

## SEROEPIDEMIOLOGIC SURVEY OF CORONAVIRUS (STRAIN 229E) INFECTIONS IN A POPULATION OF CHILDREN

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Kaye, H. S. (Respiratory Virology Branch, Center for Disease Control, Atlanta, GA 30333) and W. R. Dowdle. Seroepidemiologic survey of coronavirus (strain 229E) infections in a population of children. *Am J Epidemiol* 101:238-244, 1975.—The indirect hemagglutination (IHA) test for coronavirus 229E antibodies was used for serodiagnostic and seroepidemiologic studies in a population of children. Subjects ranged in age from 5 to 19 years and lived in a home which participated in a longitudinal surveillance of respiratory illness (1960-1968). During this period 1477 respiratory illnesses were observed; 63 (4%) were associated with sero-response (fourfold or greater antibody rises) to 229E. An additional 105 sero-responses were associated with unreported or subclinical illness. The frequency of these infections was cyclical, and 229E and coronavirus OC 43 infections peaked in different years among the same population. Sero-responses occurred mainly in the fall, winter and spring quarters. Preexisting antibody was demonstrated in one-third of the children with 229E sero-responses. Clinical studies indicated that the most frequent complaints with 229E infections were sore throat, coryza and cough, and the most frequent findings were pharyngeal injection, coryza and fever.

**antibodies; coronavirus; hemagglutination tests; respiratory tract infections; serology; sero-response**

### INTRODUCTION

In an 8-year serologic study of coronavirus 229E and OC 43 in working adults, Hendley et al. (1) found that 3 per cent of all colds were serologically related to 229E infection. This figure increased to 5 per cent if only the winter and spring seasons were considered. In a 6-year surveillance of 229E infections in young adults, Hamre and Beem (2) found 15 to 35 per cent of infections occurred during three seasons of

“high” prevalence and 1 to 5 per cent in intervening seasons of “low” prevalence. Infection also occurred in a winter and spring seasonal pattern. Similar infection rates and seasonal distributions have been demonstrated in other adult populations (3-5). Confirmation and extension of these studies to include groups of children have been limited (4, 5).

Assay procedures for 229E antibodies have not been entirely satisfactory. The complement fixation (CF) test has been used for serodiagnosis, but it is not recommended for serosurveys since 229E CF antibodies are relatively short-lived (4). Neutralizing antibodies persist longer, but the examination of large numbers of sera by neutralization (NT) test in cell cultures is tedious (6). In an earlier report (7) we described the development of an indirect hemagglutination (IHA) test for 229E antibodies. The test was shown to be sensitive

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Abbreviations: CF, complement fixation; IHA, indirect hemagglutination; MEM, minimum essential medium; NT, neutralization.

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and specific, and the results closely parallel those obtained in the NT test.

In this report we describe the application of the IHA test for a seroepidemiologic study of the role of 229E in infections occurring in a children's population over an 8-year period. The number and seasonal distribution of illnesses and infections, antibody prevalence, the relation of antibody to protection, and the clinical syndrome were studied.

#### MATERIAL AND METHODS

*Study population.* A longitudinal survey of respiratory illness was conducted from 1960 to 1968 in a church-sponsored children's home in the Atlanta area. This population has been described in detail in previous studies (8). For each year, the total number of children, median age, percentage of turnover, and the number and percentage of children from the original study group are shown in table 1.

*Collection of specimens.* All children with any symptoms of respiratory illness were sent by housemothers to the Home clinic for examination by the attending physician who recorded all signs and symptoms on a standard form. Acute and convalescent blood specimens were collected 2 to 3 weeks apart. Control sera were collected from all children three times a year

during the first 2 years of this study. At least twice yearly collections were made in succeeding years.

*Virus.* The coronavirus strain 229E which had been originally isolated in human embryonic kidney cell culture and then passaged in WI-38 cells was used in this study (9).

*Antigen production.* The preparation of antigen for the IHA test has been described in detail elsewhere (7). Briefly, RU-1 cells (diploid human fetal lung fibroblasts) were grown in 32-oz (960-ml) bottles with Eagle's minimum essential medium (MEM) containing 10 per cent fetal calf serum and aureomycin (25  $\mu$ g/100 ml) at 35 C. When monolayers were complete, two-wash cycles were performed with serum-free Eagle's MEM to reduce calf serum proteins. The cells were then inoculated with 229E, 20 ml of serum-free Eagle's MEM were added, and incubation was continued. When minimal cytopathic effect was detected, all but 2-3 ml of medium was decanted, and the cells were frozen at -70 C. The bottles were frozen and thawed three times, the contents were centrifuged lightly, and the supernatant fluid was collected and stored at -70 C.

*Antisera production.* Immune sera were prepared in New Zealand White rabbits by subcutaneous inoculations of partially pu-

TABLE 1  
*Profile of study population by year*

Year*	Total No.	Median age	Turnover		% new	Children from original study group	
			Entering	Leaving		No.	% of yearly total
1960-1961	175	9		43			100
1961-1962	173	10	41	28	24	114	66
1962-1963	153	10	8	30	5	108	71
1963-1964	147	12	24	49	16	88	60
1964-1965	126	11	28	26	22	65	52
1965-1966	128	11	28	35	22	49	38
1966-1967	121	11	18	19	15	41	35
1967-1968	120	11	8	8	7	34	28
1968-(Dec.)	134	11	14		10	19	14

\* Beginning September 1.

rified 229E virus in Freund's incomplete adjuvant (supplied by Dr. John C. Hierholzer, CDC, Atlanta, GA).

*Serologic methods.* All sera had been previously tested by CF and/or hemagglutination inhibition for diagnostic rises in antibody titer to influenza A and B, parainfluenza types 1, 2 and 3, adenovirus, mumps virus, respiratory syncytial virus, herpesvirus, *Mycoplasma pneumoniae*, and coronavirus OC 43. In this study, sera were tested only for IHA antibody to coronavirus 229E. The CF test was not performed for 229E because of frequency of serum anti-complementary activity which was probably related to length of storage and previous usage.

The IHA test has been previously described (7). The test was performed on microtiter "U" plates. A 0.05 ml volume of 1:150 dilution of normal horse serum in phosphate-buffered saline, pH 7.2, was used as the diluent. Each test serum was titrated in 0.05-ml volumes in twofold dilutions beginning with a 1:10 dilution. Controls consisted of 1) the first four dilutions of each serum plus tannic acid-treated unsensitized erythrocytes, 2) sensitized erythrocytes plus diluent, and 3) 229E rabbit antiserum. The plates were shaken and permitted to stand for 3 to 4 hours at room temperature before erythrocyte-setting patterns were read. A fourfold or greater increase in antibody titer was considered to be indicative of infection. All sera were inactivated at 56 C for 30 min.

RESULTS

*Incidence of respiratory illness and sero-response to coronavirus.* Over the 8-year period 1477 respiratory illnesses were reported; 229E IHA sero-responses were demonstrated for 63. (None of the 63 illnesses could be related by isolation and/or seroresponse to any of the other respiratory disease agents included in previous studies.) An additional 105 sero-responses

were detected by fourfold or greater increases in antibody titer between control bleedings. The total number of sero-responses was 168 (table 2). A total of 112 was recorded for the years 1961-1962, 1965-1966, and September through December 1968. Thirty-one of the 112 were associated with reported illnesses. The remainder (81) represented either unreported disease or subclinical infections. Fifty-six sero-responses were found throughout the other 5 years of the study. Of these, 32 occurred among children who reported respiratory illness. Included in the 168 sero-responses were 20 individuals who had two sero-responses during the 8-year surveillance period.

*Seasonal distribution.* One hundred and ten sero-responses to 229E occurred during three outbreaks in the fall, winter, and spring quarters of the years 1961-1962 and 1965-1966 and from September to December 1968 (figure 1). Forty-eight sero-

TABLE 2  
Yearly incidence of respiratory illness and sero-response\* by IHA test to coronavirus strain 229E

Year†	Total respiratory illness	Sero-responses		Total
		Reported illness‡	No reported illness§	
1960-1961	291	6 (2)	4	10
1961-1962	238	19 (7)	46	65
1962-1963	116	0 (0)	1	1
1963-1964	138	4 (3)	2	6
1964-1965	175	6 (3)	6	12
1965-1966	160	7 (4)	18	25
1966-1967	170	9 (5)	4	13
1967-1968	155	7 (5)	7	14
1968-(Dec.)	34	5 (15)	17	22
Total	1477	63 (4)	105	168*

\* Fourfold or greater increase in IHA titer.

† Beginning September 1.

‡ Paired acute and convalescent sera. Percentages in parentheses.

§ Detected by rises in serum antibody titer between normal bleedings.

\* Twenty patients had 2 sero-responses during the period of study.

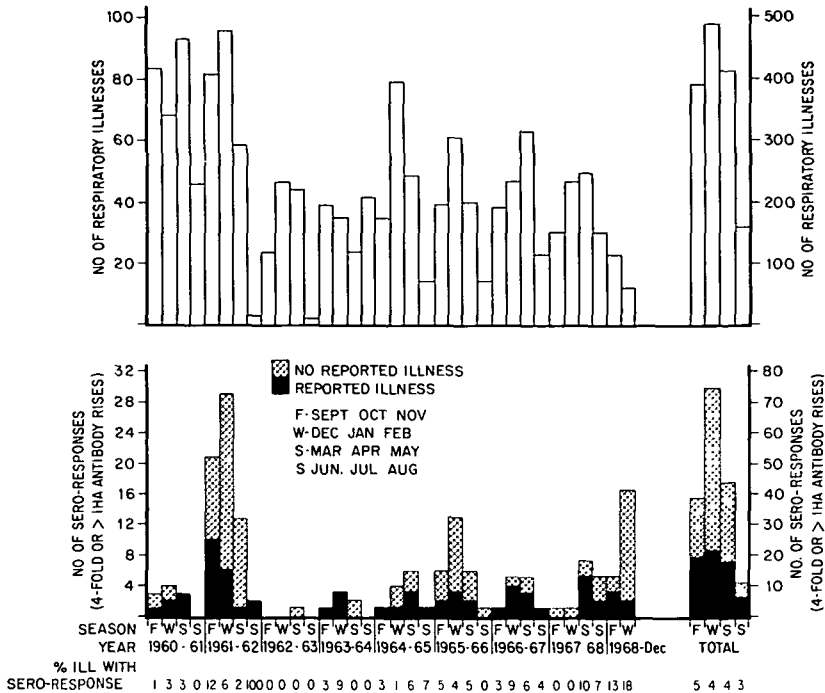


FIGURE 1. Seasonal distribution of sero-responses to coronavirus strain 229E and association with respiratory illness.

responses were found in the fall-winter-spring quarters during years other than those previously mentioned. Only 10 sero-responses were found in the summer quarter during the entire study, but 6 of these were in children who reported illness. The 229E sero-responses which could be associated with illness accounted for 5 per cent of the total illnesses seen in the fall quarter, 4 per cent in the winter and spring quarters and 3 per cent in the summer quarter. Although the largest number of sero-responses occurred during the three outbreaks mentioned above, percentages for other years are worth noting. Strain 299E accounted for 9 per cent of the total respiratory illness in the winter quarters of 1963-1964 and 1966-1967, and for 10 per cent in the spring quarter of 1967-1968.

**Prevalence of antibody.** The prevalence of 229E IHA antibody in the total population rose from 48 per cent in 1960-1961 to a high of 69 per cent in 1961-1962 and had fallen to 42 per cent in 1968 when the study

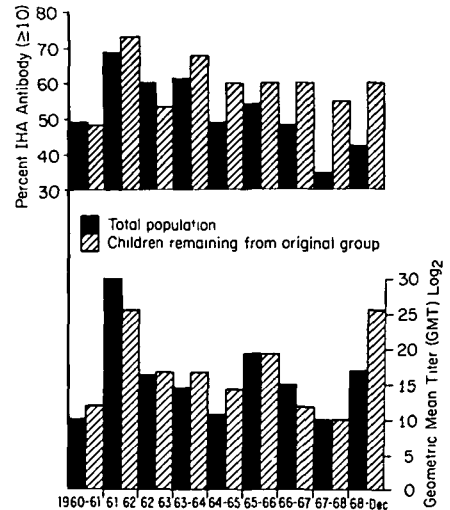


FIGURE 2. Prevalence and geometric mean titer of IHA antibody to coronavirus strain 229E in a children's population.

was terminated (figure 2). The geometric mean antibody titer rose from 10 to 30 and fell to 17 over the same period. Of the 14 children who remained in the study during

the entire 8 years, the percentage with antibody increased from 47 per cent in 1960-1961 to 75 per cent in 1961-1962 and had declined to 60 per cent by the winter of 1968. The geometric mean titer rose from 12 to 26 from 1960-1961 to 1961-1962, decreased to a low of 10 per cent in 1967-1968, and again increased to 26 when the study was terminated. Antibody in 5 of the 14 persisted throughout the study.

*Evidence of preexisting antibody.* Of 168 persons with sero-response in the population, 59 (35 per cent) had preexisting antibody titers of 10 or greater in two consecutive serum specimens collected before the rise in antibody titer (table 3). Pre-existing antibody was less frequent in the younger age group (5-9) than in the older

age group (15-19). Almost a third of the 10- to 14-year-old group, which had the highest number of sero-responses, had preexisting antibody. Thirteen of the 59 children with sero-responses to 229E had preexisting antibody titers of 20 or greater.

*Clinical syndrome.* Clinical histories were available for 61 of the 63 children with reported respiratory illnesses (table 4). The major presenting complaints were sore throat (66 per cent), coryza (52 per cent), cough (43 per cent), and fever (21 per cent). The predominant physical findings were pharyngeal injection (82 per cent), coryza (64 per cent), fever  $\geq 37.6 < 39$  C (34 per cent), fever  $\geq 39$  C (8 per cent), and cervical adenitis (30 per cent). No statistically significant differences were found in the

TABLE 3  
*Preexisting IHA antibody\* in children with sero-responses† to coronavirus 229E*

Age group	Reported illness			No reported illness			Totals		
	Sero-responses	Preexisting antibody		Sero-responses	Preexisting antibody		Sero-responses	Preexisting antibody	
		No.	%		No.	%		No.	%
5-9	17	6	35	29	7	24	46	13	28
10-14	35	17	49	55	15	27	90	32	36
15-19	11	5	45	21	9	43	32	14	44
Totals	63	28	44	105	31	30	168	59‡	35

\*  $\geq 10$  in two consecutive serum specimens prior to sero-response.  
† Fourfold or greater increase in IHA titer.  
‡ Thirteen had titers of 20 or greater.

TABLE 4  
*Clinical aspects of respiratory illness\* in 61 children with (W) or without (WO) preexisting antibody† to coronavirus strain 229E*

Presenting complaints	Total	%	Preexisting antibody				Physical findings	Total	%	Preexisting antibody			
			W (27)‡		WO (34)					W (27)		WO (34)	
			No.	%	No.	%				No.	%	No.	%
Sore throat	40	66	18	64	22	65	Pharyngeal injection	50	82	23	85	27	79
Coryza	32	52	12	44	20	59	Coryza	39	64	17	63	22	65
Cough	26	43	10	37	16	47	Fever ≥ 37.6 < 39 C	21	34	10	37	11	32
Fever	13	21	4	15	9	26	Fever ≥ 39 C	5	8	1	4	4	12
Headache	9	15	3	11	6	18	Cervical adenitis	18	30	8	30	10	29

\* Fourfold or greater rises in IHA titer between paired acute and convalescent sera.  
†  $\geq 10$  in two consecutive serum specimens prior to sero-response.  
‡ No. of children in parentheses.

clinical aspects of respiratory illness in children with or without preexisting antibody to 229E.

### DISCUSSION

The development of the IHA test for 229E enabled us to conduct a seroepidemiologic study with a population previously investigated by the hemagglutination inhibition technique for evidence of coronavirus OC 43 infections (10).

IHA antibody sero-responses to coronavirus 229E accounted for 63 (4 per cent) of the 1477 respiratory illnesses reported. Twenty-nine of the 63 reported illnesses were found in three distinct outbreaks occurring in the fall, winter, and spring quarters of the years 1961-1962, 1965-1966 and the fall and winter of 1968. Another 80 sero-responses were found in children who were asymptomatic or did not report illness during these same periods. In this study, unlike previous studies, a large number of sero-responses were found during the fall quarters. In the fall of the year 1961-1962, for example, diagnostic 229E antibody rises were detected in 12 per cent of all the reported illnesses.

The nature of the 229E outbreaks was cyclical and closely resembled that of the OC 43. Outbreaks of OC 43 were found in our previous study in the years 1960-1961, 1964-1965, and 1966-1967. Major outbreaks of 229E occurred during the year 1961-1962, 1965-1966, and 1968. Low numbers of 229E sero-responses were also found during periods of high OC 43 prevalence. This has been observed by others as well (1, 5).

In our study, the combined total of 229E (4 per cent) and OC 43 (3 per cent) infections accounted for 7 per cent of all illness reported over an 8-year period. This was higher than the 4 per cent found by Hendley et al. (1) over a comparable length of time. However, they found that OC 43 and 229E accounted for 8 per cent of all respiratory illness, if seasons other than

spring and winter were excluded. The comparative ages of the populations under study could also have accounted for these differences in findings.

The prevalence of 229E IHA antibody in the total population stayed relatively constant over the 8-year period. Previous studies have demonstrated the persistence of NT antibody to 229E, and a close relationship has been noted between IHA and NT antibody for 229E (7) or for other animal coronaviruses (11). Despite the presence of such antibody, two infections with 229E occurred in 20 individuals during our study. Reinfection with coronaviruses had also been noted in previous studies (1, 10, 12).

Previous studies of experimental and natural infections with human and animal coronaviruses have revealed heterologous antibody responses (13-18). In our earlier study (7) of antibodies to 229E and OC 43 in human sera, simultaneous sero-responses were not found among 103 of 104 paired sera with a diagnostic antibody rise to either virus, despite the fact that over 30 per cent of these pairs had preexisting antibody to the other virus. Hendley et al. (1) also reported that antibody increases to either of these two coronaviruses were not accompanied by an increase in antibody to the other. Our observations apply only to 229E and OC 43. Heterotypic antibody rises caused by unknown or uncharacterized strains cannot be excluded. Our findings, as well as those of other investigators, must be interpreted with caution, particularly in view of the absence of isolation of etiologic agents responsible for the illness, the lack of comparable serologic methods for assaying 229E and OC 43 antibodies, and the present knowledge of the serologic interrelationships of other possible human and animal coronaviruses.

In previous clinical studies reported by Kapikian et al. (3), the chief complaint of patients shedding 229E-like virus was coryza or nasal congestion followed by

sneezing, sore throat, headache, cough, muscular aches, chills and fever in that order. Hendley et al. (1) found that illness due to 229E in adults was characterized mainly by nasal symptoms with little or no fever. They also found that illnesses due to OC 43 resembled colds caused by rhinoviruses and were characterized by pharyngitis, cough and nasal congestion. Our findings, based on patients with sero-response to 229E, indicate a close parallel in regard to nasal involvement. Our attending physicians saw more fever than that described by Hendley et al. (1), although the frequency of fever with 229E was less than that described in our previous study with OC 43. The presence of preexisting antibody seemed to have no significant ameliorating effect on respiratory illness caused by 229E.

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