Community-wide Outbreak of Infection with a 229E-like Coronavirus in Tecumseh, Michigan

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In 1966, Hamre and Procknow described the isolation of an ether-sensitive, ribonucleic acid-containing virus from students with respiratory illnesses [1]. The virus, 229E, was found to be morphologically similar to another "new" respiratory virus, strain B814, reported by Tyrrell and Bynoe and Almeida and Tyrrell [2, 3]; both were shown to possess properties similar to those of viruses of the avian infectious bronchitis and mouse hepatitis groups [3–6]. Subsequently, additional isolates resembling B814 and 229E were recovered from persons with upper-respiratory infections [6–8]. The name "coronavirus" has been proposed for this group of human and animal viruses [9].

Since its original description, the 229E virus, and similar strains, have been isolated on several occasions and have been shown to be capable of causing respiratory illness in volunteers [2, 8, 10]. The studies to date have involved mainly adults in special occupational situations. Little is known of the relative importance of 229E virus as a respiratory pathogen in the general population.

In 1967, a sharp outbreak of infection with a 229E-like virus was detected in Tecumseh, Michigan by serologic surveillance. An extensive study of sera collected in Tecumseh during 1966 and 1967 was, therefore, undertaken in an attempt to determine the occurrence of infection with this agent in a natural population. The following report is a characterization of the outbreak.

Methods

Cell culture. Tubes of human embryonic lung cell strain WI-38 were purchased from HEM Research, Inc. Maintenance medium consisted of Eagle's minimal essential medium (MEM) supplemented with 2% inactivated fetal bovine serum, and 100 units of penicillin, and 100 μg of streptomycin per ml.

Virus. Coronavirus 229E, purified by terminal dilution in human diploid cell strain WI-38, was received from Dr. Dorothy Hamre. Viral antigen employed in complement-fixation (CF) and neutralization tests was prepared in our laboratory in WI-38 cell culture. Either 32-oz bottles or tube cultures were inoculated with undiluted 229E virus and incubated for 34–38 hr at 33 C. The harvested material was frozen and thawed 3 times for use in the CF test; for use in neutralization tests the harvested cultures were frozen and thawed once. Fluids were pooled and centrifuged, and the supernatant was employed as CF antigen or infective virus.

Population surveillance and collection of specimens. Since November, 1965, a system of continuous surveillance for respiratory infections has been in progress in Tecumseh, Michigan. The general aim has been to describe the behavior of respiratory pathogens as they progress through the community. This is accomplished by the isolation and identification of circulating agents, together with the testing of sera collected at set time intervals.

The families studied were recruited randomly from among those families with children whose parents were under 45 years of age. These families were followed for a period of 1 year, during which time they were questioned weekly regarding the occurrence of respiratory illnesses. Specimens for

microbial isolation were obtained when an illness was reported within 2 days of onset. These specimens were inoculated into cell culture tubes of WI-38, rhesus monkey kidney, and HL cells, a human continuous cell line.

Three blood specimens were collected from all family members. Each person was bled at the time of entering the study, 6 months later and at the end of the year of surveillance. Sera were stored at —20 C. Blood specimens from children under 5 years of age were obtained by collection of capillary blood by finger prick on filter paper disks [11, 12]. Disks were dried and stored at 4 C.

Serologic tests. Neutralizing antibody was measured in tubes of WI-38 cell monolayers. In all tests, the amount of virus employed was 10–32 50% tissue culture infective doses ($TCID_{50}$) per 0.1 ml. Equal volumes of viral dilutions and serial 2-fold dilutions (1:4–1:32) of inactivated serum were incubated at room temperature for 90 min; 0.2 ml of each of the mixtures was inoculated into each of 2 cell culture tubes. Tubes were incubated at 33 C on a roller drum and read at 2-day intervals until simultaneous titration of virus indicated maximum cytopathic effect (CPE). Serum titers were calculated by the method of Reed and Muench [13]. A complete series of 3 sera per person was run in the same test.

CF tests were performed by a microplate technique; 4 units of antigen and 2 exact units of complement were used. Blood specimens were screened initially at a 1:4 dilution. Positive specimens were rerun in serial dilutions for confirmation of the presence of antibody and determination of the titer. As with the neutralization test, a complete series of 3 bloods per person was run in the same test. For both procedures, a significant rise in antibody was considered to have occurred if antibody developed at 1:4 dilution or greater when no antibody was detected in the initial serum of a pair at a 1:4 dilution (serologic conversion). If antibody was present in the initial serum of a pair, a 4-fold increase in titer was required for significance.

When blood specimens were collected on filter paper disks, at least 4 were obtained at one time from each person. For the performance of the CF test, eluates were prepared from dried disks by addition 0.5 ml of veronal-buffered saline to 2 disks placed in a disposable plastic syringe. Disks

were held overnight at 4 C; eluate fluid was then expressed into a clean tube. This eluate was considered to represent a 1:4 dilution and was tested for CF antibody as described above. Because of the small volumes of eluate fluid obtainable, eluates were tested only for the presence of CF antibody.

Results

Epidemic curve defined by complement-fixation. Serial blood specimens have been collected from all participants in the Tecumseh study; the serologic data obtained were used to define the behavior of different viruses as they pass through the community. When the complement-fixing antigen for 229E virus became available, testing of large numbers of these sera for 229E antibody was begun. At first, little antibody was found in the population, but in 1967 the prevalence of antibody increased sharply. A total of 1,990 sera were tested by CF. They had been collected from randomly selected individuals under surveillance in 1966 and 1967, and all 3 sera obtained from a single individual in the surveillance year were tested. The schedule of serum collection had been designed by a system of random selection of families; individuals bled in any one month should, therefore, be as representative of the whole population as those bled in any other month.

Results of the CF tests are shown in figure 1. The solid line is based upon the presence of CF antibody at a 1:4 dilution, or greater; of the sera positive for CF antibody, 44.8% had a titer of greater than 1:4. The occurrence of CF antibody was less than 1% in sera collected from July to December, 1966. In January, 1967, the prevalence of CF antibody began to increase gradually and peaked in April; approximately 15% of the sera collected in that month were found to have CF antibody. The prevalence of CF antibody then declined sharply in sera collected in May and June, with a moderate increase from July to September. This increase probably does not represent a second wave, but rather the tendency of infections to cluster in families who were bled at the same time. Thereafter, the level approached that observed during the same period in 1966. These serologic data indicate that an outbreak of infections with 229E-like virus occurred during April, 1967. In
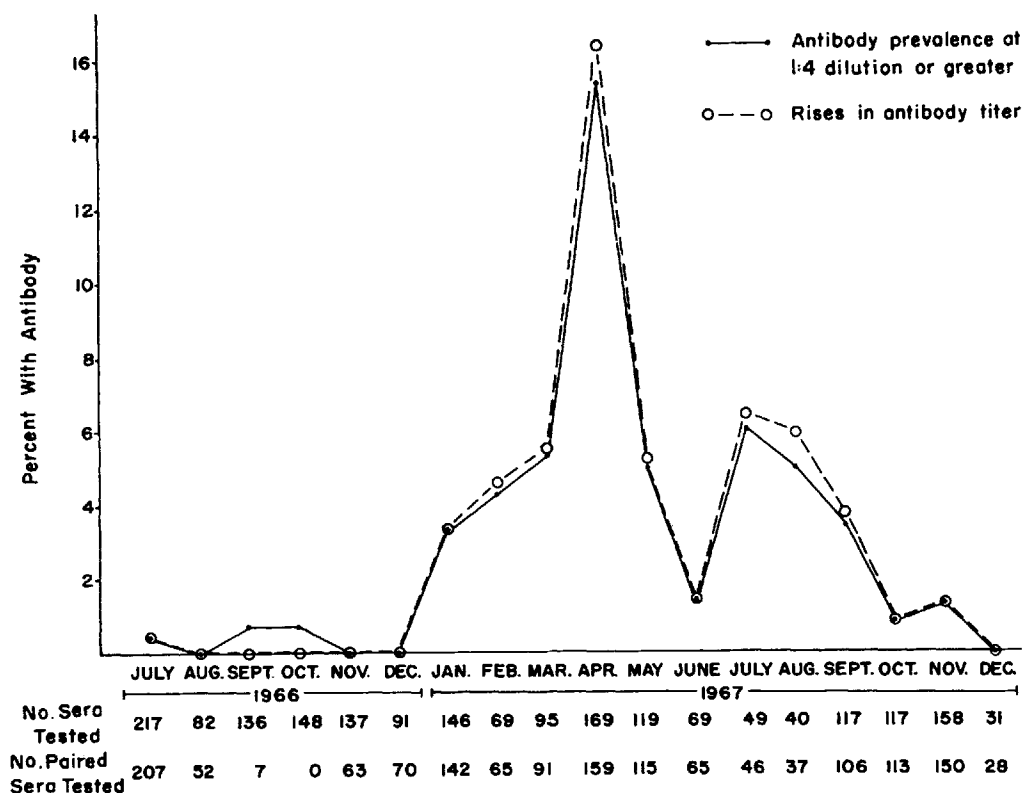
Figure 1. Prevalence of CF antibody to 229E virus and occurrence of rises in titer of CF antibody, 1966–1967.

view of the usual delay in production of antibody, an increased incidence of infection probably occurred also in March, 1967.

Calculation of results in terms of actual rises in antibody titer produced the dotted line shown in figure 1; the time indicated for each paired serum was the time of collection of the second serum in the pair. This is nearly identical with the curve of antibody prevalence because of the almost uniform absence of antibody in sera collected in 1966. The similarity also suggested that CF antibody is not of long duration, for had CF antibody persisted in those infected in the spring of 1967, it would be expected to be present in the fall. However, it was not, and, instead of deviating, the 2 curves remained virtually the same.

Relative sensitivity of CF and neutralization tests. The CF test was useful in timing the occurrence of the outbreak, partly because of its apparent lack of persistence. However, its relative sensitivity in detecting infection was unknown. It was, therefore, decided to use the neutralization test as an additional method for studying the

effect of the infection in the community. Sera from all families in which members were bled in April 1967, and from approximately 40% of families with members bled in May through July, 1967, were tested for neutralizing antibody. For each individual, a serum prior to the April to July period had been obtained so that rises in neutralizing as well as CF antibody could be identified. Specimens obtained by disks were not suitable for testing by neutralization.

Results are presented in table 1. For purposes of comparison, individuals were divided into 2 groups, those from whom sera were collected in April and those from whom sera were obtained later. For individuals with blood specimens collected in April, a rise in titer of CF antibody (a + c) occurred in 20 (21.0%) of those tested, while 23 individuals or 24.2% (b + c), showed a rise in neutralizing antibody titer. This indicates that the CF test was almost as sensitive as the neutralization test during this period. Overall, a total of 29 (30.5%) of those tested showed a rise in either CF or neutralizing antibody titer or both.

Table 1. Relative sensitivity of CF and neutralization tests performed on sera obtained during April and afterward

Serologic finding	April		After April	
	No.	%	No.	%
No. with rise only in CF antibody	6/95*	6.3	0/64	0
No. with rise only in neutralizing antibody ..	9/95	9.5	24/64	37.5
No. with rise in both CF and neutralizing antibody ..	14/95	14.7	1/64	1.6
Total no. with rise in antibody	29/95	30.5	25/64	39.1

* Number positive/number tested.

The CF test was much less sensitive in detecting infection in persons with serum collected in the period after April. None showed a change only in titer of CF antibody. A rise in CF antibody titer in conjunction with a rise in neutralizing antibody occurred in only 1 (a + c) of those tested. However, 25 individuals or 39.1% (b + c) showed a rise in titer of neutralizing antibody. Overall, a total of 25 (39.1%) of those tested showed a rise in antibody titer.

Therefore, it again appears that CF antibody persists for a considerably shorter period than does neutralizing antibody, since CF was almost useless in detecting infection in the period after April, while it was nearly as sensitive as the neutralization test in the April period. The CF test would be of value in screening a large number of sera collected shortly after suspected infections, perhaps even in the absence of prior sera. The neutralization test can be used for somewhat greater sensitivity when sera are collected immediately after an outbreak and as the only method to detect infection when they are collected later. Unlike the CF tests, paired sera are always required to detect infection by neutralization.

The only difference between the specimens obtained in April and those obtained afterward was the time of collection of the sera, not the population from which they were drawn. Combination of the total number of rises in antibody titer, whether detected by CF or neutralization, provides a serologic infection rate for the entire group sampled. Of the 159 sets of sera tested, 54 (34.0%) showed a rise in titer, thus indicating the large

impact that 229E-like infection had on the community.

Age-specific occurrence of infection. There is no knowledge of the behavior of 229E infection in various segments of a population in which all ages are represented. Therefore, the serologic infection rates were calculated by age to determine if all segments were equally affected. Results for both CF and neutralization tests are given in figure 2.

All sera under study had been tested by the CF test, and the most complete picture of the distribution of infection among the population could be obtained by examining rises in titers of CF antibody. The percentage of individuals showing a rise in titer increased gradually to age 15. The rate remained high in the 20–29-year-old group, but declined and appeared to plateau in the over 30-year-old group.

The rates for rises in titer of neutralizing antibody in the sub-sample of families are much higher than those for CF because of the greater sensitivity of the test in the period following the outbreak. For purposes of a side-by-side comparison, a different scale has been used in the figure for the CF and neutralization results. In the older age groups, the pattern of the rates is similar to that obtained by CF, even though the number of sera tested by neutralization was much less. The only discrepancy, probably related to these small numbers, is in the over 40 age group, which had the highest rate by neutralization, but the third highest by CF.

These data indicate that all segments of the population showed serologic evidence of infection by a 229E-like virus. Young children were also infected, though at a lower rate. Among those aged 1–4 years, there were 3 instances of infection, the youngest person showing a rise in CF antibody was one year old. This observation is particularly noteworthy, since infection with 229E virus has not been frequently documented in infants and young children.

Aggregation of infection within families. The role of the family unit in transmission of infection was studied by determination of the tendency of rises in antibody titer to cluster in households. Of the 38 families studied, 26 (68.4%) were found by CF and/or neutralization test to have at least one member who showed serological evidence of infection. In 8 of these 26 families, there
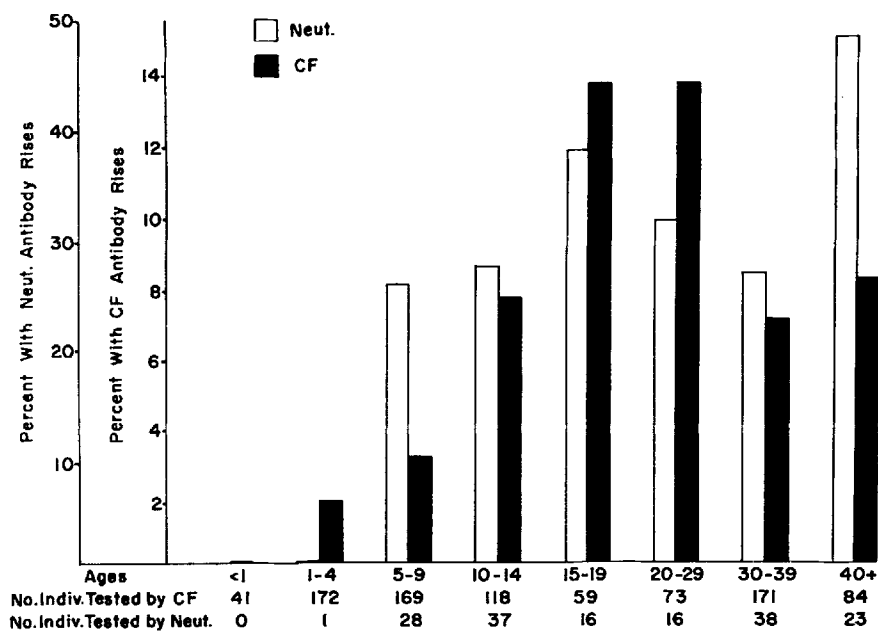
Figure 2. Age-specific rises in titers of CF and neutralizing antibody, 1967.

were 2 individuals infected; in 7 cases 3 individuals and in 2 instances 4 individuals had a rise in antibody titer.

An important observation is that in 8 of the 9 families that had only one instance of infection, the infected individuals were 15 years of age or older. The older children and adults may thus serve as index cases and introduce infection into the family. In these particular instances, secondary spread failed to occur.

Association of rises in antibody titer with illness. Individuals with significant rises in antibody titer, whether detected by the large scale CF screening tests or by the neutralization tests, were matched with individuals of the same age who showed no change in antibody titer. Matched controls had a serum specimen collected at the same time as the seropositive serum of the index individual; the same serologic tests were performed on sera from controls as from index individuals. Of the 76 persons who had rises in either CF and/or neutralizing antibody titer, 14 could not be matched with appropriate controls and were excluded from further consideration.

Based upon the CF antibody curve shown in figure 1, the occurrence of illnesses with any respiratory symptoms was compared during the 7-week period from February 26 to April 15, 1967; the number of illnesses was determined on

the basis of reports received during the weekly telephone surveillance. For both the 62 index individuals and the 62 matched controls, there were 434 person-weeks of observations. Index individuals had 42 illnesses in this period, while controls had 23; most of the excess illnesses were upper respiratory in character although 42% did have symptoms of the lower respiratory tract, including productive cough, wheezing, or pain on respiration. The difference between index individuals and controls is statistically significant at the 5% level ($\chi^2 = 6.00$, $.05 > P > .01$). Rates of respiratory illness in the index and control groups were not significantly different in the 7-week periods before and after February 26, to April 15, 1967. The occurrence of multiple illnesses in a single individual did not affect the analysis. Therefore, the infections identified serologically were related to actual clinical illnesses.

Past infection in the community. The outbreak in the spring of 1967 was rather abrupt in onset, with little or no activity of a 229E-like virus in the previous months. Because of the persistence of neutralizing antibody, it was possible to employ the neutralization test to obtain evidence of previous infection in the community. Therefore, sera collected from July 1966 to December, 1966 were tested to ascertain whether such prior infection had occurred. Results are
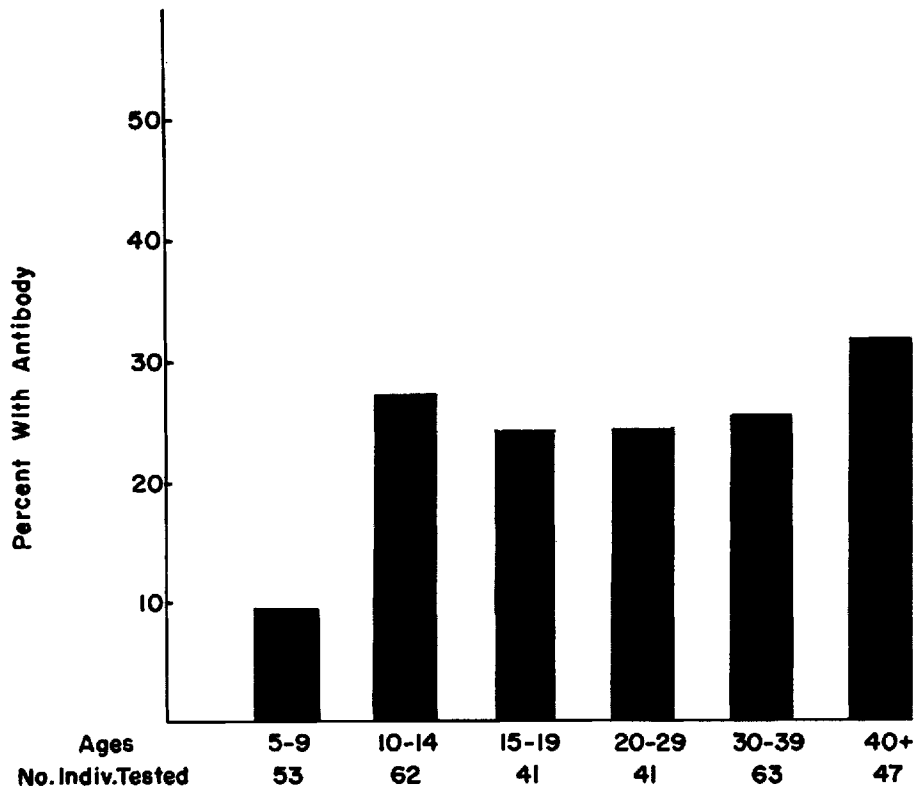
Figure 3. Age-specific prevalence of neutralizing antibody to 229E virus in sera collected in 1966 before the outbreak.

shown in figure 3. Neutralizing antibody was present at 1:4 dilution in each of the age groups tested, with only the 5–9 group showing markedly lower prevalence. Hence, the community had clearly experienced exposure to 229E-like viruses prior to 1967. The antibody in the 5–9-year age group was detected in children 6 years of age or older. Infection of the community must, therefore, have occurred in 1961 or more recently.

Discussion

Investigation of respiratory infection in Tecumseh was designed so that the behavior of respiratory pathogens could be determined in defined segments of the population of the total community. Both isolation and serologic methods were to be employed in detecting the agents. Isolation of infectious agents was intended to demonstrate their presence in the community but because of practical limitations of the technique, was never expected to define the extent of infection. For this purpose, an extensive collection of sera on a

routine basis was begun; testing of the sera would give information on the impact of a particular agent on the community and its population.

This serologic approach was used to characterize the outbreak caused by a 229E-like virus. The virus was not actually isolated in Tecumseh, since its primary isolation requires cell lines that are sensitive specifically to this virus [1, 8]. Because of the possibility that another coronavirus might have been responsible for the serologic findings in Tecumseh, it is of interest to examine the relationship of 229E to other members of this group.

Recent findings indicate that 229E virus is antigenically distinct from the known organ culture-grown coronaviruses (the National Institutes of Health organ culture strains and the B814 strain) [6, 7, 14]. The 229E virus fails to react in CF and neutralization tests with immune animal sera prepared against the organ culture strains. Likewise, sera of patients from whom the organ culture strains have been isolated do not show a CF response to 229E virus. In addition,

no cross-relationship has been found to exist between 229E and the recognized animal coronaviruses, mouse hepatitis virus (MHV) and avian infectious bronchitis virus (IBV) [6, 7, 14]. Therefore, it would appear that an antigenically similar or identical virus to coronavirus strain 229E was most likely responsible for the outbreak detected in Tecumseh in 1967. This outbreak occurred at a time when 229E was isolated in Chicago (D. Hamre, personal communication) and Washington, D.C. [8], an occurrence that increases the likelihood that it was the infecting agent in Tecumseh.

Utilization of both the CF and neutralization tests provided new insights into the nature of antibody response to this viral infection. The CF antibody response appeared to be of short duration, while the neutralizing antibody appeared to persist for a longer period of time after infection. Presence of CF antibody at 1:4 dilution or greater, even in the absence of an acute-phase serum can, therefore, be taken to indicate a recent infection. The neutralization test can be used not only as a sensitive method for detecting recent infections, but also as the only method for determining the past experience of an individual with this virus. When the test was used for the latter purpose, results from sera collected prior to the 1967 outbreak showed that much of the population had been infected previously.

Previous studies of 229E viral infections have involved mainly college students, adults in special occupational situations, and hospitalized cases of lower respiratory disease [1, 8, 10]. The outbreak in Tecumseh provided the first opportunity to determine the effect of this virus on individuals of many ages living in their own family environment. The results indicated that infection with this virus was common in all age groups. Of particular interest was the fact that young children were also infected, although at a lower rate; the lower rate may mean in part that the virus was acting as a poor antigen in children. Our data confirm the findings of Bradburne et al. [10] that such infection is possible during the first 5 years of life, and suggest that this virus or group of viruses may be responsible for some of the respiratory illnesses of unknown etiology seen in this age group. As expected, infections spread preferentially within families. There was suggestive evidence that the older children and adults

were responsible for the introduction of infection into the family. However, in a number of cases, the infection did not spread to other family members; this may be an explanation for the higher rates of infection found in the older children and adults.

On the basis of the observation that CF antibody to 229E remains elevated for only a short period, the peak of antibody prevalence in April meant that the outbreak of infection must have occurred shortly before that time. When the illnesses of persons with serologic evidence of infection were examined for the period from February 26, to April 15, 1967, a significant difference in respiratory illnesses was found to have occurred in this group as compared with a control group. One may conclude that a definite association existed between the 229E-like virus which elicited the antibody responses and the clinical illness seen in seropositive individuals. These data confirm, in a natural setting, the etiologic relationship of 229E viral infection to respiratory illness; this relation has been demonstrated previously only in studies of volunteers [2, 10].

This outbreak in Tecumseh was widespread, with 34.0% of the population tested showing serologic evidence of infection. The sampling method employed in this study insured that this rate is a true representation of the impact of 229E virus infection in the community. The agent must, therefore, be considered a major human pathogen. There is no indication whether these findings for 229E-like infection would also apply to other human coronaviruses. Should other members exhibit a similar pattern of infection, together they could be responsible for a significant portion of respiratory illnesses of man, which up to now have been of unknown etiology.

Summary

The occurrence of 229E-like virus infection in a natural population was determined by a survey of sera collected from families in Tecumseh, Michigan. Sera were studied by the complement fixation (CF) test, and a sharp outbreak of 229E-like virus infection was detected in the community in the spring of 1967. The serologic infection rate in a representative group of families, as determined by the combined results of both the CF and

neutralization tests, was 34.0% during the periods studied. All segments of the population were affected. This virus tended to spread preferentially within families. Secondary cases were found in 17 of 26 families in which infection was detected; in all but one of the families in which the virus was introduced but did not spread, the single infection was in a person 15 years or older. Serologic evidence of infection was associated with an increased number of illnesses reported during February–April 1967; most of the illnesses were upper respiratory in character.

The determination of CF and neutralizing antibody responses to 229E-like infection suggests that CF antibody to this virus remains elevated and detectable for a relatively short period of time after infection, while neutralizing antibody persists and remains detectable for a longer period of time than the CF antibody. The CF test may thus be used to detect infection with 229E virus if it is performed on sera collected shortly after infection, even in the absence of an acute-phase serum. The neutralization test may be used both to detect recent 229E infections and to determine an individual's past experience with this virus.

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