Entomological Survey Methods

By ROBERT E. SERFLING, Ph.D.

Public health entomological surveys are primarily directed toward measurement of the relative abundance of arthropod vectors of human diseases. In the United States these include the mosquito vectors of malaria and dengue, mosquitoes and other arthropods implicated in the natural history of infectious encephalitis, ticks which transmit Rocky Mountain spotted fever and tularemia and which have been implicated in Q fever, certain flies which have a role in the spread of bacteria causing diarrhea and enteritis, rat ectoparasites which transmit typhus, and ectoparasites of wild rodents responsible for sylvatic plague.

The amount of information currently available on the epidemiology of the arthropod-borne diseases varies considerably from one disease to another. The relationship of mosquitoes to transmission of malaria has been worked out in great detail, and a large body of information is available on the habits and behavior of *Anopheles quadrimaculatus*, the only important vector of malaria in the southern United States. For infectious encephalitis, on the other hand, knowledge of the natural mechanism through which the disease affects man, birds, and other animals has many gaps. *Culex tarsalis* frequently has been implicated as a carrier of the virus, but the complete story on this arthropod and others suspected of transmitting infectious encephalitis remains to be determined. In insect surveys relating to a disease for which the epidemiology has been well worked out, emphasis can be given to quantitative investigation. In a disease for which less information is available, qualitative and descriptive studies of the natural history of suspected vectors take precedence.

Dr. Serfling, an entomologist, is chief of the statistics section, epidemiology branch, Communicable Disease Center, Public Health Service, Atlanta, Ga. He presented this paper June 19 at the Second Conference of Public Health Statistics, School of Public Health, University of Michigan, Ann Arbor.

In surveys of human populations, advance information on location and characteristics of the population is available, and the subject of investigation is the species with which we are most familiar. Census lists, directories, and other sources of information provide the investigator with data from which he can estimate in advance the range and likelihood of his sampling error.

Comparable sources of information on insect populations do not exist. Collection of insects depends on response of members of the insect population to some stimulus, and the resulting sample is similar to those obtained in "mass surveys," or in some epidemiological surveys in which bubble gum is used to persuade children to be examined.

In an epidemiological study, it would be desirable to obtain quantitative estimates of the vector population to determine the number and proportion of infected vectors and their relationship to levels of human infection. Ross (*1*) and others have investigated this problem theoretically and have obtained useful general information through arbitrary estimates of the necessary constants, but direct estimates have not been attempted. In applied public health entomology, indexes of prevalence replace estimates of population, and efforts to measure the proportion of infected vectors are beset with many difficulties.

The various methods of obtaining indexes of insect prevalence take into account the insect's reaction to light and food, to suitable sites for egg-laying, and to other physiological stimuli. And reactions of closely related insects to such stimuli may differ markedly.

The success of many collecting methods depends on the skill and experience of the collector. Although tests of a collector's ability can be designed, and are sometimes employed, the entomologist usually appraises the collector's skill subjectively. Unless readily evident discrepancies occur, the entomologist accepts the collector's findings.

A description of some common methods of obtaining indexes of prevalence of insects will enable the reader to understand the sampling problems.

Collection Methods

Exposure of human bait provides a direct index of local mosquito prevalence. In one method, the collector drives his car to a collecting point at night, turns on the dome light, removes his shirt, rolls down the car windows, and captures the mosquitoes as they enter and settle upon him. A quantitative index may be obtained by exposure during some standard time interval. This and other "biting catch" methods require a detached interest usually found only in the enthusiast, and for routine collections more prosaic and less direct methods are the usual practice.

The mosquito light trap employs an electric light bulb as an attractant and an electric fan to create a current of air which forces the mosquitoes through a wire net cone into a killing jar. This is a useful device for collecting many species. A disadvantage is that the light source is also attractive to many other insects, and the mosquitoes must be sorted from a hodgepodge of moths, beetles, and bugs. The time spent in sorting out the mosquitoes and identifying the species of interest severely limits the number of collections which can be handled currently.

Anopheline mosquitoes in the United States have a habit of resting during the daytime in shelters near their breeding places or source of food. This trait enables direct collection of adults from sheds, small barns, culverts, and other structures in the vicinity of human or animal habitations.

However, different collection methods may not yield comparable indexes. Response of a mosquito to an electric light or to a source of blood may be quite different. Light traps, for example, attract anophelines in very small numbers, but they draw some of the culicine species in great abundance. A collection procedure appropriate to the survey objective must be selected and then used consistently. Although the results cannot be taken as a measure of the total mosquito population, they will provide an index of seasonal change or difference in abundance from one area to another.

Mosquitoes may also be collected in the larval stage, and for this purpose the familiar household water dipper is widely used. Use of the dipper must be adapted to the behavior of the species under study, and the collector must be trained to recognize the habitats and behavior of the various species. Some individuals develop the knack of collecting much better than others, and these differences in collecting skill may be great. Ten "dips" are often used as a standard number for inspection of a limited area, but the numbers actually taken may vary widely. The results are useful principally in determining presence or absence of larvae. Anopheline larvae can readily be distinguished from larvae of other groups of mosquitoes, but even highly skilled collectors, thoroughly familiar with the local species, can only recognize a few distinctive ones in the field. The larvae must therefore be returned to the laboratory for identification. This places a limit on the number of collections to be taken.

Flies

For domestic flies, collection of adults is the principal survey method. Although larvae may also be collected, they are difficult to identify and are usually reared to the adult stage for identification.

Flies may be captured by traps baited with materials attractive as food or as egg-laying sites. The housefly and several species of fleshflies and blowflies are of interest, and mixed baits containing substances attractive to the various species are employed. The traps are usually exposed for 24 hours. The time con-

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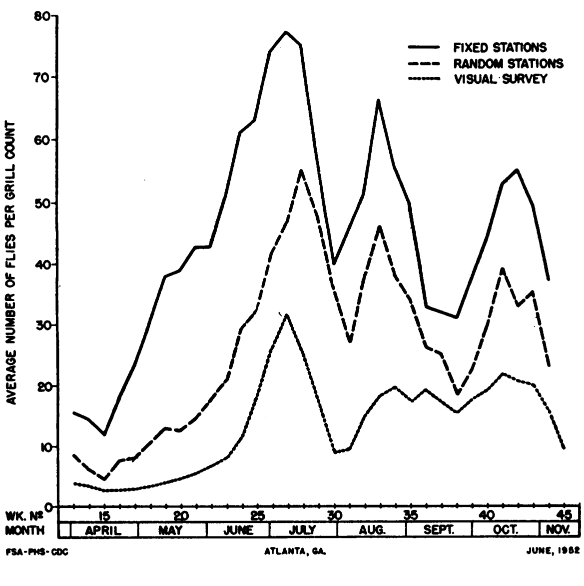
sumed in setting out the traps and in collecting and identifying the catch limits the number of traps which can be used. As the bait trap competes with natural attractants, interpretation of catch differences from one area to another may be difficult. Bait-trap catches are used chiefly to obtain qualitative information on occurrence of the various species and on broad differences in composition of fly populations.

A device which has been developed and widely used in Communicable Disease Center fly surveys is the Scudder grill (*2*). It consists of a light framework of slats 36 inches long and ¾-inch wide arranged to form a 36-inch square of parallel slats separated by ¾-inch spaces. The grill was originally designed to provide a light-colored background for estimating numbers of flies in dairies and restaurants but has been adapted by entomologists to many other situations. In use the grill is slipped over an attractant—garbage, manure, or other debris at which flies gather. The flies, disturbed by the grill, rise through the open spaces and settle momentarily on the slats. The inspector estimates the number of flies of each several kinds. Tests indicate that counts are fairly accurate up to about 25 flies. For larger numbers of flies, counts are made of a section

Figure 1. Comparison of three indexes of fly prevalence in a residential area having a low sanitation level, Phoenix, Ariz., 1950.



of the grill and the totals are estimated. Variability of estimates of larger numbers is roughly proportional to the total number of flies on the grill. Maximum estimates are of the order of 1,000 flies. In urban surveys 5 to 10 grill readings are usually made in a city block and the five highest counts are recorded. Various statistics have been used or proposed for use on these readings. These include the highest count, the third highest count, and the arithmetic mean or total of the five highest counts. A logarithmic transformation of the data has been found useful in some analyses.

A cruder index is the "visual estimate" technique, in which the inspector examines the attractants in a block and estimates the average grill count for the block. In using this method the inspector makes a grill reading in approximately 1 block out of 10 in order to stabilize his estimates.

Ectoparasites

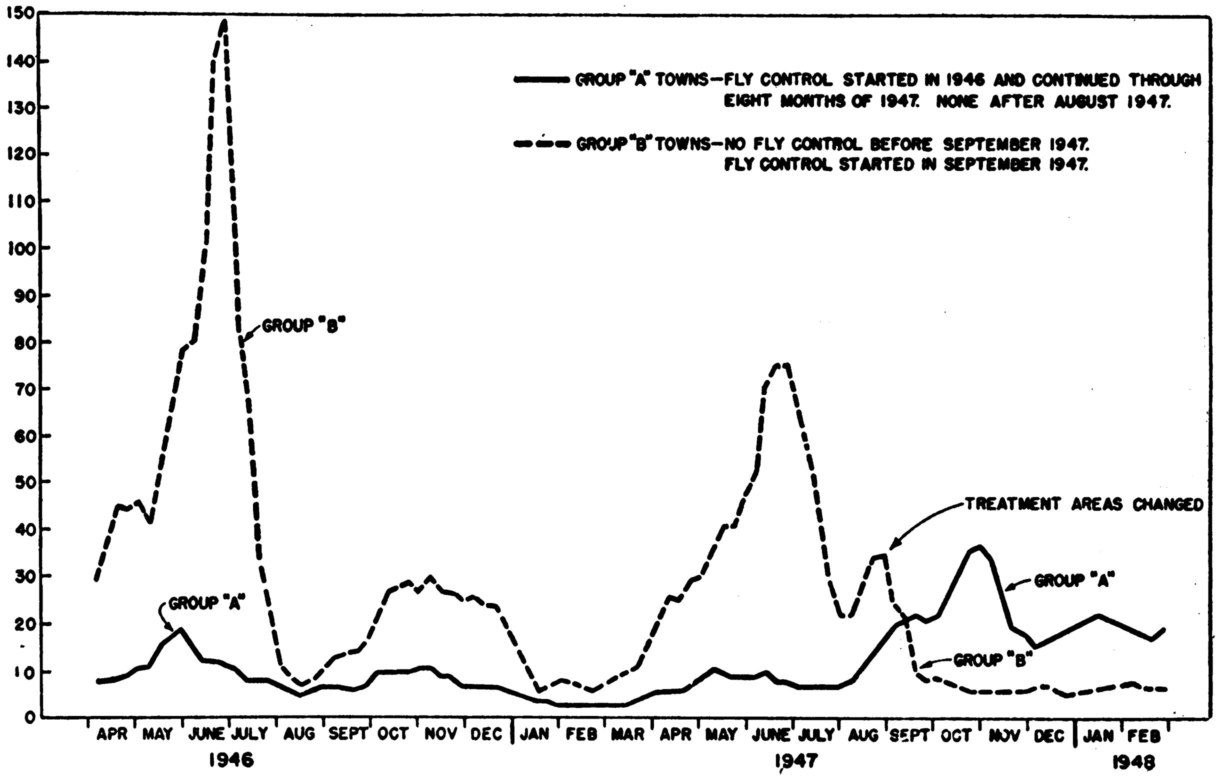
Fleas, ticks, and lice associated with rats and other rodents are collected in studies of typhus and plague. In typhus investigations the rats are trapped and anaesthetized, and their parasites are combed out, identified, and counted. In studies of sylvatic plague, wild rodents are collected and their ectoparasites pooled for laboratory determination of plague infection. The field survey problem in these diseases is essentially one of sampling animal populations.

To control the dog tick, *Dermacentor variabilis*, vector of Rocky Mountain spotted fever in the eastern United States, a direct measure is obtained of the prevalence of ticks likely to feed on man. For this purpose, a light-colored piece of flannel is drawn along the grass at the edges of pathways and roadways used by pedestrians (*3*). The ticks attach themselves to the flannel cloth and are counted, providing an index of relative prevalence which is expressed as the number of ticks per 1,000 yards dragged.

Types of Surveys

Communicable Disease Center entomological surveys fall into three classes: (*a*) exploratory surveys to determine the fauna of an area; (*b*) surveys to appraise effectiveness of insect con-

Figure 2. Average of high grill-index of total flies, 3-week moving average. Diarrheal disease control studies, Hidalgo County, Tex., 1946–48. (Redrawn from ref. 4.)



trol operations; and (*c*) surveys included as part of epidemiological field studies.

Exploratory

In faunal surveys, the contribution of formal sampling techniques is minor. Resources are usually limited, and, although the same logical principles that underlie sampling theory come into operation, decision on choice of collecting method and distribution and selection of sampling sites is made largely through the entomologist's knowledge of the behavior of the insect species and the ecology of the area to be surveyed.

Control Operations

The design of surveys required to appraise control operations must meet a twofold objective: provision of information through which control operations may be directed and appraisal of the effectiveness of control. Fly control operations illustrate the problem. An urban community may be broken into areas with different sanitary levels, but within these areas conditions may fluctuate from week to week. Cast-off garbage, animal feces, dead animals, and other sources of breeding material for flies may occur in one place one week, in a different place the next. Blocks that are relatively free of breeding places at one examination may be producing large numbers of flies a week or two later.

In 1949 the Communicable Disease Center made a study of fly-sampling methods, using the Scudder grill as an index, and collected data in Topeka, Kans., and Charleston, W. Va. In each city, a series of random samples of paired blocks was selected systematically from an ordered series of block numbers. A different series was examined each week and comparison was made of variances within pairs of blocks and among pairs within sections. The within-pair variances turned out to be of about the same magnitude as the variance among pairs within sections, and it was evident that surveillance as a guide to selective control would require weekly examination of all blocks within the area in which control was to be maintained.

Since the Scudder grill technique was too time-consuming for such extensive surveillance, the visual survey method was introduced, and in 1950 the results were compared with the results of using the Scudder grill in the field. The urban areas under study were subdivided into units of about 10 city blocks of similar type. In each unit a "fixed station" (the block in the unit which would be expected to have the greatest fly prevalence) was selected by the entomologist. A second block from each unit was selected at random. The fixed station was checked each week with the Scudder grill, and each week a different random station from each unit was also checked with the Scudder grill. Every block in each unit was examined weekly by the visual survey method. According to H. F. Schoof (unpublished data), similar results were obtained by all three methods in an area having a low sanitation level (fig. 1). In an area of better sanitation, results by the random-block grill survey and the visual survey were similar, but the fixed-block grill survey displayed peaks which were not reflected by the other two indexes.

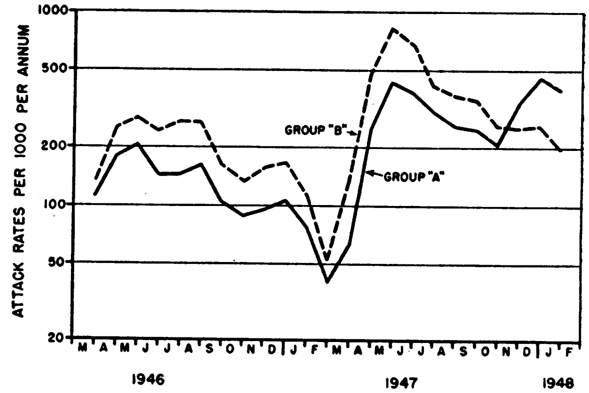
The more flexible response of the fixed-block survey to extreme conditions is an advantage in some scientific investigations, but in routine control operations it suffers from the serious disadvantage that the fixed blocks may receive greater attention than the random blocks.

Many variables influence the behavior of flies. The time of day at which a count is made, temperature, amount of cloud cover, precipitation, and wind velocity can cause marked variation in grill counts. The resulting data may seem very difficult to appraise if attention is focused on these and other sources of variability. In the aggregate, however, the data provide adequate information on points of primary importance.

Epidemiological Field Study

In an early field study in Hidalgo County, Tex. (*4*), simple indexes were used to determine whether fly control in a poorly sanitated area would lower the incidence of diarrhea and enteritis. A group of small communities was divided into two sets. For a period of several months, April 1946 through August 1947, the flies were controlled in one group but not in the other. Control was accomplished by application of residual insecticide every 6 weeks, with spot re-treatment when necessary. At that time, development of resistance of flies to DDT had not become a problem. In September 1947, fly control was discontinued in the first group of communities and begun in the second group. In each group a fixed set of blocks was examined for flies each week. The three highest grill counts in each block were recorded, and the highest reading was used as a statistic for comparison with data on diarrheal disease incidence collected from residents of the area.

Figure 3. Reported diarrheal disease in children under 10 years of age, 2-month moving average. Diarrheal disease control studies, Hidalgo County, Tex., 1946–48. (Redrawn from ref. 4.)



From these area residents, data on incidence of diarrhea was obtained through household visits by interviewers. The infection index chosen was the attack rate among children under 10 years old, and from this group monthly samples of stools were cultured for *Shigella* and *Salmonella.* A summary of results on fly prevalence and incidence of diarrhea in the treated and untreated communities is presented in figures 2 and 3. The many sources of variability in individual measurements apparently did not impede recognition of the main effects of the treatment.

Interpretation of Results

In some entomological surveys, there may be no comparable control population from which a random sample may be selected. This point may be illustrated by entomological appraisal of the effectiveness of treatment of households with residual insecticide during the final stages of malaria eradication in the United States. The counties selected for treatment were those which in the past had had the highest malaria rates. In these counties, densely inhabited areas with the heaviest *A. quadrimaculatus* breeding potential were given preference for treatment. Hence, there was no comparable control area. Selection of individual comparable houses in the treated areas and in the adjoining untreated areas was left to the discretion of the entomologist.

The index chosen was a simple one—presence of live *A. quadrimaculatus* in a home during the afternoon. Movement of these anophelines during the daytime is negligible so that it could be assumed that any found in homes during the afternoon had entered not later than the preceding night. Because the residual insecticide was slow in action, no inspections were made during the morning hours.

In the course of 4 years of work, approximately 47,000 treated and 5,000 untreated houses were inspected (*5*); live *A. quadrimaculatus* were found in 1.7 percent of the treated and in 16.0 percent of the untreated houses.

The difference between the two percentages is manifestly significant by any statistical test. However, interpretation of the significance depends upon one's judgment of the ability of the entomologist to select comparable samples of treated and untreated houses. Although this is an extreme example, it is illustrative of the qualitative judgments which enter into most entomological sampling problems.

Results of entomological surveys are influenced by many variables which cannot be controlled and by many subjective factors not amenable to routine measurement. Statistics used to describe the findings should be simple and readily grasped by the epidemiologist, the entomologist, and the engineer. By using simple statistics, qualitative interpretations may be made jointly by the statistician and his colleagues in the natural sciences. Interpretation of elaborate statistical methodology depends in many instances on the statistician's intuitive appraisal of the extent to which theoretical assumptions may be violated. On these questions the natural scientist usually has less basis for making judgments, and he may regard the methodology as an "ivory tower," and of questionable value. Therefore, the statistical presentation should be in terms of statistics whose import is fully understood by all members of the group.

Many phases of an entomological survey may give rise to special problems for which planned experiments of complex statistical design are necessary. Among these are tests of collecting devices, individual differences among collectors, and design of subsampling methods for rapid estimation of the composition of trap collections.

However, even in the planned entomological experiment, the intangibles of skill, effort, and thoroughness play a leading role, and the statistician must learn to reckon with these factors as familiarly as with the tools of his statistical methodology.

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