AMERICAN JOURNAL OF EPIDEMIOLOGY
Vol. 94, No 1

Printed in U.S.A

SEROEPIDEMIOLOGIC SURVEY OF CORONAVIRUS (STRAIN OC 43) RELATED INFECTIONS IN A CHILDREN'S POPULATION

H. S. KAYE[1], H. B. MARSH[1, 2], AND W. R. DOWDLE[1]

(Received for publication December 14, 1970)

Kaye, H. S. (CDC, Atlanta, Ga. 30333), H. B. Marsh and W. R. Dowdle. Seroepidemiologic survey of coronavirus (strain OC 43) related infections in a children's population. *Amer J Epid* 94: 43–49, 1971.—Acute and convalescent serum pairs and control sera collected from subjects living in a children's home over a 7-year period (1960–1967) were examined by hemagglutination-inhibition (HI) test with coronavirus strain OC 43. Ninety-three serologic conversions were observed; 44 were associated with reported illnesses and 49 with no reported illnesses. In three distinct outbreaks during the winter and spring quarter of 1960–1961, 1964–1965, and 1966–1967, 67 conversions occurred. Seroconversions to strain OC 43 were associated with as much as 19% of the respiratory diseases in a single season. Over the 7-year period coronavirus strain OC 43 accounted for 3% of the total 1328 respiratory illnesses. Evidence of preexisting antibody was apparent in one-third of the children showing seroconversions. The HI test was more sensitive for serodiagnosis than the complement-fixation test. The major presenting complaints of the children with respiratory disease associated with coronavirus strain OC 43 were sore throat, cough and coryza; the predominating symptoms were pharyngitis, coryza, fever and cervical adenitis.

antibodies; complement-fixation test; epidemiology; hemagglutination-inhibition test; respiratory illness; seroconversion; serology; viruses

INTRODUCTION

The newly identified coronavirus group, which includes the prototype strains B814 (1), 229E (2), and "IBV-like" viruses (3), may be emerging as an important cause of common cold-like respiratory illness in man. However, clinical and epidemiologic studies have been limited because of the fastidious growth requirement of certain of these viruses (1, 3).

The adaptation to growth in suckling mouse brain of "IBV-like" strains OC 38 and OC 43, previously grown only in organ culture, resulted in the recognition of complement-fixing (CF) antigens (4) and led to the demonstration of hemagglutinins for these apparently serologically identical strains (5, 6). The hemagglutination-inhibition (HI) test as well as the CF test are now available for serodiagnosis and seroepidemiologic surveys.

This present study was designed to evaluate the role of coronavirus strain OC 43 in a children's population by performing HI tests on acute and convalescent serum pairs and control sera collected over a seven-year

Abbreviations: CF, complement-fixation; GMT, geometric mean HI antibody titer; HI, hemagglutination-inhibition; MHV, mouse hepatitis virus.

[1] From the Respiratory Virology Unit, Laboratory Division, Center for Disease Control, Health Services and Mental Health Administration, Public Health Service, U. S. Department of Health, Education and Welfare, Atlanta, Georgia 30333. Reprints requests to Mr. Kaye.

[2] Present address: Peter Bent Brigham Hospital, Boston, Massachusetts 02115.

The authors thank Dr. Gerson H. Aronovitz, Dr. James D. Lucas, Dr. John A. Stewart and Dr. Steven R. Mostow for their cooperation, and Deborah C. Eltzroth and Avis L. Sisson for their technical assistance.

TABLE 1

Analysis of study population by year

Year*	Total no. children	Median age	Turnover			Children in original study group	
			Entering	Leaving	% new	No.	%
		yrs	*No.*	*No.*			
1960-61	175	9	—	43	—	—	100
1961-62	173	10	41	28	24	114	66
1962-63	153	10	8	30	5	108	71
1963-64	147	12	24	49	16	88	60
1964-65	126	11	28	26	22	65	52
1965-66	128	11	28	35	22	49	38
1966-67	121	11	18	19	15	41	35
1967-(Dec.)	120	11	8	—	7	34	28

* Beginning September 1.

period. The number of illnesses and infections, seasonal distribution, antibody prevalence, relationship of antibody to protection, and clinical syndrome have been determined, and the results are presented.

MATERIALS 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 was described in detail previously (7). The study group consisted of healthy Caucasian children ranging in age from five to 19 years who were admitted to the home for socioeconomic reasons. The children lived in cottages which housed from eight to 12 each. Assignment to a cottage was made on the basis of age and sex. Meals were eaten in a common dining room, recreational facilities were shared and the children attended public schools in the community. A study year covered the period between September 1 and August 31. The total number of children, median age, percentage of turnover and the percentage of children from the original study group per year are shown in table 1.

Collection of specimens. Children with symptoms of respiratory illness were sent by house mothers to the home clinic for examination and treatment by the attending physician. Throat swab specimens were collected for bacterial and viral isolation. Techniques for isolation of coronavirus were not available at the time of the study. Acute and convalescent blood specimens were collected two to three weeks apart. Control sera were collected three times per year during the first two years of this study. Less frequent collections were made in succeeding years.

Virus. The coronavirus strain OC 43, which had been isolated in organ culture and subsequently adapted to the suckling mouse brain, was used for antigen production (4).

Production of antigen. Antigens were prepared from infected and normal suckling mouse brain by making a 10 per cent suspension in phosphate-buffered saline, pH 7.2 (HI), and Veronal-buffered diluent, pH 7.3 (CF). They then were clarified by refrigerated centrifugation at 600 × *g* for 20 minutes (5). The antigens were stored at −70 C.

Production of antisera. Immune sera were prepared by intraperitoneal and intracerebral inoculation of adult Swiss white (ICR) mice (5).

Serologic tests. During the seven-year study all acute, convalescent and control sera were tested by CF for diagnostic rises in antibody titer to influenza A and B, parainfluenza types 1, 2, and 3, adenovirus, mumps virus, respiratory syncytial virus, herpesvirus and *Mycoplasma pneumoniae*. In this present study sera were tested only for antibody to coronavirus OC 43 antigens. HI tests were performed by the microtiter technique with phosphate-buffered saline diluent and 0.5 per cent adult chicken erythrocytes (8). The CF test in the present study was also performed by microtiter (9). All sera were inactivated at 56 C for 30 minutes. A fourfold or greater increase in antibody titer was considered to constitute a seroconversion and to be indicative of infection.

TABLE 2

Yearly incidence of respiratory illness and seroconversion to coronavirus strain OC 43

Year*	Total respiratory illness	Coronavirus seroconversions†		
		Reported illness‡	No reported illness§	Total
1960–61	291	19 (7)	12	31
1961–62	238	2 (0.8)	1	3
1962–63	116	0 (0)	2	2
1963–64	138	3 (2)	2	5
1964–65	175	9 (5)	14	23
1965–66	160	0 (0)	6	6
1966–67	170	9 (5)	12	21
1967–(Dec.)	40	2 (5)	0	2
Total	1328	44 (3)	49	93¶

* Fourfold or greater.

† Beginning September 1.

‡ Paired acute and convalescent sera. Percentages in parentheses.

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

¶ Three cases of apparent reinfection.

RESULTS

Incidence of respiratory illness and seroconversion to coronavirus. Among 1328 respiratory illnesses which occurred over the seven-year period (table 2), 44 seroconversions to coronavirus strain OC 43 were found. An additional 49 seroconversions were detected between normal bleedings, making a total of 93.

Seroconversions to strain OC 43 were observed each year of the study, but the largest number totalling 75 was recorded for the years 1960–1961, 1964–1965, and 1966–1967. About one-half of the 75 were associated with reported illnesses. Eighteen seroconversions were scattered throughout the remaining four years of the study, although only seven of these occurred among children reporting respiratory disease.

Sixteen other subjects with antibody titer of <1:10 in two consecutive serum specimens later acquired antibody titers of 1:10

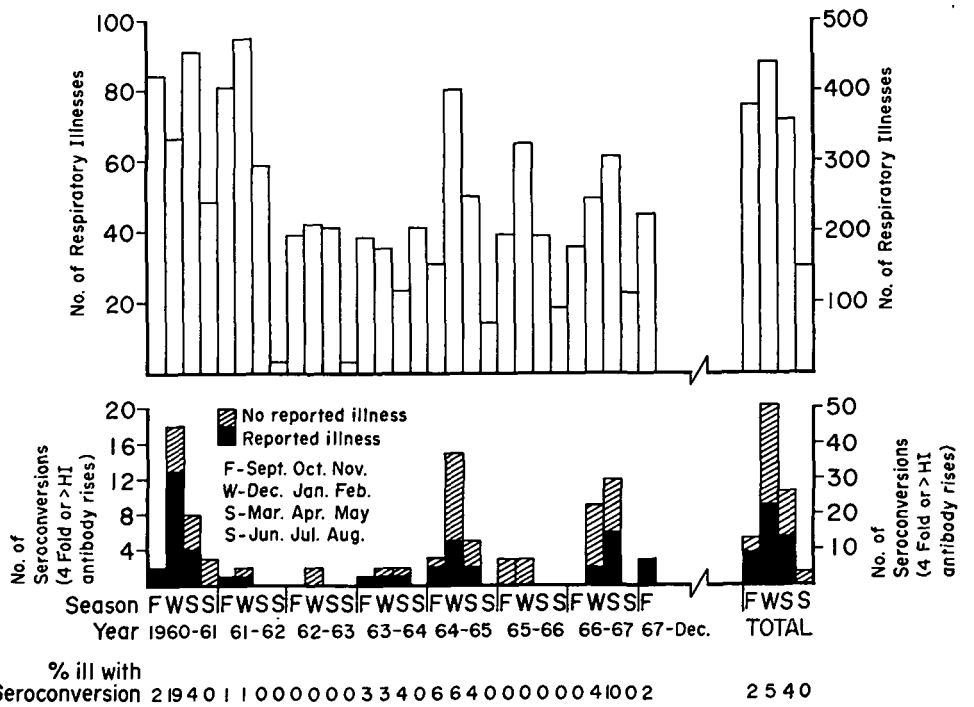

FIGURE 1. Seasonal distribution of seroconversions to coronavirus strain OC 43 and association with respiratory illness.

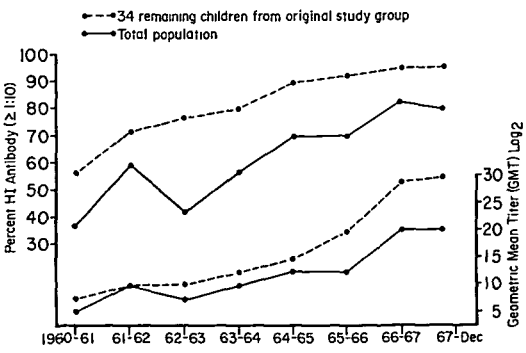

FIGURE 2. Prevalence of HI antibody to coronavirus strain OC 43 in a children's population.

TABLE 3

Evidence of preexisting HI antibody in children with seroconversions to coronavirus strain OC 43*

Age group	Reported illness			No reported illness			Totals		
	Seroconversions†	Preexisting antibody*		Seroconversions†	Preexisting antibody*		Seroconversions†	Preexisting antibody*	
		No.	%		No.	%		No.	%
yrs									
5-9	19	5	26	15	1	7	34	6	18
10-14	16	9	56	21	8	38	37	17	46
15-18	9	2	22	13	6	46	22	8	36
Total	44	16	36	49	15	31	93	31‡	33

* ≥1:10 in two consecutive serum specimens prior to seroconversion.

† Fourfold or greater.

‡ Seven had titers of 1:20 or greater.

TABLE 4

Comparison of HI and CF tests in the serodiagnosis of coronavirus (strain OC 43) related infections in children

Serology	Seroconversions*		GMT†
	Total No.	%	
HI and CF	44	100	
HI	44	100	<10:56
CF	28	64	<8:19
HI only	16	36	
CF only	0	0	

* Fourfold or greater.

† Reciprocal geometric mean titer.

in at least two consecutive serum specimens. Because these subjects had less than fourfold rises in antibody titers, they were not included in the total seroconversions.

Seasonal distribution. In three distinct outbreaks which occurred during the winter and spring quarters of 1960–1961, 1964–1965, and 1966–1967 (figure 1), 67 seroconversions to OC 43 were found. Eleven seroconversions were found in the winter and spring quarters of other years, 12 were detected in the fall quarter and three were found in the summer quarter. Illness associated with OC 43 accounted for 5 per cent of the reported respiratory disease in the winter quarter and 4 per cent in the spring, compared with 2 per cent in the fall and none in the summer. The figures for certain years were much higher. OC 43 accounted for 19 per cent of the respiratory disease reported in the winter quarter of 1960–1961 and 10 per cent reported in the spring quarter of 1966–1967.

Prevalence of antibody. HI antibody in the total populations ranged from 36 per cent in 1960–1961 to 79 per cent by the fall of 1967 (figure 2). The geometric mean antibody titer (GMT) correspondingly rose from 1:5 to 1:20 during the same period. Sera collected over the entire seven-year period were available on 34 children. The percentage with antibody increased from 56 per cent in 1960–1961 to 94 per cent in the fall of 1967. The GMT rose from 1:10 to 1:29 during the same period. All 19 of the 34 subjects with antibody in 1960–1961 continued to have measurable antibody throughout the study. The increase of antibody prevalence from 56 to 94 per cent reflects the number (13) of children with seroconversions during the seven years.

Evidence of preexisting antibody. Of the total number of the children with seroconversions (93), 33% had preexisting antibody titers of 1:10 or greater in at least two sequential serum specimens collected prior to antibody rise (table 3). The frequency of preexisting antibody among subjects with seroconversions was essentially the same for

groups reporting or not reporting illness. Only seven of the 31 cases with preexisting antibody had titers of 1:20 or greater.

Comparison of HI and CF tests. HI and CF test results were compared on 44 paired sera from children showing seroconversion to coronavirus strain OC 43 (table 4). Of 44 paired sera, 28 (64 per cent) showed fourfold or greater rises by CF compared with 100 per cent by HI. Thus, 16 cases of illness (36 per cent) would have gone undetected if only the CF test had been used. The reciprocal GMT for acute and convalescent sera for the HI test was <10 and 56 as compared with <8 and 19 for the CF test.

Clinical syndrome. Complete clinical histories were available for 43 of 44 children. The major presenting complaints of the 43 children with respiratory illness and HI seroconversion to strain OC 43 were sore throat (30 per cent), cough (30 per cent), coryza (19 per cent), and fever (9 per cent) (table 5). The predominant physical findings were pharyngeal injection (72 per cent), coryza (49 per cent), fever of 99.6 F and above (40 per cent), fever of 102.2 F and above (21 per cent), and cervical adenitis (35 per cent).

TABLE 5

Clinical aspects of respiratory illness in 43 children with seroconversions to coronavirus strain OC 43*

Presenting complaints			Physical findings		
	No.	%		*No.*	%
Sore throat	13	30	Pharyngeal injection	31	72
Cough	13	30	Coryza	21	49
Coryza	8	19	Fever 99.6 F and above	17	40
Fever	4	9	Fever 102 2 F and above	9	21
Other	5	11	Cervical adenitis	15	35
			Pulmonary rales or dullness	2	5
			Rash	1	2

* Paired acute and convalescent sera showing fourfold or greater HI antibody rises.

DISCUSSION

McIntosh et al. (4) tested paired sera from 59 adults with upper respiratory illness during the winter of 1965–1966; 18 (31 per cent) of the 59 developed a fourfold or greater rise in CF antibody to coronavirus strains OC 38 and OC 43. During this period of coronavirus prevalence, infection with other viruses causing upper respiratory illness was rare.

In our present study HI antibody seroconversions to coronavirus strain OC 43 accounted for 44 (3 per cent) of the 1328 respiratory illnesses reported. Thirty-seven of these seroconversions were found in three distinct outbreaks which occurred in the winter and spring quarters of the years 1960–1961, 1964–1965, and 1966–1967. The highest incidence of seroconversions without reported illness also occurred during these same periods. Although the average incidence of illness associated with coronavirus strain OC 43 was 5 per cent for all winter seasons, 19 per cent of respiratory illnesses occurring in the winter of 1960–1961 showed diagnostic coronavirus antibody rises. These findings suggest that a significant percentage of respiratory illness may be due to infection with this virus. Like McIntosh and associates, we also found that the highest number of seroconversions to OC 43 occurred at a time (1960–1961) when respiratory diseases associated with other etiologic agents were absent.

HI antibody prevalence in our children's population rose almost continually from 1960–1961 to the termination of the study in December 1967. The level of antibody in the home was slightly higher than has been previously reported for other populations. McIntosh and coworkers (10) found that 47 per cent of the sera collected in 1965–1967 from children between the ages of five and seven years had CF antibody titers of 1:4 or greater against OC 38 and OC 43. The measurable CF antibody in adults during 1962–1964 and 1965–1967 was 72 and 67 per cent, respectively. Unreported results from HI tests performed in our laboratory on sera collected from the general population in 1963–1965 also confirm these general findings. The high level of antibody prevailing in the children's home population at the termination of the survey may be attributed to

the increased median age of the population from age nine in 1960 to age 11 in 1967, and the outbreaks occurring in the latter part of the study. The increased prevalence of antibody among the 34 children remaining in the study over the entire seven-year period reflects both the long-term persistence of HI antibody and the number of seroconversions over the same period of time.

The significance of preexisting antibody to OC 43 is not clear. Approximately one-half of the older age groups demonstrating seroconversions were found to have preexisting antibody, and diagnostic antibody rises were recorded on two separate occasions for three subjects. These findings suggest that either protection against reinfection is short-lived or that such antibody reflects previous infection with a closely related strain. Other studies have also noted the heterogeneity of antibody responses with known or presumed coronavirus infection. McIntosh et al. (6) found that OC 38 and OC 43 were clearly related to the mouse hepatitis virus (MHV) group by CF and neutralization tests. Other types isolated from organ culture had an indefinite relationship with MHV and OC 38 and OC 43 strains. Kaye and Dowdle (5) have also shown a relationship between OC 38 and/or OC 43 strains and MHV by CF and HI tests. Antibodies to MHV in human sera from patients with respiratory illness have been demonstrated by Hartley et al. (11). McIntosh et al. (10), in a seroepidemiologic study of coronavirus infection in children, found a moderate overlap of antibody response (35–45 per cent) occurring between MHV and OC 38 and/or OC 43. Bradburne (12) reports that fourfold or greater HI antibody rises to OC 43 were found among 14 per cent of 70 paired sera obtained from volunteers before and after infection with coronaviruses other than OC 43. Extension of these observations of heterotypic rises to yet unknown strains cannot be excluded. The absence of data on isolation of coronaviruses during our study places severe restrictions on assigning OC 43 as the sole etiologic agent. Regardless of the origin of preexisting coronavirus antibody, our finding that only seven of the 31 cases with preexisting antibody had titers of 1:20 or above suggests that reinfection is limited largely to those subjects with low levels of antibody.

Because of the limited quantity of sera, CF as well as HI tests were not performed on all specimens. However, the small number of CF tests performed on sera showing HI seroconversion confirm a previous report by Kaye and Dowdle (5) that CF is less sensitive for diagnosis. The possibility that improved antigens could increase the sensitivity of the CF test should not be discounted.

The sparse amount of information available on the clinical aspects of infection with OC 38 and OC 43 does not permit a comparison of our data with those of others. Our findings, based only on patients with seroconversion to coronavirus strain OC 43, indicate a close parallel with B814 and 229E (1, 13, 14), particularly in regard to nasal involvement. However, our attending physicians saw considerably more fever than has been noted for other members of the human coronavirus group. This discrepancy may be explained by the age differences in the population under study or by the conditions of infection. Because of the absence of virus isolation, further studies are necessary to confirm the signs and symptoms of this disease under natural conditions.

References

1. Tyrrell DAJ, Bynoe ML: Cultivation of a novel type of common-cold virus in organ cultures. Brit Med J 1:1467–1470, 1965
2. Hamre D, Proknow JJ: A new virus isolated from the human respiratory tract. Proc Soc Exp Biol Med 121:190–193, 1966
3. McIntosh K, Dees JH, Becker WB, Kapikian AZ, Chanock RM: Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. Proc Nat Acad Sci USA 57:933–940, 1967
4. McIntosh K. Becker WB, Chanock RM:

Growth in suckling-mouse brain of "IBV-like" viruses from patients with upper respiratory tract disease. Proc Nat Acad Sci USA 58:2268–2273, 1967

5. Kaye HS, Dowdle WR: Some characteristics of hemagglutination of certain strains of "IBV-like" virus. J Infect Disease 119:282–290, 1969
6. McIntosh K, Kapikian AZ, Hardison KH, Chanock RM: Antigenic relationships among the coronaviruses of man and between human and animal coronaviruses. J Immun 102:1109–1118, 1969
7. Dowdle WR, Stewart JA, Heyward JT, Robinson RQ: *Mycoplasma pneumoniae* infections in a children's population: a five-year study. Amer J Epidem 85:137–146, 1967
8. Sever JL: Application of a microtechnique to viral serological investigations. J Immun 88:320–329, 1962
9. US Public Health Service: Standardized diagnostic complement-fixation method and adaptation to microtechnique. PHS Monograph 74, Atlanta, National Communicable Disease Center, 1965
10. McIntosh K, Kapikian AZ, Turner HC, Harley JW, Parrott RH, Chanock RM: Seroepidemiologic studies of coronavirus infection in adults and children. Amer J Epidem 91:585–592, 1970
11. Hartley JW, Rowe WP, Bloom HH, Turner WC: Antibodies to mouse hepatitis virus in human sera. Proc Soc Exp Biol Med 115:414–418, 1964
12. Bradburne AF: Antigenic relationship amongst coronaviruses. Arch Ges Virusforsch 31:352-364, 1970
13. Kapikian AZ, James HD Jr, Kelly SJ, Dees JH, Turner HC, McIntosh K, Kim HW, Parrott RH, Vincent MM, Chanock RM: Isolation from man of "avian infectious bronchitis virus-like" viruses (coronavirus) similar to 229E virus, with some epidemiological observations. J Infect Dis 119:282–290, 1969
14. Bradburne AF, Bynoe ML, Tyrrell DAJ: Effects of a "new" human respiratory virus in volunteers. Brit Med J 3:767–769, 1967