Epidemiological Concepts and Historical Examples

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Glossary

fomite (singular: fomes)   Articles, such as clothing, food and utensils, tools, or toys, that convey infection to others because they have been contaminated by pathogenic organisms.

incidence   The rate at which new infections or cases occur in a population. The numerator is the number of new events that occur in a defined period; the denominator is the population at risk of experiencing the events during this period.

RFLP   Restriction fragment length polymorphism, a laboratory technique for comparing DNA sequences based on the lengths of DNA fragments produced by cleavage by restriction endonucleases. Often used to identify strains of microbial pathogens to track outbreaks of infectious disease.

seroepidemiology   Epidemiologic study based on testing of characteristic changes in the serum level of specific antibodies.

vector   Any living carrier, frequently an arthropod, that transports an infectious agent from an infected individual (human or animal) or its wastes to another susceptible individual, its food or its immediate surroundings. The agent may actively infect the vector, undergoing specific life cycles in the vector, or may be carried passively from host to host.

Abbreviations

CDC	Centers for Disease Control and Prevention
EBV	Epstein–Barr virus
RFLP	Restriction fragment length polymorphism

Defining Statement

This article explains epidemiological concepts related to acute and chronic infectious diseases, epidemiological principles of causation, methods by which epidemics are studied and analyzed, and strengths and weaknesses of the epidemiological approach. Historical case-studies of both ancient and recent epidemics are used to illustrate these epidemiological concepts.

Introduction

The term 'epidemiology' (epi ($\epsilon\pi\iota$) demos ($\delta\eta\mu o\varsigma$) logia ($\lambda o\gamma\iota\alpha$) = [up]on + people + discourse) has been classically defined (see the Sydenham Society Lexicon) as disease "prevalent among a people or a community at a special time, and produced by some special causes not generally present in the affected locality". The Hippocratic corpus includes several books referring to 'epidemics' and famous authors such as Thomas Sydenham also treated the subject extensively. In current usage, there are three loosely applied terms that describe diseases that occur 'on the people': epidemic, endemic, and pandemic. These are related concepts and are not clearly delineated from one another. When a disease becomes continually present, usually at a more or less constant prevalence, it is called endemic. Examples include Epstein–Barr Virus (EBV) in human populations and plague (*Yersinia pestis*) in ground squirrels in the western parts of North America. Epidemic disease has a more sporadic character, with widely fluctuating prevalences over time and place. Classic examples include influenza, human plague, cholera, and measles. When a disease (either endemic

or epidemic) occurs very widely or nearly globally, it has been termed 'pandemic' (pan = B∀<, all).

Historically, epidemiology evolved from Hippocratic descriptions of similarities of common diseases, to the systematic collection of death rates with the first Bills of Mortality in sixteenth century England, to collections of clinical observations such as Sydenham's description of the great plague of London in 1665 and the quantitative bedside data gathered by the great French physician Pierre Louis in the nineteenth century. With the development of germ theories of disease in the late nineteenth century, epidemiological approaches gained powerful new scientific tools with which to explain and understand epidemics, the natural history of the diseases, their causes and prevention. This understanding has eventually helped to establish diagnostic criteria as well as to set public health policies and guidelines. More recently, developed tools include specific statistical approaches to complex empirical data, the availability of reliable demographic data collected by governments and private organizations, and the development of computer technologies including sophisticated statistical software which can manipulate and analyze large data sets.

Modern epidemiologic thinking is based on the triad of 'host–agent–environment', a concept that requires attention to biological, social, and physical factors. There are natural oppositions that occur in epidemiological studies: the individual versus the population; the biological versus the environmental; and the qualitative versus the quantitative.

Much of epidemiological thinking depends on knowledge of the state of a disease in a specified population. This knowledge, in turn, depends on accurate determination of who is affected, the clear delineation of the population under study, and the nature and effects of the disease. Often it is crucial to know the so-called 'natural history' of the disease, that is, the changes and expression of the disease over time in some sort of 'typical' case. Sometimes it is even important to consider and evaluate the variability of the disease process.

Epidemiologists depend on two basic quantitative measures to describe disease: prevalence, incidence. Both require knowledge of who is affected and who is at risk to be infected. The simple prevalence of a disease is the ratio of the number of individuals who have been identified as affected (not always a clear or easy task) divided by the total population under consideration (again, this number must be clearly determined). Thus, the prevalence of tuberculosis in the United States is the number of 'cases' (this must be clearly defined, for example, as all individuals with a positive tuberculin skin test, or perhaps alternately, as all individuals with a positive sputum culture) divided by the entire population of the United States. On the contrary, the incidence of a disease is a measure of the rate of appearance of new cases in a defined time interval (usually 1 year). This measure requires study of the disease

over time. Since affected individuals may die or may recover in the course of a year, the incidence is not simply the difference in prevalence from year to year. For chronic diseases of long duration and low mortality, the prevalence is related to the cumulative incidence over time. For acute diseases of short duration, the prevalence (at any one instant in time) may be less than the annual incidence. Incidence and prevalence are thus related to the time course and outcome of the disease. Advancement in diagnosis and treatment may change the incidence and prevalence of an infectious disease. For example, prevalence of HIV has increased because patients are living longer with HIV as well as because of improved sensitivity of the diagnostic tests. Both prevalence and incidence are important statistics needed to understand epidemic disease.

The Scope and Viewpoint of Epidemiology

Epidemiology first and foremost deals with populations; these can be as small as the clinical practice of an individual physician or as large as the entire population of the world. Typically, however, it deals with groups of intermediate size: a geographically defined population, an occupationally defined group, or population defined by some environmental, social, or medical characteristic. Because one cannot often study the entire population of interest, the study of a subset or sample of the population is undertaken with the intent to extrapolate conclusions based on the sample to the population as a whole (the principle of generalizability). Thus, sampling and extrapolation methods are of central importance in epidemiological studies.

While diseases of interest to epidemiologists can be studied in terms of pathophysiology and molecular mechanisms, it is the settings under which disease occurs that is often the main focus of epidemiological interest. Sometimes sets of conditions can be identified that point to necessary and/or sufficient conditions for disease to occur or to become epidemic. In addition, epidemiological evidence can, in some cases, identify disease prior to its recognition based on individual cases. Disease surveillance networks and adverse effect reporting are some of the epidemiologic approaches designed toward this end. The concern is not only with individual patients but also with the social community and its diseases. Sometimes the definition and understanding of the diseases may change over time with the advance of medical technologies and our ability to study these diseases. While it is sometimes thought that epidemiology is confined to infectious diseases, it can be applied to all types of illness, including chronic diseases such as heart disease, stroke, and osteoarthritis as well as injuries, disabilities, and social violence. The risk factors associated with the diseases are not limited to the physical (biological) or individual characteristics For example, psychological factors, attitudes and

beliefs, or multilevel determinants such as socioeconomic status, access to health care, and parental education are known to link to one's health.

In practice, epidemiology depends on quantitative data on individuals gathered through field studies as well as analysis of such data by application of various theoretical models and statistical procedures. Epidemiological data, in turn, depends on clear, precise, and relevant delineation of criteria for who is affected and who is not affected. Inclusion and exclusion criteria are the essential starting point for investigation of any disease. A precise definition of a 'case' is crucial for meaningful analyses of the collected data. Often, too, it is important to collect data on related parameters that might turn out to be confounding variables. Often, modern laboratory methods such as microbial isolation and identification, serological analysis, and radiography are involved in the definition of a case. Quantitative data and conclusions that are drawn from them depend on the validity and reliability of the endpoints, the measures employed, and the completeness of the data. The definition of the populations to be studied, the criteria for inclusion and exclusion of subjects, and the sampling procedures are all essential considerations in epidemiological studies.

Bias in Epidemiological Studies

An important consideration for many epidemiological investigations is the extent that the observed result deviates from the true value of the particular variable being studied. The general concept of bias describes some of the processes leading to such deviation. Many sources of bias have been identified in epidemiological studies of infectious diseases. Some of the most prevalent and difficult cases result from sampling bias, measurement bias, and confounding bias.

A difficult problem in any population survey, whether it be to estimate the prevalence of tuberculosis, the level of immunity to measles, or the likely outcome of an election, is to select a sample population for study that accurately represents the entire population of interest. Epidemiologists and biostatisticians have developed methods to achieve this goal by matching cases to controls, by demographic parameter sampling, and by internal validity testing, to name a few such methods. A high response rate, that is, obtaining data from a high proportion of the sample population is crucial to avoid potential bias based on responses weighted in one direction or another within the sample population. A high response rate is preferred, too, because nonresponse data maybe related to the risks or outcomes under study.

How a population is studied also can introduce bias. An inaccurate or imprecise laboratory determination, faulty clinical criteria, or a poorly designed survey questionnaire all are examples that can and have introduced measurement biases into epidemiological studies. For example, as the understanding of a disease changes with new knowledge, the diagnostic criteria by which patients are identified may change. In the case of AIDS, the major diagnostic criteria changed significantly in several revisions from 1985 to the present. Initially only a set of clinical manifestations was used; subsequent revisions incorporated laboratory determinations of the T cell count and direct detection of HIV in the blood. Epidemiological data across this time period show discontinuities because of these changes. False-negative and false-positive test results are other well-known examples of measurement bias.

A third important source of bias occurs because of the presence of multiple, sometimes interacting, causal factors. For example, the interrelation of nutrition with susceptibility to some infectious diseases can obscure or mislead a study of the causal factors of the disease. The distorting effect of such factors is called confounding bias. Initial investigations of epidemics are often hampered by many recognized and unrecognized confounding variables.

Other types of bias in clinical trials, population surveys, and some epidemiological studies include investigator-related biases such as selecting, evaluating, and treating subjects based on conscious or unconscious criteria that are not recognized in the study design. Sometimes laboratory tests have systematic and unrecognized biases; nonstandard laboratory tests are one such source of measurement bias. Reliable or at least generally accepted laboratory tests and diagnostic methods are often established as the metaphorical 'gold standard' test, and if a study fails to use such methods or at least verify its methods against the 'gold standard', bias may be introduced.

Causation and Epidemiology

The notion of 'cause' is a philosophically fraught concept that, nevertheless, is of central importance in epidemiology. In considering a disease or epidemic, both the necessary and the sufficient causes are often sought. Such knowledge can often lead to effective actions, social or biological, to control or eliminate the disease. Historically, epidemic disease was believed to be caused by social or environmental conditions, for example, malevolent government or sinful behaviors, by astrological events, by climatological conditions, or even witchcraft or magic. In more recent times, epidemics have been attributed to microbial contagion, lack of sanitation, and altered social ecology. While the well-known Koch–Henle criteria for establishing the causal role for a microorganism in a specific disease can be applied to individual cases and studied by the methods of experimental pathology and

laboratory microbiology, epidemiologists have approached the problem of causation from a different perspective. Through the study of populations and the social settings of disease, it is possible to identify factors that promote, enable, or modify a disease that cannot be established by studying an individual patient. Often the first clues as to the nature of the causative infectious agent can be obtained from epidemiological studies.

Before looking for causal factors, epidemiology usually looks for associations. What are the common attributes of all individuals who are affected? Are any social, biological, or cultural factors tightly correlated with resistance or susceptibility? Are there any predictors of the future course of the disease? In some sense, epidemiology takes the 'experiment' that nature provides and tries to extract information to make conclusions as strongly as possible from an (usually) imperfect natural experiment. Often this approach leads to more definitive laboratory study under well-controlled conditions. Epidemiologists are concerned with both the internal and external validity of a study. Internal validity is the concept that the study can stand on its own, that the variables and predicted outcomes are consistent within the subsets of the study. The external validity relates to the comparability of a specific study with other, unrelated, studies of the same dependent and independent variables.

While epidemiology suggests 'associations' between observable variables, it often attempts to suggest causal links between these variables. For example, the association between diet and heart disease observed in many population studies suggests that there is a causal connection between what we eat and our likelihood of having heart disease. Logically, however, association does not imply causation, it can only suggest a causal mechanism that must be confirmed by other investigations.

Because epidemiology is most often observational rather than experimental, a different set of causal criteria have evolved to deal with such data. In 1965, Bradford Hill was able to summarize the epidemiological approach in terms of nine 'viewpoints' that he related to the question of whether association can imply causation. These are listed in Table 1.

Table 1 Causal criteria (Bradford-Hill, 1965)

Strength of association
Consistency of association
Specificity of association
Temporality
Biological gradient
Plausibility
Coherence
Experimental support
Analogies

Epidemiology overlaps with clinical investigation in relation to the design of clinical trials and other such tests and intervention strategies. The detailed study of such trials is beyond the scope of this article, but both epidemiology and clinical trials share one key strategy in common, and that is the so-called case-control approach. Epidemiologists are often able to segregate their study population into groups based on some criteria so that one can compare 'cases' with 'noncases' or 'control' subjects. Comparing these two groups, it is often possible to find variables that are strongly correlated with specific risk factors, for example, the association between HIV infection and needle-sharing among injection drug users. However, clinical trials are known to be the best study design because unknown confounding bias can be eliminated by randomization. Clinical trials are not sometimes feasible because of the ethical concerns of withholding proven effective therapy or even giving potentially harmful intervention to human subjects.

Modes of Transmission

Epidemiological study often aims to determine the mode of transmission of disease in populations. Classically, epidemiologists recognized disease transmission from individual to individual by direct contact with persons (such as occurs in venereal diseases such as syphilis), with contaminated inanimate objects (so-called fomite transmission), and by contact with contaminated water or aerosols (such as sewage in the case of cholera, droplets produced by coughing in the case of influenza, or spray from air-conditioning cooling water in the case of Legionnaires' disease). This type of transmission from one individual to another, either directly or indirectly, has been termed horizontal transmission.

Some diseases, however, seem to spread more mysteriously. Eventually, it came to be recognized that diseases such as malaria, typhus, and yellow fever were transmitted indirectly between infected individuals, frequently by the actions of insect carriers of the infection. Because not all such vectors of disease were insects, these diseases became known more generally as vector-borne diseases. Sometimes more complex vector biology has been identified as important in disease transmission as in the case of schistosomiasis transmission through a freshwater snail as the intermediate host. The understanding of the role of vectors in the transmission of epidemic disease provided both the explanation for the transmission and the opportunity for control of the disease by approaches aimed at the vector. Thus, mosquito eradication became an effective way to control diseases such as malaria and yellow fever.

Epidemiologists now recognize a third category of transmission, that is, from parent to offspring. Congenital infections such as syphilis, herpes simplex, cytomegalovirus,

hepatitis B, and HIV are categorized as being vertically transmitted as distinct from horizontally transmitted in such cases.

Epidemiology and Public Health

While epidemiology is not synonymous with public health, it informs both the theory and practice of public health work. Once the settings for disease are understood from appropriate epidemiological investigation, medical and community actions and policies can be formulated. At risk populations can be identified, physiological and physical factors can be determined, and appropriate and practical measures for control and prevention can be developed. One of the first such actions which is often still the most effective is isolation and quarantine. Based on the understanding of the natural course of disease and its period of transmissibility, it is possible to determine the length of time needed for a diseased population to become disease-free and hence 'safe' to come into contact with. This concept led to the establishment of a period of 40 days (Italian: quaranta; 40) that ships were held at bay in the harbors of the Adriatic ports of Ragusa (1465) and Venice (1485) during plague epidemics.

Sanitation

Even before the advent of the understanding of the role of microbes in the origin of diseases, the association of cleanliness with healthy conditions was recognized. Pre-germ theory belief in 'miasmas', that is, disease-producing emanations from putrefying organic matter, swampy places, and other places with unpleasant odors, was substantiated by general observations that disease could be associated with 'filth'. In the nineteenth century, campaigns to clean up cities and to improve the environment through better water, better ventilation, and less crowding were undertaken to prevent epidemics and improve health. The so-called poor laws advocated by Edwin Chadwick in England were aimed at improving the health of the working class through sanitary reforms, albeit with increased economic productivity as the stated goal. Toward the end of the nineteenth century, sanitation became a mainstay of public health efforts and epidemiological evidence provided the justification for much of this work. Identification of tuberculosis in milk supplies, of typhoid in water, and plague in rats suggested specific public health remedies that could be instituted both locally and more broadly. Thus, in addition to quarantine, sanitation became a tool for controlling epidemic and chronic diseases.

Immunization

The third tool that provided public health with even greater power was the introduction of immunizations, that is, the deliberate inoculation of health individuals with inactive or attenuated infectious agents designed to provoke the natural immune responses of the body to induce a state of resistance to infectious disease. For centuries it was known that individuals who survived a case of smallpox did not take ill during the next epidemic, so the practice evolved of deliberate inoculation of populations with infectious material from mild cases of smallpox in the hope of inducing immunity through a mild case from which the patient was likely to recover. Smallpox inoculation (to be distinguished from vaccination) was widely practiced in Asia and the Middle East and was introduced to England in the eighteenth century. It formed the context for the subsequent observations of Edward Jenner, who in the late eighteenth century learned of the folk wisdom in his district of Gloucestershire that a benign case of cowpox (usually contracted from cows by the local milkmaids) was protective against subsequent smallpox exposure. Jenner adapted the practice of smallpox inoculation to the deliberate inoculation with cowpox material and observed in trials on his patients that cowpox did, indeed, immunize against subsequent smallpox inoculation. Because the cowpox was known as Variolae Vaccinae (pox of the cow), this procedure came to be universally known as vaccination. By the end of the nineteenth century, the use of both active and attenuated cultures of infectious agents for induction of immunity became widespread, supported by the practical experiments of Pasteur as well as the biological understanding of the immune system pioneered by Ehrlich, Metchinikoff, and Bordet.

Epidemiological Models

The fundamental conceptual model in epidemiology is based on the notion that a population can be divided into three 'compartments': susceptible individuals (S), infected individuals (I), and recovered individuals (R). This model is called the SIR model. Because experience with many infections diseases shows that recovered individuals have immunity to the disease, it is often assumed that the recovered population is distinct from the susceptible population. A more realistic variation of this model includes a compartment of latently infected individuals (E) who eventually move into the compartment of infected individuals who are actively infectious to others, a modified version of (I). This model is called the SEIR model.

The number of individuals in any compartment can vary over time, so that this simple model has been used to

predict the time course of an epidemic. Assumptions for the rate of infection (λ), the duration of the illness, or equivalently, the rate of recovery (δ) are variables that must be incorporated into any specific model. Simple differential equations describe this type of model:

$$dS/dt = -\lambda SI$$

$$dI/dt = \lambda SI - \delta I$$

$$dR/dt = \delta I$$

The basic reproductive rate of the epidemic, designated $R(0)$ is given by λ/δ. This quantity is greater than 1 when the infection rate exceeds the recovery rate and implies an expansion of the number of infected individuals. If $R(0)$ is less than 1, the epidemic is dying out. Alternately, as time increases, and $R(t)$ goes to $R(4)$, the proportion of recovered individuals equals $1 - S(0)\exp(-R(0)[R(\infty) - R(0)])$. This equation predicts that at the end of an epidemic there will remain some uninfected, susceptible individuals. While this model works reasonably well for certain acute infectious diseases such as measles, mumps, and rubella, it requires substantial modifications to account for instances where there are complexities such as significant differences between exposure rates and infection rates, a compartment of preimmune individuals, geographic or genetic variations in infection rates, and age and comorbidity factors. Modern epidemiological practice involves more complex models including both deterministic models such as the first order SIR model as well as stochastic (probabilistic) approaches.

Historical Examples

To illustrate some of these concepts, several examples from the fourth century BCE to the late twentieth century show how some of these concepts have been applied.

Hippocrates

The succinct principles of epidemiological investigation and their role in understanding of disease were put forth in the Hippocratic writings known as 'The Epidemics'. The author (usually assumed to be Hippocrates) emphasized both the general and specific features of disease and its context which are needed for full understanding:

> With regard to diseases, the circumstances from which we form a judgment of them are, — by attending to the general nature of all, and the peculiar nature of each individual, — to the disease, the patient, and the applications, — to the person who applied them, as that makes a difference for better or for worse, — to the whole constitution of the season, and particularly to the state of the heavens, and the nature of each country; — to the patient's habits, regimen and pursuits; — to his

> conversations, manners, taciturnity, thoughts, sleep, or absence of sleep, and sometimes his dreams, what and when they occur… etc. (*Epidemics*, Book I, para 10)

This classical approach to the study of disease has been adopted by medical writers ever since, and it has proven a reliable and useful conceptual framework for understanding disease.

Microbial isolation

Often a new disease first makes it appearance in epidemic form without any prior recognition of the disease, its pathophysiology or its cause. A combination of field epidemiological study and laboratory investigation is often the approach that illuminates such a new disease. The success of the laboratory work, of course, depends on the adequacy of the experimental tools available at the time. The understanding of the hog cholera epidemic in nineteenth century America is an example of one such disease. As we now know, this disease is caused by an RNA virus, but at the time, such agents were unknown and the methods for their study not yet developed. Furthermore, the overlap in symptoms and pathology of the disease caused by the hog cholera virus and that caused by inoculation with a usually nonpathogenic commensal bacterium, *Salmonella choleraesuis*, led to much confusion. This example illustrates the role of laboratory study in the elucidation of a serious animal epidemic at the dawn of the era of germ theories.

In the 1870s, American hog farmers were experiencing losses of between 5 and 20% of the hogs in the country to an acute, apparently contagious, illness called at the time, swine plague. From studies commissioned by the US Department of Agriculture it appeared that some sort of bacterium was present in the blood and body fluids of hogs that had died of swine plague. This bacterium was named appropriately, *Bacterium cholera suis*. Later in honor of Daniel Salmon's contribution to the study of this group of enteric organisms, it would be renamed *Salmonella choleraesuis* and is now taken as the type strain in defining the Genus Salmonella. Salmon, the head of the two-person Bureau of Animal Diseases, was interested in the logic of disease causation and confronted this problem directly in his study of hog cholera.

Salmon was concerned that the methods available for isolation and identification of microbes associated with a specific disease state might be misleading. In his words:

> Again, cultivation experiments are not such reliable evidence as many seem to suppose. It is true that when the eighth or tenth generation of a cultivation proves virulent, we have ample evidence that the contagion has multiplied, but does this consist of the organism to which our attention has been directed? (Salmon, 1881, p. 433)

In other words, what standards of 'purity and homogeneity' do we require to be sure that the organism we see (by various means of seeing) is the one that 'causes' the disease? In the course of his research he realized that it was easy to isolate spurious organisms, either secondary invaders or contaminants. First he and others, including Pasteur and Thuiller, believed that the agent was a micrococcus. Only 1 year later, however, he wrote "... we must say, at this point, we no longer consider a micrococcus as the cause of all the outbreaks of the disease known as swine plague". By 1885 Salmon had been joined by the young Theobald Smith and together they concluded that a short, rod-shaped, motile, bacterium which they named *Bacterium suis* was the cause of the disease called swine plague.

What happened? In their attempts to apply Salmon's criterion regarding immunization as a standard of causation, they investigated a vaccine from Pasteur that they confidently expected to confirm the causative nature of the micrococcus. France and Germany were being ravaged by a highly contagious epidemic of a lethal swine disease called *rouget* or *rotlauf*. It was generally held that this disease was the same as swine plague in North America. Pasteur and his colleagues had studied *rouget* and reported that the agent they isolated was a "microbe in the shape of a figure eight" (interpreted as a micrococcus). On the contrary, Pasteur's vaccine, as obtained by Salmon, contained bacteria in the form of a *Bacillus* (a long thin rod) "not to be mistaken for a micrococcus" as noted by Salmon. Since Koch, Löffler, and Schütz in Germany had all noted the presence of *Bacilli* in the blood of pigs with *rotlauf*, Salmon reasoned that Pasteur's original report was either wrong or that there were two different diseases involved. Indeed, as Salmon concluded from detailed study of Pasteur's vaccine, it was ineffective in protecting animals against the effects of the organism he had isolated from American swine plague. Thus, it appeared that there were two diseases: *rouget* in France and Germany, and swine plague in North America. There is a clear yet unstated inversion in the logic in Salmon's work at this point: in the course of trying to associate a specific organism in a causal way with a given disease, he inverted his argument and used the bacteriological and immunological results to redefine the diseases. Thus, *rouget* and swine plague were two different diseases precisely because they were associated with two different organisms. As an accomplished veterinary pathologist, Salmon gave detailed anatomic and pathologic descriptions of swine plague and compared them with reported cases of *rouget*. Salmon thought he was, at the very least, clearing up some of the confusion by demonstration of radical difference between these diseases.

Although inoculations with pure cultures reproduced the disease recognized as hog cholera, and although the organism *B. suis* (or *B. cholerasuis*) was easily recovered from the inoculated animals, even in 1886 Salmon and

Smith noted "the successive passage of the virus through the body of pigs by feeding diminishes its virulence and may finally destroy it" (Salmon and Smith, 1887, p. 20). This was a facet of Salmon's research program that was to occupy his laboratory for the next 15 years and eventually came to undermine their conclusions as to the causation of hog cholera.

Salmon and his colleagues at the Bureau of Animal Industry worked on ways to control the disease by inactivation of the causative agent by chemical and immunological means. He saw the specific immunization against hog cholera as the final confirmation of causation. In 1903 EA de Schweinitz and his colleague M Dorset cautiously noted that "certain out breaks of that disease [hog cholera] were met with which apparently were not produced by the hog cholera or the swine plague bacilli". In looking for yet another 'causative' factor in swine diseases, these workers used the newer bacteriological techniques of filtration to show that the disease could be transferred by fluids free of hog cholera bacilli. The disease was only produced in hogs, not in other species; in hogs, however, it was highly contagious. In contrast to the previous decade of work on immunizations with hog cholera bacilli, they were able to establish solid immunity to this filterable agent.

> On account of the often discordant results which were secured some years ago when the Bureau was treating diseased hogs with serum from animals which in their turn had received large and repeated doses of hog cholera and swine plague cultures, it appeared that some other factor must be considered in the efforts to produce immunity. (de Schweinitz and Dorset, 1904, pp. 1–2)

The use of new methods, in this case bacteriological filters, allowed identification of the true pathogen, a 'filterable' virus, later shown to be an RNA virus of the togavirus family, which was both unknown and unknowable to prior investigators. Consensus was formed, and yet constrained, by adherence to concepts and methods that limited consideration of infectious causes to cultivatable, microscopically visible agents. Salmon's somewhat idiosyncratic inclusion of additional criteria, however, appeared to give added weight to experimental anomalies that led eventually to new views of causation and eventually to the discovery of the true cause of hog cholera.

Seroepidemiology

Sometimes it happens that an infectious agent is known from laboratory isolation but its association, if any, with disease is unknown. Epidemiological investigations can sometimes provide clues and even 'proof' of the role of such an agent in causing disease. The well-known discovery of the role of EBV in infectious mononucleosis illustrates the application of epidemiological concepts to

such investigation. The combination of seroepidemiological investigations of prospectively collected patient samples with the detailed laboratory study of basic microbiological phenomena, coupled with good luck and Pasteur's 'prepared mind' illustrates the power of this approach to an important disease of unknown etiology.

In 1958, Denis Burkitt described a peculiar B cell lymphoma with very characteristic pathology and occurrence in children in Africa. This tumor became known as Burkitt's lymphoma. Epstein, Achong, and Barr reported (1964) the presence of particles with herpesvirus-like morphology in cell cultures grown from a biopsy of a Burkitt's lymphoma patient. The relationship of these particles to any known herpesvirus, let alone the lymphoma, was unknown. This was a case of an unknown, unculturable virus, which came to be called Epstein–Barr Virus, in search of a disease. At the same time, Werner and Gertrude Henle (he was the grandson of the famous nineteenth century pathologist, Jacob Henle, one of the developers of the Koch–Henle criteria for causation of infectious disease) were refining immunofluorescence microscopy as a tool for studying virus infections and analyzing serum samples for antiviral antibodies. Turning their attention to EBV, they noted that antibodies that reacted with cells containing EBV were always present in the sera of African children with Burkitt's lymphoma. Surprising, however, was their finding that 80% of healthy African children were seropositive as were many children and most adults in the United States. This finding suggested that the antibodies to EBV were related to some common childhood disease that was prevalent worldwide.

The Henles were unable to find the key to this puzzle until the serendipitous observation that one of their laboratory technicians mysteriously seroconverted from a previously EBV-negative status to seropositivity when she became ill with infectious mononucleosis. Not only did she seroconvert, but also her lymphocytes became easy to grow in culture at that time whereas previously they would not grow in culture. These cultures all showed the presence of EBV.

The next step was to survey a selected set of sera collected from Yale students who had infectious mononucleosis. All of the 42 students with infectious mononucleosis were seropositive for EBV. A randomly selected sample of health Yale students showed only 24% seropositivity. Even more importantly, there were 12 students for whom sera was available from before and after illness with infectious mononucleosis, and in all cases they seroconverted after the illness. Several large seroepidemiological studies of similar design have confirmed these initial findings and further have demonstrated that seropositive individuals do not have another episode of infectious mononucleosis. The close, almost absolute association of serological evidence of exposure to EBV and clinical infectious mononucleosis led to the conclusion that EBV is a necessary, but not sufficient cause for infectious mononucleosis. As it turns out, most cases of EBV infection are subclinical with seroconversion and subsequent development of immunity, but without overt, clinically significant, symptoms. About 85% of adults in the United States over 25 years of age are seropositive for EBV infection.

The relationship of EBV to Burkitt's lymphoma is yet another puzzle, and owes its partial solution more to laboratory work than to epidemiological investigation. Miller and his colleagues (1973) were able to produce B cell lymphomas in cotton-top marmosets by inoculation with EBV, a finding that suggested that EBV might be a human tumor virus. Again, extensive investigations have shown that EBV can be a necessary but not sufficient cause of certain specific human cancers.

Mysterious epidemic

The limits of the epidemiological approach alone can be seen in cases where a new disease appears but the agent is difficult or impossible to identify by conventional approaches or by known techniques. Such was the case of the pneumonia that later became known as Legionnaires' disease.

In the summer of 1976, pneumonia of unknown origin was observed in several men who had attended the annual convention in Philadelphia, Pennsylvania of the American Legion, an American veteran's organization. The association of this pneumonia with the conventioneers was possible because of the short interval between the convention and the onset of illness (about 2–3 days) and the efficient organization of the American Legion itself. The local leader of the organization noted he was getting more than the expected number of calls about postconvention illness and deaths. Within a week of the end of the convention, the Pennsylvania Department of Health had enlisted the US Centers for Disease Control and Prevention (CDC) in the investigation of this outbreak. It was about 6 months, however, before the origin and causative bacteria for this disease, subsequently called Legionnaires' disease, was identified. It turned out to be a previously unknown organism with an interesting ecology and biology that explained some of the specific pathophysiology and epidemiology of the disease.

Classical investigative epidemiological approaches were employed to understand this disease outbreak. Teams of epidemiologists, virologists, infectious disease specialists and bureaucrats converged upon metropolitan Philadelphia and by 7 August, the officials had provisionally ruled out most known bacteria, fungi, and viruses. The search then turned to possible toxins and poisons because infectious diseases seemed unlikely and the epidemiological data ruled out secondary spread of the disease. Case-control questionnaires sent to the 10 000

Legionnaires and wives who had taken part in the convention aimed at finding some factor common to all of the cases yet absent in noncases. Nickel carbonyl poisoning became a major focus of investigation as it was known to produce the delayed onset and respiratory lesions characteristic of the illness. Being colorless and odorless, nickel carbonyl would not have been noticed by people at the convention. The metal derivative, however, did not explain the high fever associated with the disease, and the CDC could not find a possible source.

On 4 September, the CDC announced hundreds of negative results on tests for various infectious agents and toxins but no possible leads; the nickel carbonyl hypothesis was abandoned when tests revealed nickel contamination of the samples. Theories on the disease kept coming: phosgene was proposed on 15 December and psittacosis (parrot fever) hypothesized on 7 January. However, on 18 January, just five and a half months after the outbreak was reported, the CDC triumphantly announced the discovery of a previously unknown bacterium implicated in the outbreak. In retrospect, the delay in identifying the etiological agent was due to unusual growth requirements such as high iron concentrations, and the poor staining characteristics of the organism with standard dyes.

The organism of Legionaires' disease, *Legionella pneumophila*, is a facultative intracellular pathogen, which means that it is able to grow within cells, but does not have to do so. In the natural ecology of the organism it appears that *L. pneumophila* can infect free-living amoebae in warm fresh water, especially air-conditioning cooling water. Its ability to grow inside of other cells explains some of its pathogenicity in humans. The organism invades and multiplies inside macrophage, especially those in the lung, eventually killing these immune defense cells. The bacteria inhibit the normal mechanisms the macrophage use for attacking bacteria, that is, they prevent fusion of the phagosomes with the lysosomes within the macrophage. In this way, *L. pneumophila* escapes the usual immunity provided by the macrophage system. Because of its intracellular location, drugs must achieve high intracellular concentrations to be effective. Examples of such agents include the macrolides (e.g., erythromycin), quinolones (e.g., ciprofloxicin), tetracyclines (e.g., doxycyline), and rifampin.

Prior to the outbreak in 1976, this organism and its association with human disease were unknown. In retrospect, a flu-like illness with very rapid onset (2–48 h after exposure) which was detected among people in the Health Department Building in Pontiac, Michigan in 1968 was found to be caused by *L. pneumophila*. This illness has been called Pontiac fever; it is self-limited, resolves without treatment, and does not result in pneumonia. It is considered a less severe form of infection with *Legionella*.

The CDC reports that Legionnaires' disease affects 8000–18 000 individuals per year (probably higher because of underreporting). Only about 10–20% of cases are identified with outbreaks and about 20% are nosocomial or hospital-acquired. The majority of cases are sporadic, not associated with clustered outbreaks. Case fatality rates have been reported between 1 and 40% and appear to depend on the rapidity of diagnosis, institution of preventive measures, and appropriate antibiotic treatment. No person-to-person spread has ever been noted.

Molecular epidemiology

Identification of the genetic lineages of microbes causing outbreaks of disease can be useful in determining the relatedness and transmission patterns of cases as well as understanding the particular causes for a given outbreak caused by a given strain of an organism. The complete DNA sequence of an organism, is of course, the ultimate tool for determining such genetic relationships and is now a standard method. When the laboratory tools for analysis of DNA were not adequate for complete DNA sequence determination, however, two approaches led the way demonstrating the usefulness of such genetic analyses: the study of the much smaller genomes of plasmids and the use of the limited sequence comparisons made possible by restriction endonuclease cleavage site patterns.

Salmonella typhi strains are notoriously complex in their taxonomy and classification, and the standard classification is now based on serological typing of the surface antigens. While the identification of an individual 'serovar' of *S. typhi* is useful, this classification lacks the specificity needed to test the relatedness of Salmonella isolates from some clustered outbreaks. Such specificity is important in tracing the origin and spread of epidemics. Since the late 1970s, both restriction fragment length polymorphisms (RFLP) and DNA sequence analyses have been helpful in providing more specific strain identifications useful in studying epidemic outbreaks of both viral and bacterial infections.

In early 1981, two outbreaks of Salmonella enteritis in Ohio and Michigan in the United States were observed to be related to the same serovar of Salmonella. Because this serovar was a relatively common strain in the general population, it became important to use other, more specific markers to test for the relatedness of these two outbreaks and to trace the origin of the outbreak. Because many strains of Salmonella, including the isolates from these outbreaks, harbor plasmids, the DNA of the plasmids was used as a highly specific maker of strain identity. Plasmid DNA is easy to study, in this case by RFLP patterns, and such studies showed that the isolates from the outbreaks in both Ohio and Michigan were caused by Salmonella of the same strain. Furthermore, isolates of the same serovar but

not from cases within the outbreak clusters had distinctly different plasmid RFLP profiles.

Epidemiological investigation of the households in which the cases occurred, together with control households, suggested that a common factor in the affected households (78%) was the use or presence of marijuana. Testing of samples of marijuana showed that Salmonella isolated from multiple samples were related by plasmid RFLP typing to the strain found in outbreak in both states.

Thus, the epidemiological association of marijuana exposure and the two separate outbreaks of Salmonella enteritis was confirmed and explained by the use of plasmid DNA analysis. RFLP analysis is rapid, simple, and often informative. More recently, rapid DNA sequence analysis of DNA segments that are highly polymorphic allows even more specific identification of individual strains against a background of related but noncausal organisms in study of epidemic infectious disease.

Formal modeling

In the early months of 1976, an outbreak of influenza occurred among soldiers at the US Army base at Fort Dix, New Jersey. This outbreak resulted in one death, 13 hospitalizations, and 230 total cases confirmed by serological testing. Because this epidemic was caused by a novel H1N1 type of influenza that was circulating in swine, but which had not circulated in the human population since the 1957–58 pandemic, there was concern that this strain might escape into the general population and cause another pandemic. After much debate, US federal health officials initiated a national immunization program in which over 40 million people were vaccinated. This national influenza immunization program turned out to have several unfortunate collateral consequences. First was the occurrence of over 500 cases of Guillain–Barre syndrome with 25 deaths among vaccinees in at least ten states. Second, was the longer term distrust of national health policymakers that resulted from this hasty and controversial program which no doubt hampered the national response at the beginning of the AIDS epidemic 5 years later.

Because the swine flu epidemic at Fort Dix occurred in a homogeneous age group which was both relatively isolated and well studied, it has been possible to apply formal epidemiological models to this epidemic and better understand why this particular epidemic failed to become a generalized pandemic and, in retrospect, why the concerns at the time were misplaced.

Using the simple deterministic epidemiological model for spread of an infection in a population, the basic reproduction number $R(0)$ (the number of infections caused by a single infectious individual introduced into a totally susceptible population) and the serial interval (the average time between an individual becoming infected and transmitting the infection to another individual) can be estimated from the medical history data of the soldiers at Fort Dix. Lessler and colleagues (2007) calculated $R(0)$ this way to be 1.09. Another way to model the data is to apply the SEIR model in a stochastic way to examine which values of $R(0)$ and serial interval best fit the observed data of the epidemic. By varying $R(0)$ systematically from 0.5 to 3.0 and the serial interval between 1.6 and 10.0 days, Lessler and colleagues (2007) made such calculations for hypothetical individuals in the Fort Dix population for each value of these two parameters applied in a probabilistic way. Ten thousand such hypothetical calculations were then averaged to predict the overall likely population behavior. The actual epidemiological data from the Fort Dix outbreak best fit an estimate of $R(0) = 1.2$ and a serial interval of 1.9 days.

Similar analyses of other influenza outbreaks as well as some theoretical arguments suggest that there is a threshold for transmissibility below which a strain will not be able to establish itself as a circulating variant in the population, that is, the outbreak will abort and will not become epidemic. This threshold is affected, too, by interventions such as antiviral drugs and quarantine. The $R(0)$ values determined for previous flu pandemics suggest this threshold is above 1.5 (1918 epidemic: $R(0) \approx 1.8$; 1957 epidemic: $R(0) \approx 1.6$; 1968 epidemic: $R(0) \approx 1.9$). Analysis by formal modeling of the Fort Dix influenza outbreak suggested the reason for its failure to develop into a full-blown pandemic was because of its low transmissibility, that is, it was not sufficiently infectious. Even though this strain of influenza had the capacity for human-to-human spread, it did not reproduce efficiently enough to sustain an epidemic outside of the very favorable population of the Fort Dix army platoons. The depletion of susceptible individuals in this population coupled with low reproductive rate, $R(0)$, resulted in the epidemic running its course in this population. Its low $R(0)$ was insufficient to sustain transmission outside of these special environment of the army camp.

Formal modeling of epidemics requires high quality demographic data as well as relevant biological data. Sufficient computational power is also required to provide probabilistic solutions to problems that cannot be solved analytically. Formal models are increasingly important both in analyzing epidemiological data from past outbreaks and in predicting the outcomes of interventions such as mass immunization campaigns, prophylactic versus therapeutic drug treatments, and behavioral changes such as promotion of needle-exchange programs for injection drug users aimed at reducing HIV transmission.

See also: Emerging Infections; Global Burden of Infectious Diseases; Plague, Historical; Polio; Smallpox,

Historical; Surveillance of Infectious Diseases; Sexually Transmitted Diseases; Water, Drinking

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