DISCUSSION

EPIDEMIOLOGY OF SAINT LOUIS ENCEPHALITIS[1]

PREVIOUS studies of the 1933 epidemic of St. Louis encephalitis failed to reveal a "concentration of cases in any sharply localized section of the city and suburbs."[2] By a statistical method which will be described in detail elsewhere it has been possible to detect a concentration of cases in a number of areas in St. Louis City and County for the epidemics of 1933 and 1937. Briefly the method consists of plotting on a given map not only the encephalitis cases but also an additional number of control cases. The control cases in the present study were taken from the only two sources known to be available.

The first controls were from general mortality records on file in the departments of vital statistics of the county and city. The sample consisted of the home addresses of all persons dying in St. Louis City and County in 1935 who were 5, 15, 25, 35, 45, 55, 65, 75, 85, 95 years of age. The second control series was taken from the city and county directories of the appropriate years so that 22.5 per cent. of the home addresses were from the county and 77.5 per cent. from the city (estimated population of county and city). Sampling was effected by selecting home addresses situated in a constant position on successive or alternate pages of the directory (*e.g.*, first home address in upper right-hand corner, etc.). There were 1,212 home addresses in the mortality sample, 2,453 in the directory samples, 1,130 reported encephalitis cases in the 1933 epidemic and 518 reported cases in the 1937 epidemic. The mortality and directory samples of populations from the twenty-six health districts in St. Louis, eighteen incorporated and twenty-one unincorporated communities of St. Louis County were tabulated and tests of homogeneity calculated.[3] Using the chi-square test no lack of homogeneity between the two control series was detected and the two control series were further checked by comparing the variation of the sampling per district and community with the United States Census figures for 1930. The next step was a systematic canvass of the map with a compass constructing circles with half-mile radii. In the area of each circle a test was made to determine whether a statistically significant preponderance ($P = 0.01$ or less) of control over encephalitis cases or *vice versa* occurred. Thus it was possible to disregard city and district lines and to shade those areas with a preponderance of encephalitis and to cross-hatch those in which a significant scarcity of encephalitis occurred. Statistically significant concentrations of reported encephalitis cases occurred in the two epidemics. In both epidemics the disease appeared first and simultaneously in these same widely separated areas and continued to be most prevalent there during the remainder of the epidemic.

After a study of the terrain it became obvious that all foci of encephalitis were situated within a mile and all except one within a half mile of the small streams in St. Louis and St. Louis County. The streams all or nearly all carry sewage. The encephalitis shading ended sharply in West End district, where the River des Peres is buried, and began sharply in the Oakland district where the same stream rises to the surface again. The survey revealed a fairly close correlation between the occurrence of epidemic encephalitis foci and the lack of outdoor sanitation in an area as judged by the presence of weeds, open sewage, ponds, streams, garbage and tin-can dumps. Ninety-five per cent. of the inhabited weed, open sewage, stream and pond area of St. Louis showed a significant ($P = 0.01-$) or probably significant ($P = 0.05$ to 0.01) preponderance of encephalitis and 87 per cent. of all cases occurred within a mile of such an area. Every district in the St. Louis area which was without weeds, open sewage and ponds and which was separated a mile or more from such an area was not only without encephalitis foci but actually showed a statistically significant scarcity of encephalitis. About 40 per cent. of the population of St. Louis lived under these fortunate conditions. In this connection St. Louis City had for the two epidemics a combined rate of 109 cases per 100,000 population, incorporated communities in St. Louis County the rate of 216, and unincorporated communities a rate of 420 per 100,000 persons.

The prevalence of encephalitis in sewage, stream, pond and weed areas could not be explained on the presence of more old people in such regions, since the mortality sample used as a control was largely made up of old persons and had the same age distribution as the encephalitis cases. Nor could poverty and poor indoor sanitation explain the prevalence in sewage, stream, pond and weed areas, since the areas in St. Louis with greatest poverty and most unsatisfactory indoor sanitation had among the least encephalitis in the St. Louis area. On the other hand, encephalitis foci did occur in districts where the population was either wealthy or above the average in wealth. The disease did not tend to predominate in the most congested areas and in general actually predominated in the most sparsely settled areas of city and county. The disease has no epidemiological feature in common with known contagious diseases (such as congested housing, prevalence in cold season, multiple cases in

[1] From the Departments of Pathology and Medicine, Saint Louis University. The work was supported by a grant from the Encephalitis Fund.

[2] J. P. Leake and others, *Public Health Bulletin*, No. 214, 1935.

[3] R. A. Fisher, "Statistical Methods for Research Workers," Oliver and Boyd, London, 1932.

the same family or household, etc.) and every known feature of its epidemiology is common to the mosquito-borne diseases, such as yellow fever,[4] malaria[5] and equine encephalomyelitis.[6,7] It shows "a lack of obvious connection between the cases as to contagion, water supply, food supply, or milk supply." In 1933 and in 1937 the disease seemed to appear simultaneously in widely separated areas in St. Louis County and City. The same areas were involved in the two years. Throughout both epidemics the predominance of the cases was in those areas in which it first appeared. It does not occur every year, occurs only in the period of the year between July and October and ends with cold weather. The disease is concentrated in the vicinity of the small streams in St. Louis and St. Louis County in areas characterized by proximity to weeds, refuse dumps, open sewage and ponds.

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LIFE-CYCLE OF A SPOROZOAN PARASITE OF THE OYSTER[1]

SINCE 1930 serious oyster mortalities have occurred in five different coastal regions from Mobjack Bay, Va., to Lake Barre and vicinity in Louisiana. In each instance the oysters have shown an unusually weak condition of the adductor muscle and inability to maintain closure of the shell during dredging, transplanting and shipping operations. Though the exact cause of these epidemics has not been definitely established the microscopical examination of weak and dying specimens from each region has disclosed a heavy concentration of spores in the tissues of the muscle, gills and mantle, the number per oyster frequently amounting to several million. These resistant, thick-walled spores, ovoidal in form, are generally grouped in variable numbers (1 to 16) in hypertrophied host cells and surrounded by a crescent-shaped epispore. The mature spore (length 20 μ, diameter 11 μ) contains a single vermiform sporozoite folded twice on itself and is the resting or final developmental stage in the oyster.

Studies conducted under natural and laboratory conditions show that the meats of dying, infected oysters are readily devoured by the common mud crabs, *Panopeus herbsti* and *Eurypanopeus depressus,* in the intestine of which hatching of the spores takes place. The sporozoite escapes through a micropyle and migrates to the epithelium of the gut, where it becomes attached by a globular epimerite. In 15 to 20 hours it develops into a very small, delicate, cephaline gregarine which soon detaches and undergoes precocious coupling with other sporonts (primary sporadins), forming syzygies of 2 to 10 individuals in chain-like formation. The chains break up and the young gregarines, after another short period of attachment, develop into short, cylindrical sporonts (hypersporadins) having a length of 18 to 30 μ. The characteristic gregarine stage in the crab has a granular, spherical protomerite with a lens-shaped, epimeric cap of hyalin material. The long deutomerite, rounded at the end, has a more dense, granular cytoplasm containing a large vesicular nucleus with single nucleolus. A clear, well-defined cuticle surrounds both parts and forms a wedge-shaped "muscular collar" or septum at the point of junction.

The sporonts grow to a considerable size and form syzygies of two individuals of unequal length in which the primites attain a length ranging from 275 to 342 μ and the satellites from 220 to 286 μ. These migrate to the rectum of the crab, and after becoming attached to the cuticle by an adhesive disc, coil up and form strong, thin-walled gametocysts, varying in diameter from 81 to 192 μ. The two gregarines in each gametocyst undergo extreme nuclear division into gametes which, after a period of mixing, fuse in pairs forming zygotes that become arranged radially in small groups around slightly larger central cells. These rosettes of zygotes, or gymnospores, 4 μ in diameter, are released into the water with the rupture of the gametocysts and are carried into the shell of the oyster by the feeding current. The possibilities of heavy oyster infections are great considering the general abundance of the crabs and their close association with the mollusc, and the fact that 40 to 86 gametocysts, containing from approximately 8,000 to 90,000 gymnospores each, may be produced in a single crustacean host.

Experiments with vitally stained gymnospores show that they attach to and penetrate the epithelium of the oyster gill by means of a pseudopod projected from the central cell. Though the zygotes may develop here to maturity they generally are picked up by the phagocytes and transported in the circulatory system to nearly all parts of the body. In the blood vessels and sinuses of the gills, mantle and muscle the infected phagocytes accumulate in large numbers and increase to a considerable size (diam. 30 to 100 μ) with the rapid growth of the zygotes and their transformation into sporozoites. After each sporozoite has surrounded itself with a heavy, double-walled sporocyst, the parasite has reached the characteristic resting or dissemina-

[4] Walter Reed and others, "Yellow Fever, A Compilation of Various Publications," Government Printing Office, Washington, 1911.

[5] Sir Ronald Ross, "The Prevention of Malaria," John Murray, London, 1911.

[6] C. Ten Broeck, F. W. Hurst and E. Traub, *Jour. Exp. Med.*, 62: 677, 1935.

[7] M. H. Merrill and C. Ten Broeck, *Jour. Exp. Med.*, 62: 687, 1935.

[1] Published by permission of the U. S. Commissioner of Fisheries.