

SEROEPIDEMIOLOGIC STUDIES OF CORONAVIRUS INFECTION IN ADULTS AND CHILDREN¹

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McIntosh, K., A. Z. Kapikian, H. C. Turner, J. W. Hartley, R. H. Parrott and R. M. Chanock. (Lab. of Infectious Diseases, NIAID, NIH, Bethesda, Md. 20014) Seroepidemiologic studies of coronavirus infection in adults and children. *Amer. J. Epid.*, 1970, 91: 585-592.—A seroepidemiologic study of infection by coronavirus strains 229E, OC38, OC43, and mouse hepatitis virus (MHV) strain A-59, is described. In adults with upper respiratory disease, two "outbreaks" of coronavirus infection occurred, one during the winter of 1965-1966 associated with complement fixing (CF) antibody responses to OC38, OC43 and MHV, and the other during the following winter associated with CF antibody responses to 229E. In hospitalized children, infection with 229E was rare; infection with OC38, OC43, and MHV occurred less often in hospitalized children with lower respiratory tract disease (3.5%) than in a control group with non-respiratory tract disease (8.2%). The limitations of the CF test using available coronavirus antigens are discussed.

antibodies; complement fixation tests; coronaviruses; respiratory tract diseases; serology; viruses

INTRODUCTION

Several investigators have recently reported the recovery from adults with upper respiratory infection of ether-labile viruses morphologically indistinguishable from mouse hepatitis virus (MHV) and avian infectious bronchitis virus (IBV) (1-6). The distinctive appearance of these viruses by

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electron microscopy suggested that they be designated as a new group, the "coronaviruses" (7). Members of the group are medium-sized, RNA-containing, and ether-labile, and bear characteristic club-shaped surface projections. Nine of the 23 strains recovered from the human respiratory tract were originally isolated in human embryonic tracheal organ culture (HETOC) (1,3,6,8). Three of the nine were subsequently adapted to growth in monolayer tissue cultures and two others (OC38 and OC43) were successfully adapted to growth in suckling mice and shown to be antigenically related to several strains of MHV by CF tests and fluorescence staining (6,9,10). The 14 remaining human strains were originally recovered in tissue culture monolayers and appeared antigenically similar or identical to the prototype tissue culture strain, 229E (2,5).

Little is known of the epidemiology of human infection with coronaviruses. CF and neutralizing antibodies to several strains of

MHV were found in military recruits before any coronavirus strains had been recovered from man (11). Epidemiologic analysis of these findings indicated that, although four-fold rises in antibody were frequent, there was no discernible association with disease. Both strain 229E and strain B814 have been shown to cause colds in adult volunteers (1,12). Preliminary seroepidemiologic surveys performed in this laboratory suggested that 229E, OC38, and OC43 infection occurred with sporadic frequency in adults with upper respiratory tract disease (5,9). This report describes a more extensive seroepidemiologic study using the available CF antigens from human strains and an antigen of MHV, strain A-59, shown by Hartley and others to detect CF antibody in human sera (11).

MATERIALS AND METHODS

Source of specimens

Adults. Nasopharyngeal washings and acute phase sera were obtained from employees of the National Institutes of Health with upper respiratory tract disease on or before the fourth day of illness, and convalescent phase sera three weeks later. There were 466 patients admitted to the study, some of them on multiple occasions, so that 577 serum pairs were available for study. The periods covered were October, 1962 through May, 1964, and September, 1965 through September 5, 1967. Some aspects of the study have been published (5,13).

Infants and children. Throat or nasal swabs and acute phase sera were obtained from pediatric patients on admission to Children's Hospital, Washington, D.C., and convalescent phase sera about three weeks later. A diagnosis of lower respiratory tract disease (LRTD) indicated croup (laryngo-tracheobronchitis), bronchitis, pneumonia, or bronchiolitis. Control sera were obtained at similar intervals from hospitalized patients with non-respiratory tract disease. Individuals with incidental respiratory tract disease at the time of selection were excluded

from the group. There was difficulty in finding sufficient control infants under 12 months of age (see table 1). Members of the control group were not matched with respect to their length of hospital stay. The sera chosen for study were obtained from October, 1965 through April, 1966, and from October, 1966 through April, 1967 (14).

Tissue culture and organ culture techniques

The preparation and maintenance of human embryonic intestine (HEI) diploid cell cultures, and their use in the recovery of coronaviruses have been described (5). Human embryonic tracheal organ cultures were prepared and maintained as previously reported (3). The methods used for recovery of coronaviruses in organ culture have also been described (3).

Complement fixing antigens

Dr. Hamre kindly supplied coronavirus strain 229E which had been purified by the terminal dilution technique. It was passaged several times in human diploid cell strain WI38 in this laboratory. CF antigen was prepared as a single lot of infected WI38 cells (5). Eight units of antigen as measured with a standard convalescent human serum were used in all tests.

CF antigen of MHV, strain A-59, was prepared from infected mouse liver NCTC 1469 tissue culture as previously described (11). A single lot of antigen was used for the study reported here. Four to eight units of antigen as measured with hyperimmune mouse serum were used.

The adaptation of organ culture grown coronavirus strains OC38 and OC43 to Swiss mice and the preparation of CF antigens from infected mouse brain have been described (9). The antigens used in this study were from a single lot for each virus and were free of contamination with mycoplasma and cytopathic agents. Moreover, screening CF tests were performed for possible contamination with Theiler's agent, lymphocytic choriomeningitis virus, Sendai virus, mouse leukemia, reoviruses, rubella, mumps,

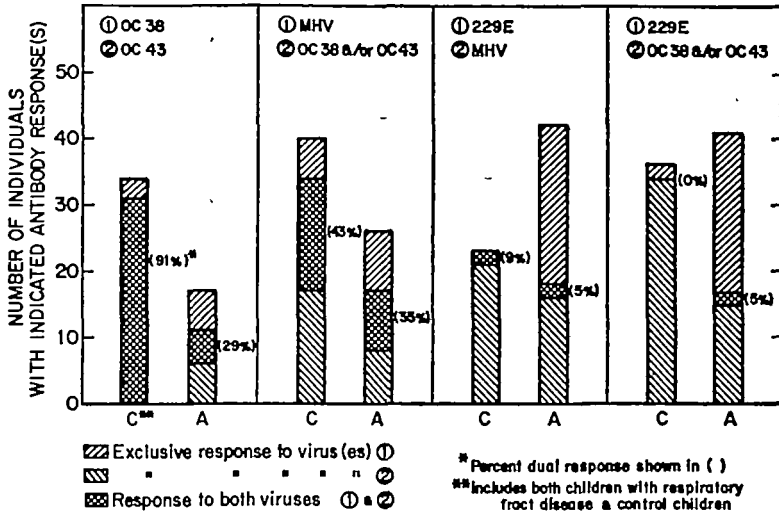


FIGURE 1. Single and dual antibody responses to various coronavirus strains in children (C) or adults (A).

measles, respiratory syncytial (RS) virus, parainfluenza virus types 1, 2, 3, and 4, and influenza virus types A, B, and C; all such tests were negative. The techniques of insuring that the antigens were also free of MHV strains have been previously described (9). Eight to 16 units of antigen as measured with hyperimmune mouse serum were used.

Control uninfected WI38 cell, NCTC 1469 cell and mouse brain antigens were prepared in parallel to infected antigen lots and were included in each test. Sera reacting with control antigens were not included in the data reported. These omissions account for the differences in the total number of serum pairs analyzed for each antigen (see table 2).

Complement fixation tests

CF tests were performed by the microtiter technique using overnight fixation at 4 C and 1.7–1.8 units of complement as previously described (15).

RESULTS

Shared antibody responses between coronavirus strains

Figure 1 is a diagram representing the number of and per cent antibody responses to the various coronavirus antigens tested in

TABLE 1
Complement fixing antibody responses to coronaviruses in infants and children with and without lower respiratory tract disease*

Age (months)	Respiratory tract disease		Non-respiratory tract disease	
	No. Tested	No. with CF antibody rise†	No. Tested	No. with CF antibody rise†
0-12	238	10 (4%)	55	1 (2%)
12-24	120	3 (3%)	19	4 (21%)
25-36	65	4 (6%)	33	5 (15%)
37-48	36	0	26	3 (12%)
49-60	32	0	26	3 (12%)
61-84	32	1 (3%)	42	3 (7%)
>85	42	2 (5%)	44	1 (2%)
Total	565	20 (3.5%)	245	20 (8.2%)

* Results shown here include antibody responses to OC38, OC43, and/or MHV, strain A-59.
† Fourfold or greater.

children and adults. The highest percentage of dual responses (91) involved strains OC38 and OC43 in children. Moderate overlap of response (35–45 per cent) also occurred between MHV and OC38 and/or OC43. Few or no dual responses were found involving 229E and MHV, or 229E and OC38 and/or OC43. Because of the frequent sharing of antibody response between OC38, OC43, and

TABLE 2
Prevalence of CF antibody to coronaviruses

A. Children*

Age (months)	OC38 and/or OC43		229E		MHV (A-59)	
	No. Tested	No. Positive†	No. Tested	No. Positive†	No. Tested	No. Positive†
0-6	188	11 (6%)	186	0	186	3 (2%)
7-12	104	14 (13%)	102	1	102	1 (1%)
13-18	80	23 (29%)	80	0	80	5 (6%)
19-24	57	14 (25%)	57	0	57	2 (4%)
25-36	95	48 (51%)	98	0	98	15 (15%)
37-48	60	27 (45%)	61	0	61	15 (25%)
49-60	57	31 (54%)	57	2	57	13 (23%)
61-84	72	28 (39%)	74	0	74	13 (18%)
>85	85	34 (40%)	85	2	85	13 (15%)
Total	798	230 (29%)	800	5 (0.6%)	800	80 (10%)

B. Adults‡

Period of study	OC38 and/or OC43		229E		MHV (A-59)	
	No. Tested	No. Positive‡	No. Tested	No. Positive‡	No. Tested	No. Positive‡
1962-1964	220	159 (72%)	222	43 (19%)	222	82 (37%)
1965-1967	242	161 (67%)	241	99 (41%)	242	78 (32%)
Total	462	320 (69%)	463	142 (31%)	464	160 (34%)

* Combined results from control patients and children with lower respiratory tract disease.

† Number of individuals with complement fixing antibody level of 1:4 or greater in acute serum sample.

‡ Antibody levels were measured once for each individual, on his first admission to the study.

§ Complement fixing antibody level of 1:4 or greater measured in acute serum sample.

MHV, and because of their reported close antigenic relationships (9), in certain analyses these agents are grouped together.

Association of antibody responses with disease in children

Table 1 shows the proportion of infants and children with and without LRTD showing fourfold or greater antibody responses to the three related coronavirus antigens OC38, OC43, and MHV, strain A-59. When all age groups were combined, a positive correlation between coronavirus infection and LRTD did not exist. However, the youngest age group (under one year) with LRTD tended to have more coronavirus infections than the control group, but the difference was not

statistically significant ($p > .05$). In all age groups taken together there was a significant negative correlation with LRTD ($\chi^2 = 7.4$, $p < .01$). This negative correlation was particularly striking in the children over the age of one ($\chi^2 = 10.6$, $p < .01$).

Prevalence of CF antibody in children and adults

In table 2 is shown the proportion of individuals tested who had measurable (1:4 or greater) CF antibody to coronavirus antigens. During the period 1965-1967 CF antibody to strain 229E was rare in children (0.6 per cent) and quite common in adults (41 per cent). CF antibody to OC38 and OC43 was increasingly common in children

up to the age of three years, when it was measurable in approximately 50 per cent of those tested. In adults, 72 per cent of those tested had measurable CF antibody to one or both strains during 1962-1964, and 67 per cent during 1965-1967. Antibody to MHV, strain A-59, was of somewhat lower prevalence (approximately 10 per cent in children and 34 per cent in adults during the periods noted above).

Incidence of coronavirus infection in children and adults

Information obtained from serologic, tissue culture, and organ culture studies is summarized in figures 2 and 3. Among adults (figure 2) coronaviruses appeared to be rarely associated with upper respiratory tract disease during 1963 and 1964. The frozen nasopharyngeal washings of a small group of patients, who in preliminary studies had shown CF antibody responses to strain 229E, were examined in HEI tissue culture. Three of these yielded coronaviruses serologically similar to strain 229E. Sporadic frozen specimens were examined in tracheal organ

culture, and one coronavirus was recovered. Apart from these isolations and a few scattered CF responses there appeared to be little detectable coronavirus activity in the adult population. During 1965-1967, however, two small "outbreaks" of coronavirus-associated colds occurred in adults. In the first of these, during the winter of 1965-1966, five coronaviruses were recovered in organ culture, two of which were serologically identical, and numerous responses to OC38, OC43, and MHV CF antigens were measured. Infections with strain 229E were either absent or very rare. During the winter of 1966-1967, however, six strains of 229E-like viruses were recovered in HEI cells, and CF antibody responses to 229E were frequent. In the second "outbreak" antibody responses to OC38, OC43, and MHV antigens were rare, and no coronaviruses were recovered in organ culture.

In children, on the other hand, (figure 3) there appeared to be no meaningful temporal pattern of coronavirus CF antibody responses, and no strains were recovered in organ culture.

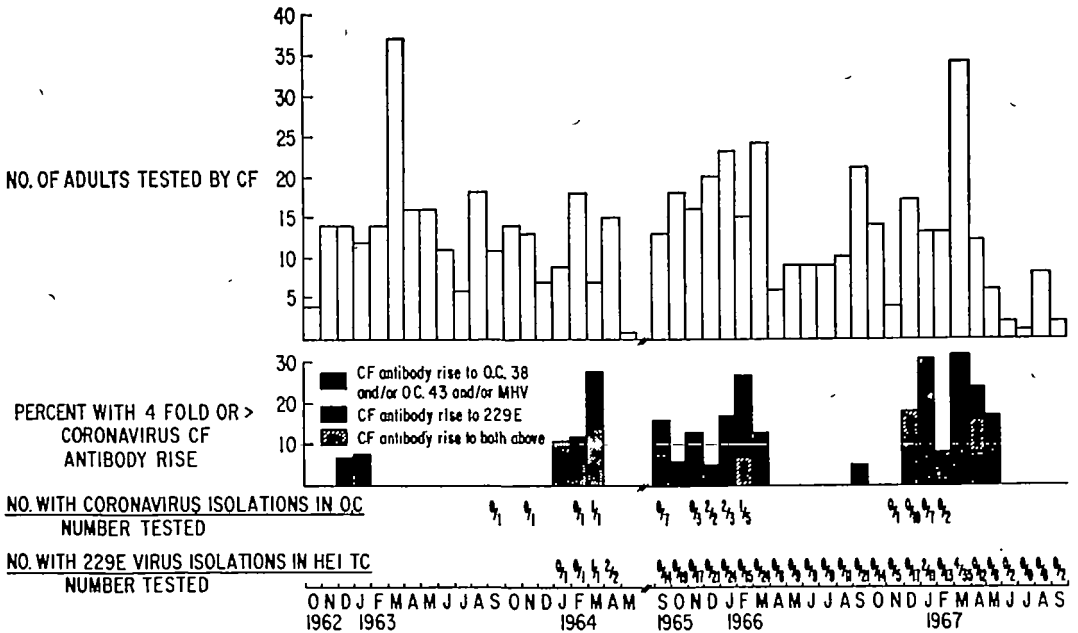


FIGURE 2. Coronavirus isolations and antibody responses in adults with upper respiratory tract disease.

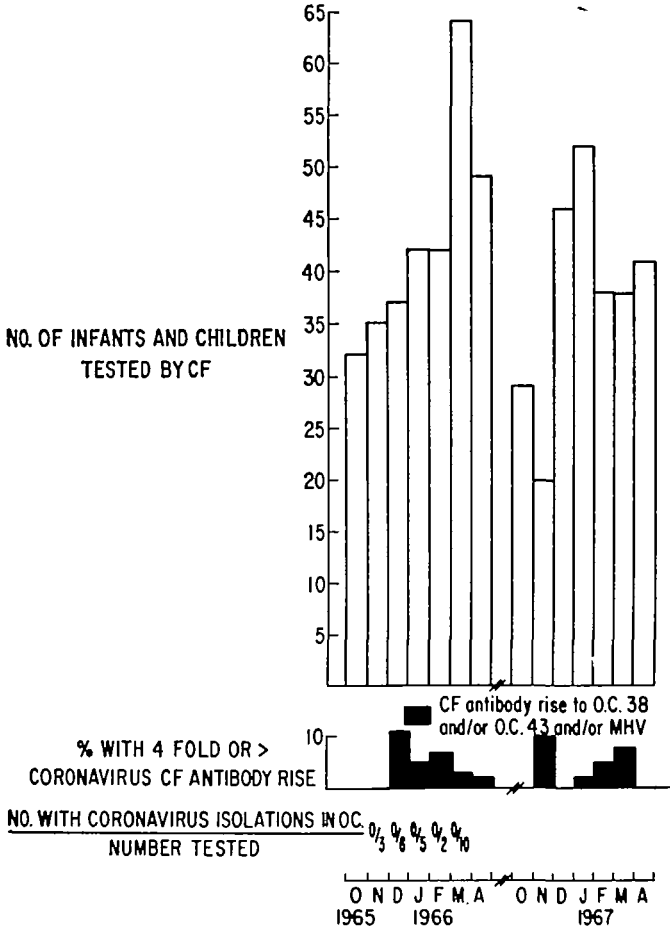


FIGURE 3. Coronavirus isolations and antibody responses in children with lower respiratory tract disease.

Significance of dual infection with coronaviruses and paramyxoviruses in children

The children under study were examined for evidence of infection by other respiratory viruses, and a certain number of dual infections with myxo- or paramyxoviruses and coronaviruses were found. There was no evidence that coronavirus infection potentiated the pathogenic effect of infection by either parainfluenza virus 3 or RS virus, the two viruses found most often in conjunction with coronaviruses (table 3).

DISCUSSION

These studies were undertaken to define more clearly the seroepidemiology of coronavirus infection in adult and pediatric popu-

lations. Serum samples and nasopharyngeal washings were studied from adults with colds occurring in two two-year periods. During the first period (1962-1964) detectable coronavirus infection was uncommon. However, in the winter of 1965-1966, members of the OC38-OC43-MHV group were prevalent and detectable both by serologic and organ culture techniques. One year later members of the 229E group were prevalent and again detectable by both serologic responses and virus isolation. It is of interest that among adults all the coronavirus recoveries and most of the coronavirus serologic responses occurred in the four winter months of December, January, February, and March. In several surveys of colds in adults, respira-

tory disease was common during these winter months but rhinovirus infection, though frequent in the fall and spring, was rare (13,16,17). This pattern of rhinovirus infection prevailed in this study during the period 1965-1967. Thus, during these winter months coronaviruses although still accounting for less than 50 per cent of colds, became the predominant identifiable organisms associated with adult upper respiratory disease.

It is evident from the studies of hospitalized children that infection with coronaviruses was not significantly associated in this survey with pediatric lower respiratory tract disease. Indeed, a negative correlation of coronavirus infection with LRTD was found. It may be that the presence of severe LRTD requiring hospitalization interfered in some way with infection of the respiratory epithelium by coronaviruses. On the other hand, it seems more likely that this negative correlation reflects the fact that it was not possible to select a completely comparable control group. Control children were drawn from different pediatric wards, and their lengths of stay in the hospital frequently differed from those of the test group. In any case, the data reported suggest that the coronaviruses tested were not an important cause of LRTD in infants and children during the period covered by this study.

Our studies did not provide information on the etiologic association of coronavirus infection with respiratory tract disease in adults. Two coronavirus strains, 229E and B814, were administered to adult volunteers and in both instances a significant number of common colds occurred (1,12). Data are still lacking, however, to show that members of the OC38-OC43 serologic type cause disease under natural conditions. It is of interest that in the seroepidemiologic studies of Hartley and others, where several strains of MHV were tested with sera from military recruits, no epidemiologic association of MHV or MHV-like virus infection with respiratory tract disease was made (11).

The infrequency of detectable CF antibody to 229E in the pediatric population

TABLE 3
Incidence of dual infection with coronaviruses and viral respiratory tract pathogens in children*

A. Parainfluenza virus 3†			
Children tested	No. of children with infection by:		
	Parainfluenza 3 alone	Parainfluenza 3 and coronavirus	Neither
LRTD‡	57	3	505
Non-LRTD	19	5	221

B. Respiratory syncytial (RS) virus§			
Children tested	No. of children with infection by:		
	RS virus alone	RS virus and coronavirus	Neither
LRTD	125	6	240
Non-LRTD	18	1	130

* Either virus isolation or 4-fold or greater rise in antibody or both.

† Periods tested were those of the entire survey.

‡ Lower respiratory tract disease.

§ Periods tested were those of RS prevalence: January-April, 1966; December, 1966-March, 1967.

studied here was surprising. Although 229E virus infection may indeed be rare in children, several other explanations for this anomalous finding are possible. The 229E antigen, in contrast to the other coronavirus antigens, may have reacted with CF antibody to a narrow range of coronavirus serologic types, owing either to the type of tissue in which it was made, or to some characteristic of the virus itself. Possibly serum CF antibody (as opposed to serum neutralizing, or secretory antibody) did not develop in children in response to 229E virus infection. Studies of 229E neutralizing antibody in different age groups would contribute to the clarification of this finding.

Antigenic studies of human coronaviruses have shown that those members of the group which were originally recovered in tissue culture are all closely related to the prototype virus strain 229E (2,5). In contrast, the strains isolated in organ culture include at least two and probably several more anti-

genic types (10). One type, exemplified by strains OC38 and OC43, is clearly related to the MHV group. The other type or types have an indefinite relationship with MHV and with the OC38-OC43 group. In particular, patients yielding coronaviruses in organ culture varied markedly in their antibody responses to the CF antigens used in this study. One such patient showed in multiple tests no rise to any of the coronavirus antigens. Two others showed responses in some tests and not in others. Infection with strain B814 was likewise difficult to detect serologically, using OC38, OC43, MHV and 229E antigens (10). The heterogeneity of antibody response in subjects with known or presumed coronavirus infection, and the probable insensitivity of presently available serologic tests in the detection of coronavirus infection indicate that the serologic studies reported here do not describe a complete picture of coronavirus infection. It is probable that undetected coronavirus infections occurred in these populations during the study periods. CF antigens from the other known coronavirus strains, and further efforts to isolate and characterize new strains will contribute to further definition of the epidemiology of coronavirus infection.

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