH <7.35, or apnea, 2 points for mechanical ventilation) [McConnochie et al., 1990]. Severe disease was defined as an index ≥2 [McConnochie et al., 1990].

We obtained a nasopharyngeal swab (NPS) specimen using a nylon flocked swab (Copan Diagnostics Inc®, Murrieta, CA) that was placed immediately into 2 ml of stabilizing buffer containing guanidinium thiocyanate (MagNA pure LC Total Nucleic Acid Isolate Kit; Roche). We placed residual clinical nasopharyngeal wash (NPW) sample that had been collected for clinical RSV/flu testing in the same buffer. An NPW sample was collected on 72.7% (320/440) of cases. Samples were frozen at −80°C and transported on ice to the Arctic Investigations Program—CDC Laboratory.

Study Design—Community Control Children

To determine the prevalence of circulating viruses in YK Delta we recruited unmatched community control children <3 years of age. During recruitment we excluded children with new onset of respiratory symptoms within 3 days before recruitment. For case–control analysis, we excluded children with any respiratory symptoms in the 2 weeks prior to enrollment. During October 4, 2005 through September 30, 2006 (Year 1), we recruited control children from the Bethel area in a pilot phase. During October 1, 2006 through September 30, 2007 (Year 2) we recruited control children regionwide during monthly village recruitment trips. During each monthly trip we enrolled children from three villages in three of six YK Delta subregions for a total of 36 villages. Although control children were not matched for village with cases, each subregion was visited six times during Year 2. We obtained informed consent, administered the questionnaire, reviewed individual medical records, and obtained a NPS using the same method as for hospitalized children.

Respiratory Pathogen PCR Assays

Nucleic acids were isolated from all NPW and NPS. Ribonucleic acid (RNA) was isolated using the QIAmp viral RNA Mini Kit (Qiagen, Inc., Valencia, CA); DNA was isolated using the BioRobot EZ1® with Bacterial DNA card (Qiagen, Inc.). Specimens were tested for RSV; influenza A and B; PIV 1, 2, and 3; hMPV; coronavirus (229E, NL63, OC43, and HKU1), adenovirus, adenovirus 14, and rhinovirus using single-plex real-time PCR assays. PCR amplification and analysis

was performed on the Stratagene MX3000P® and MX3005P® real-time detection systems using Stratagene's Brilliant QRT-PCR One-Step Kit (RNA viruses) and Brilliant QPCR Kit (adenovirus; Stratagene, La Jolla, CA). To ensure that negative test results were not due to poor extraction, the human B2MG [Watzinger et al., 2004] and β-actin [Taylor et al., 1997] genes were extracted and amplified before or during sample testing. Samples with a negative result for B2MG or β-actin were extracted a second time. No sample had a second negative result.

The primers and probes to detect RSV [Kuypers et al., 2004], PIV1 [Kuypers et al., 2006], CoV [Kuypers et al., 2007], RV [Lu et al., 2008], adenovirus [Kuypers et al., 2006], and adenovirus 14 [Lu and Erdman, 2006] have been described previously. Procedures to detect influenza A and B, and hMPV were provided by the CDC (influenza A and B, J. Lindstrom, D. Erdman, personal communication; hMPV, Erdman, personal communication). Primers and probes for detection of PIV2 (sense primer, 5′-CTG GAG TCA TGC CAT GCA AT; antisense primer, 5′-CTG CGT ACA CCC CTG TGA TG; probe, 5′-CAA CAA GTT TTT GCC CTG CTA ATT) and PIV3 (sense primer, 5′-TGGAGGTCTTGAACATCCAATAAA; antisense primer, 5′-TGCAGTCTCTCTGCGTTTTCC; probe, 5′-AGAATGCAATCTGCAACACAACTGGGTG) targeted the hemagglutinin-neuraminidase gene and were designed using Primer3 software from sequences obtained from the National Center for Biotechnology Information database. They were screened for potential cross-reactivity with non-homologous viral and bacterial sequences using the available data (November 2005) in BLAST alignment software (available from http://blast.ncbi.nlm.nih.gov/Blast.cgi) [Robinson et al., 2005]. *Bordetella* spp. PCR was performed on study samples by the State of Alaska Public Health Laboratory.

Data Analysis

Proportions were compared using a chi-squared, Fisher's exact, or contingency table randomization test as appropriate. *P*-values <0.05 were considered significant. If a pathogen was detected by PCR in either the NPS or NPW specimen, the case was considered to be positive. Virus-specific attributable fraction among the exposed (AFE) was determined by comparing the proportion of PCR positives for a given virus among cases (the exposed group) with that of control children [Kleinbaum et al., 1982]. AFE is defined as $(RR-1)/RR$ for $RR \geq 1$, where RR is calculated as the proportion of hospitalized cases positive for a given organism divided by the proportion of control children positive for that organism. The AFE estimates the proportion of cases positive for a given virus for which that virus is responsible for their illness [Kleinbaum et al., 1982]. To assure comparability between case and control children, the analysis to calculate AFE was restricted to Year 2, when control children were recruited region-wide, and to positive PCR results obtained from the NPS specimen (since only NPS specimens were obtained on control children). To estimate the proportion of all hospitalized cases attributable to a given virus over both study years, the AFE was multiplied by the overall proportion of hospitalized cases positive for that virus by NPS or NPW in both years.

RESULTS

Recruitment and PCR Testing Results

Overall 1,073 children were recruited; including 440 hospitalized children and 633 control children. During the Year 1, 229 (72%) children were recruited from 313 eligible patients hospitalized with LRTI. During Year 2, 211 (60%) children were recruited from 352 eligible hospitalized children. Reasons for non-recruitment included parent refusal, parent not available, and research staff not available. There were no significant differences between recruited and non-recruited children with respect to age, gender, or region (Table I). Among the recruited hospitalized cases 37% were <6 months of age; 64% were <12 months of age (Table I). During Year 1, 67 control children were recruited from the Bethel area; during Year 2, 566 control subjects were recruited throughout YK Delta. Among all control children, 18% were <6 months of age, and 35% were <12 months of age. One hundred thirty-five (22%) of the 633 control children had respiratory symptoms at the time of enrollment, with predominant symptoms: stuffy nose (14%, 89/633) and cough (14%, 90/633). An additional 73 control children had respiratory symptoms in the 2 weeks prior to enrollment. When we excluded these 208 symptomatic control children from the case–control analysis (Table II), the proportion positive for a given virus was the same as the proportion with that virus when symptomatic controls were included in the analysis.

Overall, one or more respiratory viruses were detected by PCR in 90% (395/440) of the hospitalized cases and 52% (221/425) of asymptomatic controls. Rhinovirus was detected in 44% (194/440) of all hospitalized cases, but was a single pathogen in only 87 (20%). Similarly, adenovirus was detected in 30% (131/440) of all hospitalized cases, but was a single pathogen in only 15 (3%; Table II).

During Year 2, RSV, hMPV, PIV, and influenza (referred to as Group 1 viruses) were detected in 55% (115/208) of cases and 15% of asymptomatic controls; while 6% of cases and 1% of control children had multiple Group 1 viruses detected (Table II). Group 1 viruses had statistically significant positive AFEs in cases versus asymptomatic control children, indicating associations with the illness (Table II, Fig. 1). Although control children were older than cases, adjustment for age did not make a difference in the AFE. Based on the AFE, 45.2% of hospitalized cases were attributable to a Group 1 virus (Table II).

By contrast, patients with rhinovirus, adenovirus, and coronavirus (referred to as Group 2 viruses) did not have significantly positive AFEs during Year 2, indicating they were less likely to be the cause of the

TABLE I. Total Number of Children Hospitalized With Acute Respiratory Infection and Number Enrolled in the Hospitalized Cases Recruited, by Demographic Information, Yukon Kuskokwim Delta Region, October 2005–through September 2007

	Children with acute respiratory infection hospitalizations		
Characteristic	Total no. (%)	Not enrolled No. (%)	Enrolled no. (%)
Age group (months)			
0–5	238 (36)	74 (33)	164 (37)
6–11	188 (28)	70 (31)	118 (27)
12–23	182 (27)	57 (25)	125 (28)
24–35	57 (9)	24 (11)	33 (7)
Hub residence			
Bethel	83 (12)	28 (12)	55 (12)
Village	582 (88)	197 (88)	385 (88)
Regional Residence			
Bethel Town	83 (12)	28 (12)	55 (12)
Bethel Region	127 (19)	46 (20)	81 (18)
S. Coastal	117 (18)	41 (18)	76 (17)
N. Coastal	177 (27)	55 (24)	122 (28)
Lower Yukon	84 (13)	36 (16)	48 (11)
Upper River	77 (11)	19 (8)	58 (13)
Sex			
Male	344 (52)	119 (53)	225 (51)
Female	321 (48)	106 (47)	215 (49)
Year/Season			
Year 1/October–May	244 (37)	58 (26)	186 (42)
Year 1/ June–September	69 (10)	26 (12)	43 (10)
Year 2/October–May	281 (42)	109 (48)	172 (39)
Year 2/June–September	71 (11)	32 (14)	39 (9)
Total	665	225 (34)	440 (66)

hospitalized illness (Table II). During Year 2, rhinovirus was detected at the same rate among cases (32%) and control children (32%); adenovirus was detected at a lower rate in cases (9%) than in control children (16%). Coronavirus was infrequently detected in hospitalized and control children (Table II).

Blood culture results are available for 409 (93%) of the hospitalized case children. Twelve cases had positive blood cultures, including 9 *Streptococcus pneumoniae*, 1 *Haemophilus influenzae*, and 2 *Staphylococcus aureus*. Three of these case children, all positive for *S. pneumoniae*, also had viruses detected by PCR—2 with RSV and

TABLE II. Positive PCR Testing Results in Hospitalized Cases and Asymptomatic Control Children by Organism, Yukon Kuskokwim Delta Region, October 2005–September 2007

	Combined years		Year 2, nasopharyngeal swab results			
Organism+	Cases, includes wash+	Control children	Cases	Control children	Attributable fraction in cases Year 2 (95% CI)[a]	Estimated percent of total cases attributable to pathogen (%)
Group 1 viruses						
RSV+	102 (23%)	18 (4%)	35 (17%)	18 (5%)	72% (52, 84)	16.6
hMPV+	66 (15%)	29 (7%)	46 (22%)	27 (7%)	68% (50, 79)	10.2
PIV+	77 (18%)	13 (3%)	37 (18%)	13 (3%)	81% (65, 90)	14.1
Flu+	23 (5%)	3 (1%)	9 (4%)	3 (1%)	82% (34, 95)	4.3
No Group 1 Virus+[b]	202 (46%)	366 (86%)				
Single Group 1 virus+	207 (47%)	55 (13%)				
Multiple Group 1 viruses+	31 (7%)	4 (1%)				
Group 2 viruses						
Rhinovirus+	194 (44%)	140 (33%)	66 (32%)	122 (32%)	−1% (−23, 27)	
Adenovirus+	131 (30%)	68 (16%)	18 (9%)	62 (16%)	−47% (−68, −13)	
Coronavirus+	25 (6%)	15 (4%)	9 (4%)	14 (4%)	15% (−93, 63)	
No virus+	45 (10%)	204 (48%)				
Total cases	440	425	208	381		

[a]Attributable fraction among the exposed is calculated using only results from the nasal swab, and eliminating control children who had symptoms within 2 weeks (n = 135).
[b]Polymerase chain reaction not positive for RSV, hMPV, PIV, or Flu.

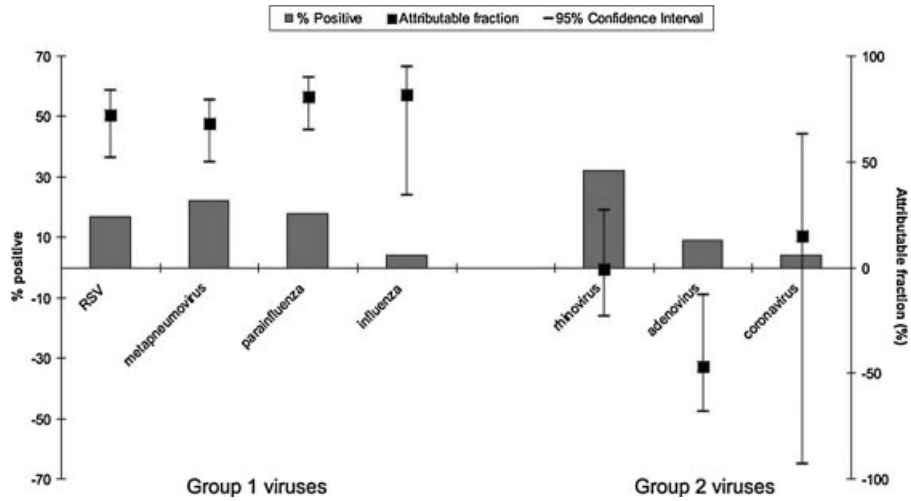

Fig. 1. Percent of cases by virus and estimated percent attributable to virus (attributable fraction among the exposed) with 95% confidence intervals, Year 2, Yukon Kuskokwim Delta region, October 2006–September 2007.

1 with influenza. *B. pertussis* was detected in 7 cases during Year 1.

The mean age of cases was 10.3 months. Cases positive for influenza were significantly older (mean 13.6 months) than other hospitalized cases (mean 10.1 months; $P = 0.049$). Cases positive for rhinovirus were significantly younger (mean 9.4 months) than other hospitalized cases (mean 11.1 months; $P = 0.048$); although cases with RSV had similar mean age (9.5 months).

Seasonality

The seasonality of individual viruses was similar in control children and hospitalized cases. All Group 1 viruses varied significantly in number and percent by month over the study years. During both years, RSV and hMPV demonstrated a seasonal peak in February/March (Fig. 2). RSV and hMPV were the most frequently detected Group 1 viruses during Years 1 and 2, respectively. PIV1 was the only PIV identified during December through June of Year 1, while PIV3 predominated during Year 2 with peaks in September and April. Influenza A was present in January/February and August of Year 1 and March/April of Year 2. Influenza B was present in April/May of Year 2.

Rhinovirus was detected during all study months with the highest proportion among cases in the summer and fall months (Fig. 3). During summer months, rhinovirus was more common in cases than control children, and there was a non-significant trend toward positive AFE (15%). The adenovirus percent positive did not vary significantly by month, and there was no seasonal trend in AFE (Fig. 3). Coronavirus was present in low numbers during fall and winter months of both years.

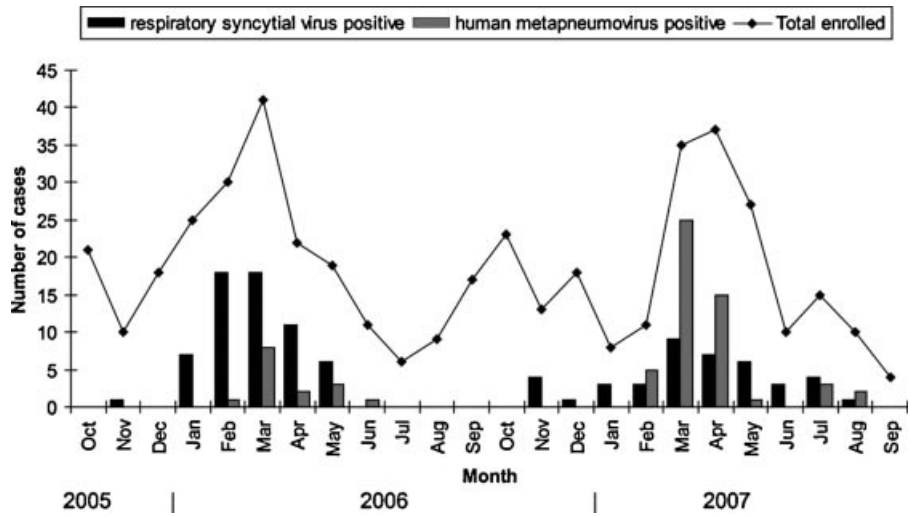

Fig. 2. Hospitalized cases who were positive for respiratory syncytial virus or human metapneumovirus by month, Yukon Kuskokwim Delta region, October 2005–September 2007.

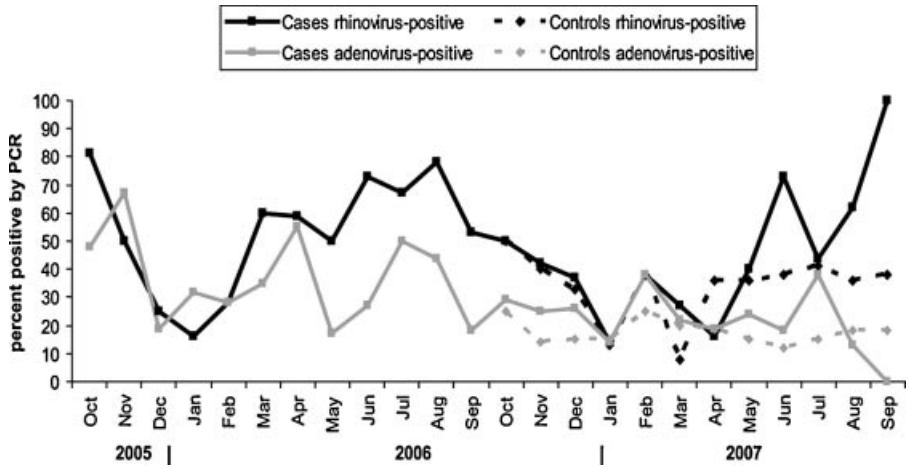

Fig. 3. Proportion of hospitalized cases and control children who were PCR-positive for rhinovirus and adenovirus by month, Yukon Kuskokwim Delta Region, October 2005–September 2007.

Adenovirus 14 Cluster

After an adenovirus serotype14 cluster was detected in southeast Alaska in Fall 2008 [State of Alaska Epidemiology Bulletin, 2009], we tested 249 adenovirus-positive samples from this study and identified 26 as adenovirus 14 (20 cases and 6 controls). Three-fourths (20/26) of the adenovirus 14 in study subjects were identified during a 5-week period in October through December, 2005. During this period, adenovirus 14 was identified in 78% (14/18) of adenovirus-positive cases and in 75% (6/8) of adenovirus-positive control children, but the overall AFE for adenovirus 14 during Year 1 was −20% (95% CI: −45%, 18%) and illness severity was not higher in adenovirus 14 cases than in other adenoviruses.

Clinical Diagnosis Associated With Viruses

Among all hospitalized participants, the most common discharge diagnoses were "pneumonia" with or without bronchiolitis (68%) and "any bronchiolitis" without pneumonia (28%). A higher percent of RSV positive children were diagnosed with bronchiolitis ($P < 0.001$), and a higher percent of hMPV+ ($P = 0.008$) and PIV+ ($P = 0.040$) children were diagnosed with pneumonia compared with cases not positive for a Group 1 viruses (Table III). A higher percent of PIV positive children were diagnosed with croup ($P = 0.012$) than children with no Group 1 virus however, only 6 of 13 cases with croup were positive for PIV (Table III).

Severity of Illness

Hospitalized cases with only RSV or only hMPV detected were more likely to require mechanical ventilation/oxygen ($P < 0.001$) than children with no Group 1 virus or other Group 1 viruses (Table IV). Cases with only RSV were more likely to have length of stay >5 days ($P = 0.018$) and severity index ≥2 ($P = 0.026$) than children with no Group 1 virus. Cases with multiple Group 1 viruses did not have higher severity than other cases. No deaths occurred in children positive for Group 1 viruses.

TABLE III. Single Organism Positive Cases by Clinical Diagnosis Compared With Other Group 1 Single Organism Positive and No Organism Positive, Hospitalized Children, Yukon Kuskokwim Delta Region, Alaska, October 2005–September 2007

		Pneumonia[a]		Bronchiolitis[a]		Croup[a]	
PCR+	N	# (%)	*P*-value[b]	# (%)	*P*-value[b]	# (%)	*P*-value[b]
RSV+	80	56 (70)	0.258	50 (63)	**<0.001**	0 (0)	0.580
hMPV+	51	42 (82)	**0.008**	15 (29)	0.053	1 (2)	1.0
PIV+	61	47 (77)	**0.040**	10 (16)	0.865	6 (10)	**0.012**
Flu+	15	10 (67)	0.769	0 (0)	0.138	1 (7)	0.304
Overall 2 × 4 table[c]			0.365		**<0.001**		**0.025**
No PCR+	202	127 (63)		35 (17)		4 (2)	
Multiple Group 1 viruses+	31	19 (61)		13 (42)		1 (3)	
Total	440	301 (68)		123 (28)		13 (3)	

[a]Cases were diagnosed with pneumonia if pneumonia was included in any of the discharge diagnoses. Cases were diagnosed with bronchiolitis if bronchiolitis was included in any of the discharge diagnoses.
[b]*P*-value = single Group 1 virus+ only versus no Group 1 virus+.
[c]Comparing the above 4 organisms.
Bold text indicates *P*-value <0.05.

TABLE IV. Polymerase Chain Reaction Positives for Group 1 Single Virus-Positive Cases by Severity Indicators Hospitalized Cases, Yukon Kuskokwim Delta Region, Alaska, October 2005–September 2007

		Mechanical ventilation or O_2		Length of stay >5 days		Severity Index ≥2	
	N	N (%)	*P*-value[a]	N (%)	*P*-value[a]	N (%)	*P*-value[a]
RSV+	80	57 (71%)	<**0.001**	32 (40%)	**0.018**	10 (13%)	**0.026**
hMPV+	51	39 (76%)	<**0.001**	19 (37%)	0.102	7 (14%)	0.053
hPIV+	61	33 (54%)	0.073	12 (20%)	0.333	2 (3%)	0.739
Flu+	15	5 (33%)	0.600	4 (29%)	1.0	1 (7%)	0.554
2×4[b]			<**0.001**		0.058		0.195
Multiple Group 1 viruses+ above	31	22 (71%)	0.054	11 (35%)	0.452	1 (3%)	0.713
No pathogen	202	83 (41%)	<**0.001**	52 (26%)	0.107	10 (5%)	0.136
Bacterial culture+	12	5 (42%)	0.395	9 (75%)	**0.001**	3 (25%)	**0.045**
Total	440	238 (54%)		130 (30%)		31 (7%)	

[a]*P*-value = single Group 1 virus+ only versus no Group 1 virus+.
[b]Comparing the above 4 organisms.
Bold text indicates *P*-value <0.05.

DISCUSSION

During 2005–2007, we conducted prospective surveillance for LRTI hospitalizations among Alaska Native children <3 years of age from YK Delta, and compared viral detections from hospitalized cases with those from asymptomatic community control children. Viruses were detected in 90% of hospitalized cases. While rhinovirus and adenovirus were the most frequently detected viruses, their rate of detection was similar in hospitalized cases and control children, so their contribution to hospitalized illness was uncertain. In contrast, RSV, hMPV, PIV, and influenza (Group 1 viruses) were detected in 55% of hospitalized cases, and few community controls, and the estimated percent of hospitalized cases attributable to these viruses was 45.2%.

Sensitive PCR assays make it possible to consistently detect a wide range of respiratory viral pathogens, but a detected virus may be associated with persistence of virus after an earlier illness. This reinforces the importance of a control group [Pierangeli et al., 2007]. Quantifying the viral genome detected may help to link detections with illness [Kuypers et al., 2004].

RSV was the most commonly identified virus associated with LRTI hospitalization similar to other studies (12–60%) [Freymuth et al., 2006; Iwane et al., 2004; Pierangeli et al., 2007; Weigl et al., 2007]. During 1994–2005 there were wide yearly variations in RSV hospitalization rates in YK Delta infants (41.6/1,000/year–245/1,000/year). The 2 years in this study had low RSV hospitalization rates for YK Delta infants as compared to overall annual rate from 2001 to 2004 (54.5 hospitalizations/1,000/year during 2005–2007 compared with 111.7/1,000/year in 2001–2004 [Singleton et al., 2006]. However, even in these low incidence years, the RSV-positive hospitalization rate in YK Delta infants was twofold higher than the estimated RSV hospitalization rate in US infants [Holman et al., 2004]. Although RSV hospitalization rates have decreased in YK Delta children, LRTI hospitalization rates have remained relatively stable, suggesting an increasing role of other pathogens. During study years, peak seasonal RSV activity occurred later and continued for a longer period than in the United States [Centers for Disease Control and Prevention, 2007a; Singleton et al., 2006].

In this study, PIV was the second most common virus associated with LRTI hospitalization and was detected in a larger proportion of hospitalized children (18%) than in other similar LRTI studies (range 0.4–9%) [Iwane et al., 2004; Aberle et al., 2005; Freymuth et al., 2006; Pierangeli et al., 2007; Thomazelli et al., 2007; Weigl et al., 2007]. However, our finding was consistent with 1990–2004 National Respiratory and Enteric Virus Surveillance System data showing PIVs second only to RSV as being detected in children <5 years of age with a respiratory illness in children [Fry et al., 2006].

Human metapneumovirus was detected among a higher proportion (15%) of hospitalized children than in most other studies (3–5%) [Iwane et al., 2004; Freymuth et al., 2006; Pierangeli et al., 2007] except for a study in Brazil (17.8%) [Thomazelli et al., 2007]. hMPV was the most frequently detected Group 1 virus during Year 2. The peak occurrence of hMPV mirrored that of RSV and was similar to that reported in other studies [Van den Hoogen et al., 2003; Mullins et al., 2004; Robinson et al., 2005]. In contrast to some studies, hMPV infection was associated with higher severity of illness in hospitalized children [Klein et al., 2006].

Seasonal influenza was identified in a relatively small proportion (5%) of hospitalized children during both seasons; however, that proportion was similar to that reported in other studies (0.8–8%) [Iwane et al., 2004; Aberle et al., 2005; Freymuth et al., 2006; Pierangeli et al., 2007; Thomazelli et al., 2007; Weigl et al., 2007]. Hospitalized children with influenza were older and less likely to have a bronchiolitis diagnosis than children with other viruses. According to unpublished YKDRH immunization coverage data, approximately half of YK children <3 years of age received influenza vaccine during the study seasons; study numbers were too small to evaluate influenza vaccine protection.

Rhinovirus was commonly detected in both hospitalized (44%) and control (33%) children, and the non-positive AFE indicated a lower likelihood of rhinovirus being the causal pathogen. The clinical significance of a positive rhinovirus PCR result has been questioned [Makela et al., 1998] since rhinovirus is detected in asymptomatic children [Nokso-Koivisto et al., 2002; Freymuth et al., 2006], commonly found as a co-pathogen [Aberle et al., 2005] and has been shown to persist in 50% of cases 2 weeks after an acute infection [Jartti et al., 2004]. However, rhinovirus has been identified as the single virus in some LRTIs [Papadopoulus et al., 2002; Guittet et al., 2003], and in our study there was a trend toward positive association with hospitalization for rhinovirus during summer months.

Adenovirus was also commonly identified but not associated with hospitalized illness. We identified a cluster of infections caused by adenovirus 14, a virus which has emerged as a cause of severe and fatal infection in some settings; however, in our study adenovirus14 was not associated with severe illness [Centers for Disease Control and Prevention, 2010].

Papadopoulos and Aberle reported that co-infections were associated with an increase in disease severity [Papadopoulus et al., 2002; Aberle et al., 2005]; while other studies did not support this finding [van Woensel et al., 2006; Pierangeli et al., 2007]. We did not find an association between dual infections and higher disease severity.

A strength of this study is the simultaneous testing of community control children which allowed us to calculate the AFE of hospitalized LRTI illness for the viruses detected. Although control children were recruited in a systematic fashion to represent the entire region, a recruitment strategy with matching by time and village may have given a better AFE estimate, but was impractical. Control children were older than case children. We did not do frequency matching; however, age adjustment did not make a difference in the AFE supporting the validity of these results. Since this was a consented study with a recruitment rate of 66%, the enrolled cases may not be representative of all cases. Additionally, the 2 years of the surveillance may not be representative of the overall viral epidemiology.

CONCLUSION

In this study, RSV, hMPV, PIV, and influenza were found to account for half of LRTI hospitalizations. RSV and hMPV were associated with higher severity of illness in hospitalized children. Although RSV hospitalization rates have decreased in YK Delta children, LRTI hospitalization rates remain similar to 1994–1997, suggesting a larger role for other viruses. Rhinovirus or adenovirus were detected in over two-thirds of hospitalized children; however, their frequent detection in control children made their role in LRTI hospitalization less clear.

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