

Coronavirus Infections in Working Adults

Eight-Year Study with 229 E and OC 43¹⁻³

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SUMMARY

Respiratory infections due to coronaviruses 229 E and OC 43 in insurance company employees occurred almost exclusively in the winter and spring. Four per cent of all colds and 8 per cent of winter and spring colds were serologically related to infection with one of the two viruses. Studies of a group of families of employees showed that the prevalence of antibodies to 229 E and OC 43 increased with age. The infection rate for both coronaviruses in employees observed for one year or longer was 14.5 infections per 100 persons per year, and approximately one-third were reinfections rather than primary infections. Reinfections were commonly associated with respiratory symptoms. Colds associated with coronavirus 229 E were mild and nasal symptoms were predominant. Infections due to OC 43 coronavirus were associated with cough and pharyngitis as well as coryzal symptoms.

Introduction

The coronaviruses are a new group of viruses that have been isolated from patients with upper respiratory infections, particularly in the winter. Certain coronavirus

strains, serologically similar to the original 229 E (1), can be detected in cell cultures by the production of cytopathic effect (2). Other coronaviruses require some variant of human embryonic tracheal organ cultures for primary isolation. Confirmation of the presence of the organ culture strains has required either inoculation of volunteers with fluids from infected cultures (3) or demonstration by electron microscopy of virus particles in culture sediments (4). Attempts to estimate the importance of coronaviruses as a cause of respiratory disease have been hampered by these methodologic difficulties, and large scale epidemiologic studies must currently rely on serologic methods.

The 229 E strain has a complement fixing (CF) antigen but no known hemagglutinating (HA) antigen. Two of the seven organ culture (OC) strains of coronavirus, OC 43 and OC 38, have been adapted to growth

(Received for publication October 14, 1971)

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² This work was sponsored in part by Contract No. PH-43-63-1128, Vaccine Development Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health and the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiological Board, and in part by the U. S. Army Medical Research and Development Command under Research Contract No. DADA 17-69-C-9010.

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in suckling mouse brain (5) and are serologically identical. Suckling mouse brain antigen has been used in both CF (5) and hemagglutination inhibition (HI) tests (6). In the current study, sera collected during a period of eight years from young adults with colds were tested for evidence of infection with two serologically unrelated coronavirus strains, 229 E and OC 43. Illnesses due to coronavirus were detected in the winter and spring months and were found in several different years. Attack rates for the two coronaviruses were calculated from illness rates obtained by continuous close surveillance of the population since 1963. Serologic evidence of repeated infection with these or related viruses was seen in a substantial number of the employees.

Materials and Methods

Surveillance and collection of specimens: Surveillance methods and the criteria used for diagnosis of a respiratory illness in the population of young adult insurance company employees in Charlottesville, Virginia have been detailed previously (7). Swabs of nose and throat secretions and pairs of acute and convalescent serum were obtained from persons with colds. In the first year of the study (1963 to 1964), specimens were also obtained from asymptomatic employees selected at random. In the fall of 1966, serum pairs were obtained from the families of 69 employees as part of a study of viral transmission within the home (8). Serum was obtained from specimens of venous blood in most instances, but some sera from family members were from capillary blood obtained from a finger by previously described methods (8).

Viral antigens and serologic techniques: Coronavirus strain 229 E was supplied by Dr. Dorothy Hamre. A CF antigen was prepared from virus grown in the WI-38 strain of human diploid fibroblasts. Four units of antigen and two exact units of complement were used in a standard CF test done in the microtitration system. Since the present work was done, it was shown that this test is more sensitive if only 1.8 units of complement are used (9).

Coronavirus strain OC 43 adapted to grow in suckling mouse brain was supplied by Dr. Walter Dowdle. The virus had been passaged five times in human embryonic tracheal organ culture and 12 times in suckling mouse brain.

A mouse brain harvest was used as the antigen in the HI test developed by Kaye and Dowdle (6).

Sera were inactivated at 56° C for 30 minutes before use in both tests. A fourfold or greater increase in titer from acute to convalescent serum was accepted as serologic evidence of infection; convalescent sera were obtained from three to ten weeks after the acute specimen. A reinfection was judged to have occurred if titers before the antibody increase were more than 4 to 229 E, or greater than 10 to OC 43, or if there was serologic evidence of a previous infection. In most instances, initial titers were measured on preinfection rather than acute phase sera. Titers were expressed as the reciprocals of the initial dilutions of serum.

Results

Occurrence of illness: During the eight-year period from 1963 to 1970, 4,259 respiratory illnesses occurred in the average population of 433 people. Serum pairs available from 592 (14 per cent) of these illnesses were tested for CF antibody to coronavirus strain 229 E, and 620 serum pairs (15 per cent) were tested for HI antibody to coronavirus strain OC 43 (table 1). Five hundred and sixty pairs of serum from asymptomatic control subjects were tested for antibody increases with 229 E antigen, and 579 control pairs were tested with OC 43 antigen.

Serologic evidence of infection with 229 E virus was found in 3 per cent of persons with colds in the winter and spring (December through May) and in 0.4 per cent of persons with summer and fall colds. During both periods, one per cent of the control subjects had an antibody increase to 229 E virus; this proportion was not statistically different from that for ill employees. Virus infections due to OC 43 were related to 5 per cent of winter and spring colds; no antibody increases to this antigen occurred in control subjects during these seasons ($\chi^2 = 8.76$; $P < 0.005$). Taken together, infections by these two coronaviruses were associated with 4 per cent of all colds and 8 per cent of winter and spring colds. The number of detectable infections during the winter (December through February) was approximately equal to that in the spring season (March through May).

TABLE 1
SEROLOGIC RESPONSES TO CORONAVIRUSES IN PAIRED SERA
FROM EMPLOYEES AND FAMILY MEMBERS

Coronavirus	Season	1963 to 1970	
		III	Asymptomatic
		No. positive/ No. tested	No. positive/ No. tested
229 E	Summer-fall	1/275 (0.4)*	4/309 (1)
	Winter-spring	9/317 (3)	3/251 (1)
	Total	10/592 (2)	7/560 (1)
OC 43	Summer-fall	0/295	1/320 (0.3)
	Winter-spring	15/325 (5) [†]	0/259 [†]
	Total	15/620 (2)	1/579 (0.2)

*Numbers in parentheses denote percentages.

[†]P = < 0.005.

The occurrence of infections with coronaviruses 229 E and OC 43 was analyzed by year (table 2). No sera were available from 1967, and a total of only 11 pairs were studied from the winter and spring of both 1966 and 1968. In the years 1963 to 1965 and 1969 to 1970 in which adequate numbers of serum pairs were tested, 229 E activity was detected in three and OC 43 in four of the five years. During these five winter and spring seasons, the highest proportion of illnesses related to either agent was 13 per cent for 229 E in 1970.

Antibody prevalence: The prevalence of antibody by age was analyzed using the titer of the first serum sample obtained from each subject (table 3). Children younger than ten years had antibody to both viruses less frequently than did teenagers and adults, and the prevalence of antibody

tended to increase with age. Ten per cent of children less than ten years of age had CF antibody titers to 229 E of ≥ 4 whereas 24 per cent of subjects older than ten years had similar titers ($\chi^2 = 5.34$; $P = < 0.05$). Titers to 229 E, when present, were low; in persons ten years and older, approximately 19 per cent had a titer of 4; 5 per cent, a titer of 8, and only one per cent had a titer of ≥ 16 . Titers of HI antibody to OC 43 of ≥ 10 were present in 21 per cent of children less than ten years of age and in 60 per cent of older children and adults ($\chi^2 = 22.4$, $P = < 0.0005$). The titers of antibody to OC 43 were greater than those to 229 E, perhaps reflecting greater sensitivity of the test. In subjects ten years of age and older, 30 per cent had an HI titer of 1.0, 20 per cent had a titer of 20, 7 per cent had a titer of 40, and 4 per cent had a titer of ≥ 80 . No dif-

TABLE 2
SEROLOGIC RESPONSES TO CORONAVIRUSES IN ILL
EMPLOYEES AND FAMILY MEMBERS BY YEAR

Corona-virus	Season	1963	1964	1965	1966	1968	1969	1970	1963-1970
229E	Summer-fall	1/89 (1)*	0/26	0/10	0/114	0/5	0/31	—	1/275 (0.4)
	Winter-spring	2/109 (2)	3/60 (5)	0/18	0/5	0/6	0/89	4/30 (13)	9/317 (3)
	Total	3/198 (2)	3/86 (4)	0/28	0/119	0/11	0/120	4/30 (13)	10/592 (2)
OC 43	Summer-fall	0/96	0/31	0/11	0/122	0/5	0/30	—	0/295
	Winter-spring	2/112 (2)	2/62 (3)	2/18 (11)	0/5	0/6	9/95 (9)	0/27	15/325 (5)
	Total	2/208 (1)	2/93 (2)	2/29 (7)	0/127	0/11	9/125 (7)	0/27	15/620 (2)

*Number positive/number tested. Numbers in parentheses denote percentages.

TABLE 3
PREVALENCE OF ANTIBODY TO TWO
CORONAVIRUSES IN SERUM OF
EMPLOYEES AND FAMILY MEMBERS

Age (years)	Reciprocal of Titer to 229 E ≥ 4	Reciprocal of Titer OC 43 ≥ 10
0-9	4/41 (10)*	8/38 (21)†
10-19	22/101 (22)	60/106 (57)
20-29	60/292 (21)	183/289 (63)
30-39	35/127 (28)	80/128 (62)
40+	25/66 (38)	31/65 (48)
Total	146/627 (23)	362/626 (58)

*P = < 0.05.

†P = < 0.0005.

ference was seen in antibody prevalence between male and female subjects with either virus.

Serologic relationships and reinfection: A longitudinal view of an employee's experience with these two coronaviruses was provided by study of multiple sera obtained over several years; selected examples are shown in table 4. In this study, increases of antibody to one of the coronaviruses were never accompanied by an increase to the other. Employee CG had two antibody increases to OC 43 whereas antibody titers to 229 E remained at ≤ 5 . Employee DR had a titer of 40 to OC 43 with a subsequent fourfold or greater increase in titer to this virus; however, his titer to 229 E was stable at ≤ 4 . Employee CH, on the other hand, had two increases to 229 E although his titer to OC 43 remained at ≤ 10 . Employee WM had an antibody response to OC 43 in 1965 and an increase to 229 E in 1970.

Serologic evidence of reinfection with the same or a related coronavirus was seen with some employees. Employee CG had two increases to OC 43 with an intervening decline in titer. Employee DR had a titer of 40 to OC 43, suggestive of prior infection, and a subsequent increase in titer to ≥ 160 . Employee CH had a fourfold increase over a two-month period to 229 E in 1963 and a second increase in 1970.

Infection rates: Data from employees under seroepidemiologic surveillance for one year or longer were used to derive corona-

virus infection rates. Each period spanning 12 months in which specimens were collected from a subject was designated as a person-year. A fourfold or greater increase of antibody, regardless of the interval between serum samples, was accepted as evidence of infection.

Two hundred and six employees under study for 229 E infections were observed for a total of 683 person-years. Fifty-one antibody increases occurred in 46 subjects, giving a rate of 7.5 infections per 100 person-years. Ten of these increases represented reinfections with 229 E or a related virus. Similarly, 211 employees were observed for evidence of OC 43 infections for 693 person-years. Forty-eight antibody increases were detected in 43 persons, for an infection rate of 7 per 100 person-years. Eighteen of these 48 increases were reinfections. The total rate for both coronaviruses was 14.5 per 100

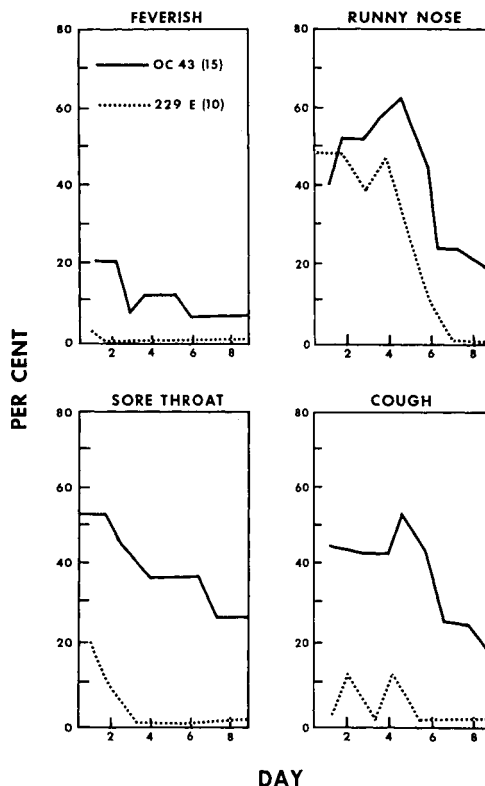


Fig. 1. Selected symptoms in coronavirus illnesses in subjects studied.

TABLE 4
ANTIBODY TITERS TO CORONAVIRUSES IN MULTIPLE SERUM
SPECIMENS FROM SELECTED EMPLOYEES

Employee Virus		Reciprocal of Titers									
CG	229 E OC 43	Date: 5/63	6/63	12/63	12/63	3/64	3/64	6/64	6/64	1/65	2/65
		<5	<5	4	5	<4	<4	<5	<5	<4	<4
	229 E OC 43	10	10	<10	10	10	10	20	40	20	10
		Date: 9/66	10/66	11/68	12/68	2/69	10/69	11/69			
	229 E OC 43	<4	<4	<4	<4	<4	4	<4			
		10	10	10	10	≥160	80	80			
DR	229 E OC 43	Date: 4/63	5/63	10/63	11/63	2/69	5/69	11/69	5/70		
		<4	<4	<4	<4	<4	<4	<4	4		
		40	40	40	40	≥160	≥160	≥160	≥160		
CH	229 E OC 43	Date: 7/63	7/63	9/63	10/63	12/68	1/69	6/69	11/69	5/70	
		<5	5	10	10	8	4	4	4	16	
		10	10	10	10	<10	<10	10	<10	<10	
WM	229 E OC 43	Date: 4/63	4/63	10/63	10/63	11/63	12/63	1/64	3/65	4/65	
		<4	<4	<4	<4	<4	<4	<4	<4	<4	
	229 E OC 43	<10	<10	<10	<10	<10	<10	<10	<10	40	
		Date: 8/66	10/66	2/69	6/69	11/69	1/70	5/70			
	229 E OC 43	<4	<4	<4	<4	<4	<4	16			
		10	10	20	40	20	20	20			

person-years, or 14.5 per cent of the employees under surveillance had serologic evidence of primary or repeat infection with one of these or a related coronavirus per year of observation.

Virus isolation attempts: Viral isolation was attempted in WI-38 cells with the frozen specimens of nose and throat secretions obtained from 10 persons who had serologic evidence of infection with strain 229 E. Despite prior familiarization with the 229 E cytopathic effect, these isolation attempts were unsuccessful. Initial testing of the fresh specimens in WI-38 cells had also been reported as negative.

Symptoms associated with coronavirus infection: The daily symptoms of the ten persons with 229 E infections and the 15 persons with infections due to strain OC 43 were compared (figure 1). Colds associated with 229 E infection were mild; only nasal symptoms were reported with any frequency. Colds associated with OC 43, in contrast, resembled those due to infections with rhinovirus in that in addition to nasal complaints, sore throat and cough occurred in approximately 50 per cent of those infected.

Discussion

In this serologic study of colds in young adults, infections with coronavirus occurred almost exclusively in the winter and spring, periods of low prevalence of rhinovirus. This is in accord with previous studies of coronaviruses using both culture (1-4) and serologic (2, 10-12) methods. In adults, 40 to 50 per cent of fall colds and a significant proportion of colds in the late spring can be related to rhinoviruses (7). Few virologic diagnoses of colds were made in the winter and early spring before the discovery of coronaviruses, and therefore this group is of special interest because it helps fill this "cold weather gap."

Knowledge about the serologic characteristics of coronaviruses remains fragmentary. Of 21 isolates from natural respiratory disease in humans discussed by McIntosh and associates (13), 14 were found to be serologically similar to 229 E and to grow in cell culture. Most of seven organ culture viruses were unrelated to the 229 E group. Two of the organ culture strains, OC 43 and OC 38, were serologically similar and a third, OC 44, was probably related to these two. Strains OC 37 and B814 were weakly re-

lated to the OC 43 group, and only OC 48 was possibly related to 229 E. The seventh isolate, OC 16, was apparently not related to any of these viruses. Bradburne (9), on the other hand, studied this same problem and found more important serologic relationships between the organ culture-grown coronaviruses and 229 E.

Information about the relative importance of coronaviruses as a cause of respiratory disease is even less complete than knowledge about the serologic status of the viruses. Only 229 E and OC 43—OC 38 antigens have been adapted to methods suitable for large scale seroepidemiologic studies. Outbreaks of illness have been observed with 229 E (1, 2, 10, 11) and OC 43 (10, 12). In the current study, illness related to the two viruses was not limited to outbreaks because illnesses due to OC 43 were seen in four of five years and to 229 E in three of five years. Together the two viruses accounted for 4 per cent of all respiratory illnesses and for 8 per cent of winter and spring colds. No antibody increases to one of these two coronaviruses was accompanied by an increase to the other. This finding is in contrast with that of Bradburne (9) who found HI antibody increases to OC 43 in 14 per cent of 70 volunteers inoculated with other coronaviruses.

Each year 14.5 per cent of employees under serologic surveillance had evidence of infection with one of these two viruses or a closely related virus. This high attack rate can be partially explained by the fact that 28 per cent of the observed antibody increases were related to reinfection. Kaye and associates (12) recently reported a 33 per cent reinfection rate with OC 43 virus in children. The true reinfection rates for coronaviruses may be even greater than reported; this is particularly so for 229 E because CF antibody to these viruses may decrease after infection (11). It was important to find that reinfections with coronaviruses were associated with illness because asymptomatic reinfections would not be of clinical importance. Seven of 25 (28 per cent) infections due to coronavirus in persons with colds were of the reinfection type.

Explanations for the frequency of colds

have included the theories that a large number of antigenically distinct viruses exist or that multiple infections occur with a few agents. The discovery of the numerous rhinoviruses has proved the first theory correct. The high rate of reinfection seen for coronaviruses provides more support for the second theory, which is already applicable to respiratory syncytial and parainfluenza viral infections. The fact that reinfections were common and were associated with symptomatic illness is of note because relatively few coronavirus types could account for a large portion of undiagnosed colds under these conditions. The total proportion of colds that can be related to coronaviruses will not be known until better methods are developed of testing for other coronavirus types, both known and unknown.

The symptoms of colds due to OC 43 in the young adults in this study were similar to natural illnesses in children due to OC 43 (12), being characterized by pharyngitis, cough, and nasal symptoms. Thus, illnesses due to OC 43 resembled rhinovirus colds (14) in that they produced symptomatic involvement of the entire respiratory tract. On the other hand, employees with colds related to 229 E reported mainly nasal symptoms with nasal discharge being the prime complaint. The mild character of the natural illnesses associated with 229 E is in keeping with the description of experimental 229 E colds in volunteers (15).

RESUMEN

Infecciones por coronavirus en adultos trabajadores

Infecciones respiratorias debidas a coronavirus 229E y OC 43 en empleados de compañías de seguros ocurrieron casi exclusivamente en el invierno y en la primavera. Cuatro por ciento de todos los resfriados y 8 por ciento de los resfriados de invierno-primavera estuvieron relacionados serológicamente con uno de los dos virus. Estudios de un grupo de familias de los empleados mostró que la prevalencia de anticuerpos 229E y OC 43 aumentó con la edad. El porcentaje de infección para ambos coronavirus en empleados observados durante un año o más fué de 14.5 infecciones por 100 personas por año, y aproximadamente un tercio fueron reinfecciones más

que primera infección. Las reinfecciones estuvieron comunmente asociadas con síntomas respiratorios. Los resfríos asociados con el coronavirus 229E fueron leves y los síntomas nasales fueron los predominantes. Infecciones debidas al coronavirus OC 43 estuvieron asociadas con tos y faringitis así como con síntomas corizales.

RESUME

Infections par le coronavirus chez des travailleurs adultes

On a observé que des infections respiratoires causées par les coronavirus 229 E et OC 43, chez des employés d'une compagnie d'assurances, survenaient presque exclusivement en hiver et au printemps. Quatre pour cent de tous les refroidissements, et 8 pour cent des refroidissements survenant en hiver et au printemps, étaient sérologiquement en relation avec une infection par l'un ou l'autre de ces deux virus. Des études menées dans un groupe de famille d'employés a montré que la prévalence des anticorps 229 E et OC 43 augmentait avec l'âge. Le taux d'infection pour ces deux coronavirus chez des employés observés pendant un an ou davantage, était de 14,5 infections pour 100 personnes par an; environ un tiers de ces infections consistait en réinfection plutôt qu'en infection primaire. Les réinfections étaient habituellement associées avec des symptômes respiratoires. Les refroidissements associés avec le coronavirus 229 E étaient bénins; les symptômes nasaux étaient prédominants. Les infections causées par le coronavirus OC 43 étaient associées avec de la toux et de la pharyngite, outre les symptômes du corvza.

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