Inapparent Infection with Western Equine Encephalitis Virus: Epidemiologic Observations

GERALD D. LaVECK, M.D.; JOHN F. WINN, D.V.M., M.P.H.; and SARAH F. WELCH

Any communicable disease against which antibodies can be demonstrated in more than 10 per cent of the population becomes a matter of immediate public health concern. So interest in this study—confined to a part of one western state—will extend far beyond the confines of that state and that section of the country.

In recent years Weld County, Colo., has been established as an endemic focus of western equine encephalitis (WEE). Because human and equine cases were reported in this area yearly from 1937 through 1949, Weld County was selected by the U. S. Public Health Service as an area in which to conduct special encephalitis studies on various facets of the epidemiology of WEE including arthropod vectors, birds, and vertebrate hosts. During the past five years the virus has been isolated numerous times from mosquitoes and birds.[1-4]

In the spring of 1954 a survey of human sera for WEE antibodies was carried out in Weld County with the following three major objectives in mind: (1) to perform a general survey in an endemic area in order to determine the past experience of a population group with WEE infection; (2) to delineate those groups at greatest risk of infection with respect to age, sex, place of residence, length of residence, and occupation; and (3) to determine by paired sera the number of individuals who developed inapparent infections with WEE virus over a given period of time. In order to fulfill this latter objective, it was necessary to bleed again a number of individuals in the winter of 1954–1955. Therefore, by collecting specimens in the spring and second specimens from the same individuals in the winter, the number of conversions from negative to positive during the summer months might give an estimation of the activity of the virus in the area.

Methods and Material

Selection and Collection of Sera for Survey—From February through June, 1954, human blood samples were drawn from 614 inhabitants of Weld County, Colo. Blood was collected from four sources, the majority of samples being obtained from apparently healthy, adult, volunteer blood donors at the Weld County Blood Bank. The age groups 18–50 were well represented from this source. A few second specimens were obtained from persons who returned to the blood bank. There were 74 specimens from first, second, and third grade

Dr. Winn and Miss Welch are with the Public Health Service, Communicable Disease Center, U. S. Department of Health, Education, and Welfare, Atlanta, Ga. Dr. LaVeck, who had been with the department at the time this paper was prepared, is now in Seattle, Wash.

children. Of these, 63 gave second specimens in the following winter, constituting the principal source of paired sera. A small number of blood samples were from children admitted to the Weld County Hospital for various afebrile illnesses. The fourth source of specimens was from premarital and prenatal bloods submitted to the Weld County Health Department Laboratory for routine serologic determinations for syphilis. This is not intended to represent an unbiased sample of the population of Weld County: findings from this study merely reflect the trends noted in the population sampled and not necessarily the county as a whole.

Approximately 10 ml of blood were collected aseptically by a laboratory technician from each individual in the study. At the same time, the technician completed a brief questionnaire for each donor. Sera were removed aseptically and stored at —40° C. until tested.

Serologic Test—The presence of antibodies was determined by means of the serum neutralization test employing half-day-old chicks [5] obtained from a local hatchery. The L2 – 34a strain of WEE virus obtained from the Communicable Disease Center Virus and Rickettsial Laboratory at Montgomery, Ala., was used. The virus was titrated, using 20 per cent horse serum as a diluent, to find the chick LD_{50}. Each serum specimen was tested at two levels. The virus inoculum varied from 6.8 to 32 LD_{50} at the lower level and from 68 to 320 LD_{50} at the higher level. Inoculum containing an appropriate LD_{50} was added to an equal volume of serum to be tested, and the mixture incubated at 4° C. overnight. For each level, four chicks were inoculated subcutaneously, each with 0.03 ml of virus-serum mixture. The chicks were observed for 72 hours and the number of specific deaths was recorded.

The presence of specific antibodies against WEE virus was demonstrated by protection of those chicks inoculated with the virus-serum mixture. If no antibodies were present in the serum, three or four chicks would become weak and prostrate and die within 24–40 hours. In contrast, if three or four chicks lived for 72 hours, sufficient antibodies were present in the serum to neutralize the amount of virus inoculated. A serum containing enough antibodies to neutralize from 68 to 320 chick LD_{50} is considered positive, while a specimen with antibodies that neutralizes from 6.8 to 32 chick LD_{50} but fails to neutralize the higher concentration of virus is weakly positive. A result is considered equivocal when two out of four chicks survive after receiving the low concentration of virus and all four chicks die with the higher concentration.

All persons with a positive or a weakly positive serum were queried to determine whether there was a past history of clinical encephalitis.

Results

Of 614 sera tested, 30 sera protected against both levels and are considered strongly positive (++), while 37 neutralized the low level but not the high level virus and are classified as weakly positive (+). Twenty-one were found to be equivocal for antibodies. By combining ++ and + sera, 10.9 per cent of the population sampled were positive for WEE antibodies, Only one of the 67 individuals with positive serum gave a history of having had encephalitis in the past (Table 1.) This 49-year-old farmer, whose serum is now weakly positive for WEE, lives on irrigated land 15 miles south of Greeley and was hospitalized in 1940 with clinical encephalitis. The remaining persons with positive sera had subclinical or unrecognized infections with the WEE virus.

Table 1—Inapparent Infections with Western Equine Encephalitis, as Measured by Neutralizing Antibodies, Weld County, Colo., 1954, by Specified Classifications

Classification Groups	Number Tested	Results of Neutralization Tests: Number ++	Number +	Number Equivocal	Per cent Positive (++ and +)
Total 1954 Sera	614	30	37	21	10.9
A. Age (years)					
0–14	88	5	0	0	5.7
15–29	184	18	19	8	20.1
30–44	209	5	7	10	5.7
45–59	87	0	6	1	6.9
60 and over	47	2	5	2	15.2
B. Length of Residence (years)					
less than 1	44	1	1	2	4.5
1–4	76	5	3	1	10.5
5–9	116	6	0	2	5.2
10–14	30	0	3	0	10.0
15–19	44	3	5	2	18.2
20–24	59	4	6	3	16.9
25 and over	241	11	19	11	12.4
Unknown	4	0	0	0	..
C. Age and Length of Residence (years)					
Age Residence					
4–14 Less than 5	24	2	0	0	8.3
5 and over	64	3	0	0	4.7
15–29 Less than 10	72	6	4	4	13.9
10 and over	112	12	15	4	24.1
30 and Less than 10	81	1	0	1	1.2
over 10 and over	257	6	18	12	9.3
Unknown	4	0	0	0	..
D. Urban or Rural					
Urban	323	14	17	9	9.6
Rural	291	16	20	12	12.4
E. Place of Residence					
Greeley proper	323	14	17	9	9.6
Greeley fringe-area	76	4	5	3	11.8
Irrigated land:					
north of Greeley	95	6	7	6	13.7
south of Greeley	107	5	7	3	11.2
Nonirrigated land	13	1	1	0	15.4
F. Residence on Irrigated or Nonirrigated Land					
Irrigated	601	29	36	21	10.8
Nonirrigated	91*	11*	2*	1*	14.3*
G. Place of Employment					
Outdoors	149	13	13	6	17.4
Indoors	120	1	4	3	4.2
Similar times of both	345	16	20	12	10.4

Table 1 (Continued)

Classification Groups	Number Tested	Results of Neutralization Tests: Number ++	Number +	Number Equivocal	Per cent Positive (++ and +)
H. Occupation					
Farmers	121	9	10	6	15.7
Skilled and unskilled manual workers	79	5	7	2	15.2
Housewives	131	7	9	6	12.2
Students	110	5	2	2	6.4
Professional and semiprofessional	112	1	4	2	4.5
All others	61	3	5	3	13.1

* Includes 78 specimens collected in 1953 but tested in 1954.
++ Serum neutralizes 68 to 320 chick LD_{50}
+ Serum neutralizes 6.8 to 32.0 chick LD_{50}

Age Distribution—Individuals from 15 to 30 years of age had a prevalence rate of 20.1 per cent for WEE antibodies, which is higher than those of other age groups, as shown in Table 1-A. A second peak in the relative frequency of positive tests was noted in persons over 60 years of age among whom 15.2 per cent were positive. Although rates for persons over 60 were relatively high, a low percentage of positives occurred in those from 30 to 60 years of age.

Sex Distribution—In Weld County, 11.7 per cent of 384 males and 9.6 per cent of 230 females were positive for antibodies as given in Table 2-A. The percentage of positive sera was higher in males than in females in the age group 15 through 29 years, but in those over age 45 the relationship was reversed.

Length of Residence—Individuals who had been residents of Weld County for the past 15 to 24 years had an appreciably higher rate of WEE positive sera than all other residents in the same county (Table 1-B).

By considering both age and length of residence at once, some of the confounding of each in tabulating the other can be resolved (Table 1-C). Of the 88 children, 32 could be listed as definite immigrants with three positives, which cannot be regarded as different from the 56 resident children. Young adults (15–29) still have the highest percentage of positives no matter how they are classified. In this group, residents of at least 10 years revealed 24.1 per cent positive, whereas those with less than 10 years' residence had 13.9 per cent positive. Adults over 30 years of age with at least 10 years' residence had 9.3 per cent positive, and those with less than 10 years' residence had only 1.2 per cent positive.

A study of the sex distribution as related to length of residence revealed that, among those who had lived in the area for less than 20 years, the rate of positives was appreciably greater in males than in females. However, the rate of positives was higher in females than in males for persons in residence 25 years or more (Table 2-B).

Geographic Distribution — Neutralizing antibodies against WEE were detected in 12.4 per cent of the rural and 9.6 per cent of the urban population

Table 2—Inapparent Infections with Western Equine Encephalitis, as Measured by Neutralizing Antibodies, Weld County, Colo., 1954, by Sex and by Age or Length of Residence

Classification Groups	Males					Females				
	No. Tested	No. ++	No. +	No. Equivocal	Per cent Positive (++ and +)	No. Tested	No. ++	No. +	No. Equivocal	Per cent Positive (++ and +)
Total Sera	384	20	25	13	11.7	230	10	12	8	9.6
A. Age (years)										
0–14	40	2	0	0	5.0	48	3	0	0	6.2
15–29	109	14	14	4	25.7	75	4	5	4	12.0
30–44	156	4	5	7	5.8	53	1	2	3	5.7
45–59	57	0	3	1	5.3	30	0	3	0	10.0
60 and over	22	0	3	1	13.6	24	2	2	1	16.7
B. Length of Residence (years)										
less than 1	17	1	1	0	11.8	27	0	0	2	..
1–4	47	4	2	0	12.8	29	1	1	1	6.9
5–9	65	3	0	2	4.6	51	3	0	0	5.9
10–14	17	0	3	0	17.6	13	0	0	0	..
15–19	25	3	3	2	24.0	19	0	2	0	10.5
20–24	43	4	4	2	18.6	16	0	2	1	12.5
25 and over	168	5	12	7	10.1	73	6	7	4	17.8
Unknown	2	0	0	0	..	2	0	0	0	..

++ Serum neutralizes 68 to 320 chick LD_{50}
+ Serum neutralizes 6.8 to 32.0 chick LD_{50}

sampled (Table 1-D). According to descriptions of past epidemics,[6] clinical case rates were greater in rural populations, where exposure to the mosquito vector is presumably greater. The results of this study of inapparent infections in rural and urban populations do not parallel the findings of past outbreaks of clinical infections. The division into rural and urban groups in Weld County may be artificial since the only urban district is Greeley, a town of 21,000 people. Although this is defined as an urban area by the U. S. Bureau of the Census, it may well be classified ecologically as a rural area; thus, the entire population studied might be considered rural and the separation into rural and urban groups of no significance.

Weld County was divided into five regions to determine in which area the population was at greatest risk of exposure to the virus. Table 1-E shows that the sampled populations of all areas had similar percentage positives for WEE antibodies.

Past surveys in 1950 and 1951[7] demonstrated that antibodies were found relatively more frequently in persons living on irrigated land (12.5 per cent) in Weld County than in those living on nonirrigated land (5.7 per cent). This may be due to the lower Culex tarsalis population in nonirrigated areas. Unfortunately, the present survey did not include a sufficiently large sample from nonirrigated land to re-evaluate this relationship. However, for a comparison, laboratory tests were

performed on 78 specimens which had been collected in 1953 from inhabitants of Nunn, Colo. This small town of 395 population is located 20 miles north of Greeley in nonirrigated territory. By including these samples with those obtained in 1954, 10.8 per cent of sera from persons residing on irrigated land were positive as compared with 14.3 per cent on nonirrigated land, Table 1-F. Therefore, it would appear from this survey that inhabitants of both irrigated and nonirrigated areas have been subjected to similar risks of developing inapparent infections with WEE virus.

Occupation—In order to determine the effect of occupation on inapparent WEE infections, the sampled population was classified into three groups. Table 1-G reveals that 17.4 per cent of the population whose occupations required a great deal of outdoor activity had antibodies while only 4.2 per cent of those employed indoors had demonstrable antibodies. The group that worked both indoors and outdoors was intermediate with 10.4 per cent positives. The three groups are similar in regard to sex distribution and place of residence, but the group that worked both indoors and outdoors contains a number of students which lowers the age distribution in that group. However, the comparison between the indoor and outdoor categories is probably valid. As might be expected, farmers and manual laborers lead other occupational groups in percentage of positive reactions (Table 1-H).

Incidence of Inapparent Infections—To determine the incidence of inapparent WEE infections developing during the summer of 1954, sera were obtained in the following winter from 78 volunteers who had been bled in the spring. Sixty-three paired sera were from pupils in one school in Greeley, and 15 pairs of sera were collected from donors at a blood bank.

Five individuals with paired sera had WEE antibodies in both specimens. The remaining 73 pairs were negative for specific antibodies. From this small and biased sample there was no evidence of inapparent WEE infections acquired during the summer of 1954. Just one clinical case of WEE was reported in Weld County during the year. Additional sera from a number of individuals tested at regular intervals might clarify the relationship between clinical and subclinical infections and reveal the patterns in which subclinical infections may be manifested in the host.

Discussion

In this study sera that neutralize 6.8 to 32.0 LD_{50} and sera that protect against 68 to 320 LD_{50} are both considered positive. A two-level screening procedure was used rather than determining a neutralization index, because of the simplicity and the sensitivity of the former. A preliminary survey was conducted in Weld County in 1950 and 1951[7] in which 10.6 per cent of the population sampled had WEE antibodies. Mice were employed as the laboratory animals and a neutralization index of 50 or greater was considered positive; an index of 32 to 50 was classified as equivocal. By including both $++$ and $+$ sera as positive in the 1954 survey, 10.9 per cent of the sera had antibodies. Therefore, this 1954 study, which employed chicks, is in close agreement with the 1950 and 1951 survey in which mice were used.

A serum which neutralizes no more than 6.8 to 32 chick LD_{50} of virus may represent a waning antibody titer from a previous infection with WEE virus. An equivocal result may denote an even lower antibody titer due to a still longer period of time since infection. Higher rates of weakly positive and equivocal sera are noted in individuals who have

resided in Weld County for more than 15 years.

Outbreaks of clinical arthropod-borne encephalitis were reported each year in Weld County during the summer seasons from 1938 through 1941 and to a lesser extent in 1944 and 1949. Past epidemics in California and elsewhere indicate that the attack rates for clinical WEE infections are frequently highest in infants, young children, and elderly people.[8, 9] An explanation for Weld County's high rates in groups under age 30 might be resolved by the fact that the severe outbreaks from 1938 through 1941 resulted in subclinical or undiagnosed WEE infections in children and in older individuals in their fifties and sixties. After 14 to 17 years high rates are still present in these same persons now 15 to 30, and 60 years of age and over. Residents of Weld County who have lived in the area for the past 15 to 24 years showed a higher rate of positive sera than other residents. This is in accordance with the premise that many of these people were infected during 1938 to 1941 outbreaks and have retained serum neutralizing antibodies since that time.

Several hypotheses may be proposed from these results. The young adult immigrants in this study apparently had an appreciable exposure before moving to Weld County. Older immigrants either came from clean areas and were not subjected to exposure, or had reverted to serologic negatives and were not re-exposed. Older adults in general had lower percentages of positives. The total data lend credence to a theory of Weld County being an enzootic area with age-sex-occupational hazards defining the exposure and re-exposures, and WEE being a disease which produces an immunogenic response that may not be serologically evident after a period in the magnitude of 1–10 years without re-exposure. For example, the predominance of positive sera from individuals under age 30 years as compared to those over age 30 might be due to an increased exposure to mosquitoes because of occupational differences. It is known that Culex tarsalis bites human beings most frequently just prior to darkness.[10] Many farmers, filling station attendants, ranchers, and others employed outside work in the evenings and are exposed to bites at that time. Although this undoubtedly is a factor, indoor as well as outdoor workers under age 30 had higher rates of positive sera than indoor or outdoor workers over age 30. From these data it is our impression that the age distribution of persons with positive sera is not entirely a function of occupation but may be the effect of unknown factors.

Observations from past experience in California [8] and Colorado [7] indicate that clinical attack rates for WEE are about twice as high in males as in females. It has been suggested that this may be a reflection of occupational differences and the opportunity for exposure. Lennette and Longshore [6] have stated that such an explanation is not entirely satisfactory because the differences in sex distribution are also noted in childhood age groups. In this study 11.7 per cent of males and 9.6 per cent of females had had inapparent infections as evidenced by WEE antibodies. The percentage of positive sera was higher in males than females in the 15- to 30-year-age group, but the relationship was reversed in those over 45 years of age. This is not in complete agreement with the published sex distribution of clinical infections.

Although the sample of paired sera is inadequate to make an evaluation, the lack of conversions and the rarity of clinical encephalitis cases emphasize the low risk of infection for human beings during the summer of 1954.

Because of unfavorable climatic factors the mosquito population was low in Weld County in 1954. A more extensive study would be necessary to estimate the conversion rate of inapparent infections.

Summary

Sera obtained from 614 inhabitants of Weld County, Colo., during 1954 were tested for neutralizing antibodies against WEE to determine the frequency and epidemiologic pattern of inapparent infections. Antibodies were demonstrable in 10.9 per cent of the sampled population. For persons under 30 years of age the rate of positive sera was twice as high as that for persons over age 30. Although males and females had similar rates when all ages were considered, the percentage of positives was greater in males than females in the age group 15 to 30 years, but was higher in females over age 45. Residents who had lived in the county for the past 15 to 24 years had higher rates than other residents. No significant difference was noted in the frequency of inapparent infections in rural and urban areas nor in irrigated and non-irrigated land. A higher per cent of persons with outdoor employment had positive sera than those with indoor employment. Farmers and manual laborers led other occupational groups in percentage of positive reactions.

Seventy-eight individuals were bled in the spring of 1954 and again in the fall to determine the incidence of inapparent WEE infections during the summer months. None converted from negative to positive.

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