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Coronavirus Infection in Acute Lower Respiratory Tract Disease of Infants

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A serologic surveillance of lower respiratory tract disease in 417 hospitalized children under 18 months of age revealed infection with coronaviruses (strains OC43 and/or 229E) in 34 (8.2%). During the same interval, one of 13 control infants was infected. There were two distinct periods lasting six and 14 weeks, respectively, during which the incidence rose to as high as 18.9% of patients with lower respiratory tract disease. The incidence of coronavirus infection in patients with pneumonia and bronchiolitis was higher than the incidences of adenoviruses, influenza, parainfluenza viruses types 1 and 2, and rhinoviruses, and lower only than the incidences of parainfluenza virus type 3 and respiratory syncytial virus. Coronaviruses serologically similar or identical to strain 229E were recovered from frozen nasal washes obtained during the acute phase of pneumonia in two children.

Continuing programs of surveillance during the past decade have provided evidence that viral infection is the major identifiable cause of acute lower respiratory tract disease (LRTD) in children. Nevertheless, in every published study between one-third and two-thirds of serious illnesses cannot be associated with infection by known viruses [1–4]. When the human coronaviruses were first discovered [5–7] and were shown to be of importance in upper respiratory disease in adults [8, 9], it was hoped that they might account for a proportion of these unexplained illnesses. However, a seroepidemiologic study of their role in LRTD showed no statistically significant correlation with illness [8]. Nevertheless, in the youngest group examined (children less than one year old), there was a suggestion that coronaviruses might play a small but definite part in the etiology of bronchiolitis and pneumonia. Because of the great difficulty in cultivation of coronaviruses in vitro, no attempts were made in these studies to corroborate serologic data with recovery of virus. Indeed, to our knowledge, coronaviruses have not previously been isolated from young children.

Because of this paucity of information, and because of the suggestive evidence that infants might be subject to lower respiratory infections with coronaviruses, we undertook an examination of coronavirus infections in children less than 18 months old who were admitted to Cook County Hospital with acute LRTD. Conventional studies of respiratory virus in this group of children have been reported elsewhere [4].

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Materials and Methods

Patients. Children less than 18 months old admitted on Sunday through Friday mornings to Cook County Hospital in Chicago were included in the study. They were housed in a single ward and were tested for shedding of virus within 24 hr of admission. Acute and convalescent sera were obtained from every child.

The diagnostic definitions in this study were

those commonly used by others. Pneumonia required the demonstration by chest X ray of an infiltrate; bronchiolitis was characterized by cough, tachypnea, suprasternal and subcostal retractions, expiratory wheezes, and, often, rales; in laryngo-tracheo-bronchitis there was a harsh "croupy" cough, with stridor; and bronchitis was used to define a lesser disease with cough, rhonchi, and, sometimes, wheezing.

"Control" children with adequate serum samples were few. Such infants were admitted for noninfectious disorders and had not experienced an acute respiratory illness during the previous 10 days.

Virologic methods. Conventional methods for recovery of virus and serodiagnosis have been described [4]. These methods would detect infection by respiratory syncytial virus, parainfluenza viruses types 1, 2, and 3, adenoviruses, influenza virus types A and B, and *Mycoplasma pneumoniae* [10]. In this study, antibodies to three coronavirus serotypes were measured. Human coronavirus type 229E [6] CF antigen was made in L-132 cells [11]. Strain OC43 antigen [7] was produced in brains of suckling mice [12]. Hemagglutination inhibition was measured with chicken erythrocytes as described by Kaye and Dowdle [13]. A multivalent CF antigen containing four serotypes of mouse hepatitis virus (Microbiological Associates, Bethesda, Md.) was included in our tests because of the known antigenic relationship of mouse hepatitis virus to the human coronaviruses [14, 15] and because of its demonstrated capacity to react with antibodies to coronavirus in human sera [16].

We attempted recovery of coronavirus from frozen oropharyngeal swab specimens obtained from selected children during acute illness with serologic evidence of 229E infection. Specimens were thawed and inoculated onto roller-tube monolayers of the human embryonic intestine fibroblast cell line MA-177 (Microbiological Associates). This line previously had exhibited unusual sensitivity in the recovery of 229E-like viruses [17]. Material from the first or second passage was then transferred to and further propagated in WI-38 cells. Unconcentrated sonicates from infected roller-tube cultures were stained with phosphotungstic acid and examined in an RCA EMU-4A electron microscope.

Results

Epidemiology of coronavirus infection. During the study period 417 children with acute LRTD were examined for serologic evidence of coronavirus infection. Only 13 serum pairs from control patients were available during this period, but these had an age distribution proportionally similar to that of the LRTD serum pairs. About 80% of the children were under one year old; more than 90% of the infants were black.

Coronavirus infection was detected serologically by a fourfold or greater rise in antibody in 34 infants (8.2% of all those with acute LRTD) and in one of the 13 control infants. The incidence of infection in relation to age is shown in table 1. There is some evidence that the youngest group shown, those under two months old, were infected less often than older children.

The seasonal incidence of acute LRTD and of coronavirus infection is shown in figure 1. Numbers of hospital admissions for patients with acute LRTD show the expected peaks during the winter months. Coronavirus infections tended to occur in small outbreaks but with little seasonal preference. This finding is in contrast to most other studies, which have shown distinct increases in coronavirus infections in the winter and early spring [8, 18].

Two periods of increased coronavirus incidence occurred (table 2). Both small outbreaks involved infants who had either pneumonia [6] or bronchiolitis [1, 2].

During both these periods infections with coronavirus were more common than those due to other viruses. The first period followed a large epidemic of respiratory syncytial virus, but during the two months of coronavirus activity only 8.9%

Table 1. Relation of age to coronavirus infection.

Clinical category, age (months)	No. with serologic evidence of infection/no. tested (%)
Lower respiratory tract infection	
0–1	2/49 (4.9)
2–6	13/137 (9.5)
7–12	10/143 (7.0)
13–18	9/88 (10.2)
Total	34/417 (8.2)
No respiratory disease (total)	1/13 (7.7)

Table 2. Periods of increased incidence of coronavirus infection.

Period	No. with serologic evidence of coronavirus infection/ no. tested (%)	Prevalent strain	No. with evidence of simultaneous infection with other viruses	
			By isolation	Serologic
Feb. 1967–Mar. 1967	7/37 (18.9)	229E-like	0	0
Oct. 1968–Jan. 1969	8/49 (16.3)	OC43-like	0	3*

* Respiratory syncytial virus and parainfluenza viruses 1 and 3.

of the children were infected with respiratory syncytial virus. During the second period there were cases of respiratory syncytial virus, influenza A, and parainfluenza types 1 and 3, but none of these individual viruses was found in more than 11.6% of infants, whereas 16.3% of the infants had coronavirus infections.

The incidence of coronavirus infection in the four clinical LRTD syndromes is shown in table 3. Coronavirus infection in pneumonia and bronchiolitis (table 4) ranks in incidence higher than adenovirus, parainfluenza viruses types 1 and 2, influenza A, and rhinovirus, and lower than respiratory syncytial virus and parainfluenza type 3.

Clinical aspects of pneumonia and bronchiolitis associated with coronavirus infection. Of the 30 infants with coronavirus infection during bronchiolitis or pneumonia, 13 were simultaneously infected with one or more other viruses. However, the single infections did not differ clinically from the multiple infections. Eighteen children required oxygen or were thought to be in respiratory distress with intercostal retractions. Vomiting occurred in nine infants and otitis media in six. Diarrhea was seen only once. The right lower lobe was the site of most frequent involvement, as shown by radiographic examination. The average length of hospital stay was 4.6 days, with a range of one to 16 days.

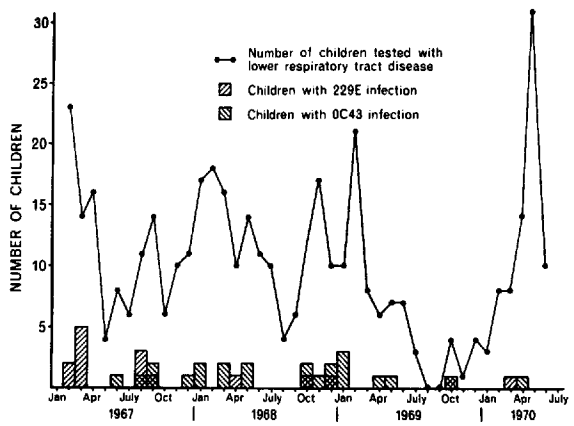

Figure 1. Incidence of coronavirus infection in acute lower respiratory tract disease during the period of study.

Shared serologic activity among the human coronaviruses. The rises in antibody to coronaviruses in paired sera of infants are analyzed in detail in table 5. All such rises were reproduced in replicate testing or were eightfold or greater. Serologic responses were shared between OC43 and mouse hepatitis virus in seven serum pairs. Cross-reactivity between OC43 and 229E was found in five infants. It is of interest that the seven infants included in the first outbreak of coronavirus described in table 2 all showed infection with 229E alone. At other times the character of the rises in antibody to coronavirus was more heterogeneous.

Recovery of coronaviruses from infants with acute LRTD. Frozen oropharyngeal swab specimens from nine infants with serologic evidence of infection with 229E alone were thawed and inoculated onto MA-177 monolayers. Seven specimens were from children involved in the outbreak in February and March 1967. Despite multiple blind passages of these specimens, and despite the appearance of suggestive cytopathic changes at certain passage levels, consistent CPE failed to develop.

Table 3. Relation of clinical category of lower respiratory tract disease in infants to coronavirus infection.

Category	No. with serologic evidence of coronavirus infection/ no. tested (%)
Pneumonia	20/231 (8.7)
Bronchiolitis	10/149 (6.7)
Laryngo-tracheo-bronchitis	2/29 (6.9)
Bronchitis	2/8 (25.0)
Control	1/13 (7.7)

Table 4. Relative incidence of various respiratory virus infections in infants with pneumonia or bronchiolitis.

Virus	No. infants with indicated condition who were positive for virus (%)		
	Pneumonia	Bronchiolitis	Total
Respiratory syncytial	58 (25.1)	48 (32.2)	106 (27.9)
Parainfluenza 3	54 (23.4)	28 (18.8)	82 (21.6)
Coronavirus	20 (8.7)	10 (6.7)	30 (7.9)
Adenovirus	11 (4.8)	15 (10.1)	26 (6.8)
Parainfluenza 1	11 (4.8)	9 (6.0)	20 (5.3)
Influenza A	10 (4.3)	5 (3.4)	15 (4.0)
Rhinovirus	6 (2.6)	2 (1.3)	8 (2.1)
Parainfluenza 2	3 (1.3)	3 (2.0)	6 (1.6)
Total	126 (55.0)	83 (55.7)	209

NOTE. Infants were considered positive if virus was recovered or if there was serologic evidence of infection; 231 infants with pneumonia and 149 infants with bronchiolitis were tested. Some infants were positive for more than one virus.

Specimens from two infants (ages, one and two months) hospitalized with pneumonia on August 11 and August 28, 1967 yielded cytopathic agents on second blind subpassage. The

Table 5. Shared serologic activity of coronavirus in 34 infants with evidence of coronavirus infection.

No. of children in indicated category	Fourfold or greater rise in antibody to coronavirus strains		
	229E	OC43	MHV*
11	+	−	−
14	−	+	−
0	−	−	+
2	+	+	−
4	−	+	+
0	+	−	+
3	+	+	+

* Mouse hepatitis virus.

CPE in WI38 cells was typical of that described for strain 229E [6]. Sonicates of infected cells showed typical particles of coronavirus (figure 2) by electron microscopy. Guinea pig antiserum to strain 229E neutralized both viruses, and guinea pigs antiserum to one of the two strains neutralized both test viruses and strain 229E. The viruses were chloroform-labile and insensitive to the action of 0.05 M iodo-deoxyuridine. Both viruses were successfully isolated again from the original specimens. CF antigen made from one

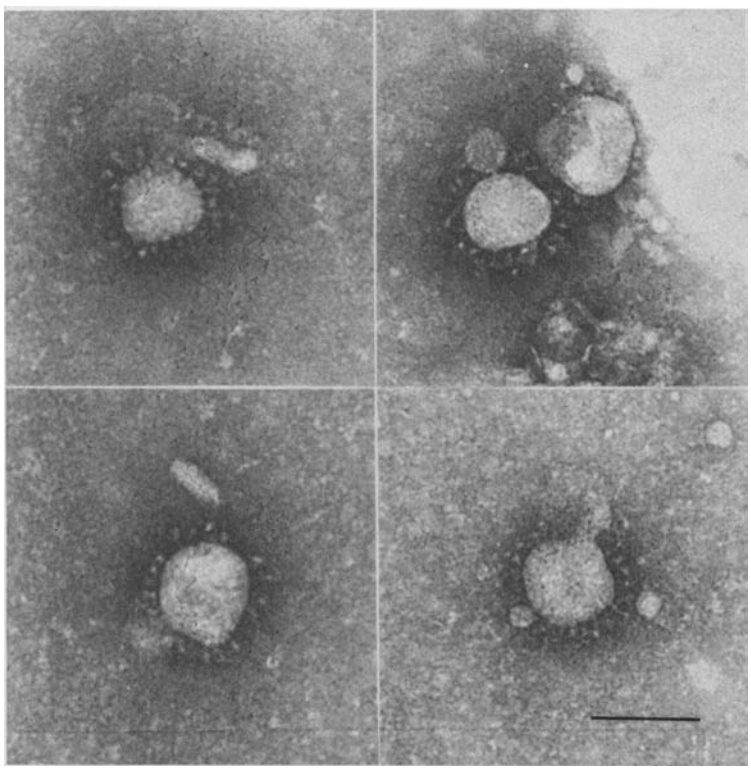

Figure 2. Electron micrographs of characteristic coronavirus particles (strain 1428) recovered from a child with pneumonia (phosphotungstic acid staining, ×129,540). Several particles from the same specimen are shown. Small tail-like protuberances in all four pictures may be artifacts. Bar represents 100 nm.

of the two isolates of virus (strain 1428) detected rises in antibody in nine serum pairs that exhibited rises to strain 229E CF antigen and to no other viral antigens tested. The two homologous serum pairs were included.

Discussion

Coronavirus infections are common in young children hospitalized with pneumonia and bronchiolitis, ranking in frequency only behind respiratory syncytial virus and parainfluenza type 3 infection. This high rate of prevalence clearly does not constitute proof that coronaviruses are causes of bronchiolitis and pneumonia in infants. Indeed, infection might have been incidental to LRTD caused by another agent. In the one previous study that examined this problem [8] infections in control children were common, but the number of such children in the group aged less than 18 months was, as in the present study, too small to be of significance, and the suitability of the "control" group was also in question. In the absence of statistically valid data, therefore, other considerations are of importance.

Two aspects of this study are unique, and provide some argument for the etiologic importance of coronaviruses in LRTD in infants. First, two small outbreaks (i.e., circumscribed periods of increased incidence) of coronavirus infection were defined. In both, coronavirus infections were clearly more common than those due to other respiratory viruses. In the first outbreak, children infected with strain 229E showed no evidence of infection with other respiratory viruses, including other serotypes of human coronavirus. In the second, despite activity of several common respiratory viruses, coronaviruses were predominant.

Second, coronavirus strains were recovered from two children during the acute stage of pneumonia. The strains were antigenically similar or identical to strain 229E, and antibody responses to these strains were demonstrated in the homologous paired sera. These two recoveries of coronavirus are the first reported from infants.

Further studies to identify the role of coronaviruses in infant LRTD seem indicated. At the present time, their role is uncertain. The present study provides data that may implicate coronaviruses in at least a portion of pneumonia and bronchiolitis of young children. The pathogenicity of coronaviruses, which has been demonstrated in adults beyond reasonable doubt by studies in volunteers [19], is more difficult to prove in children. It is likely that as techniques for recovery and recognition of coronavirus are improved, and as other coronavirus serotypes are discovered, infection will be found more frequently, and the etiologic role of coronaviruses in LRTD of children will be better defined.

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