# 6
# PLAGUE SURVEILLANCE

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Plague pandemics of past centuries illustrate how quickly plague can spread through human populations when medical services and control measures are inadequate. Although no one expects to again see the massive deaths observed during past pandemics, plague continues to pose a threat to human health in certain regions of the world where natural foci still exist. Effective plague prevention and control programmes require up-to-date information on the incidence and distribution of the disease. The best means of gathering this information is through a surveillance programme that collects, analyses, and interprets clinical, epidemiological, and epizootiological data on plague. Surveillance should identify cases and epizootics as quickly as possible so that steps can be taken to control disease spread. Systematic collection of surveillance information over many years will provide information that can be used to:

(1) predict areas where future human cases and rodent epizootics may occur;

(2) identify the most common zoonotic sources of human infection;

(3) identify the most important rodent and flea species maintaining a given focus of *Y. pestis*;

(4) indicate the hosts and flea species that should be targets for control measures;

(5) assess the effectiveness of plague prevention and control measures;

(6) identify local ecological factors or human activities that may result in increased plague exposure risks for humans; and

(7) detect trends in the epidemiology and epizootology of plague in a given region.

Many years may elapse between the occurrence of isolated cases or epidemics. Continuous surveillance of rodent and vector populations is therefore important even during periods when no human cases are reported. This chapter describes a comprehensive plague surveillance

programme including human, rodent and vector surveillance. The unique needs and resources of each country will determine the actual organization of national surveillance programmes.

## Human surveillance

### Reporting human cases

At present, plague is one of only three infectious diseases subject to the International Health Regulations, which stipulate that all confirmed cases of human plague be investigated and reported through appropriate authorities to the World Health Organization. Whenever clinical symptoms or laboratory results suggest that a patient is infected with *Y. pestis*, the suspect case should be reported immediately. This will allow public health authorities to:

(1) advise on treatment and management of human plague cases;

(2) initiate efforts to identify the source of infection;

(3) determine the extent of any epizootic activity;

(4) assess the potential for additional human cases;

(5) disseminate information on plague to health care personnel; and

(6) implement emergency prevention and control measures.

Prompt reporting is especially important for cases of pneumonic plague because this form of the disease can be transmitted directly from person to person via infectious aerosols. Emergency procedures as described below must be implemented immediately to prevent further human infections.

Local physicians and other health care workers must be familiar with the symptoms of plague and consider it in the differential diagnosis. If a patient's symptoms suggest human plague, samples should be collected for diagnostic confirmation at a microbiological laboratory. If local laboratory facilities are inadequate, health care workers should know where to send samples for bacteriological or serological confirmation. The plague surveillance programme should be prepared to provide this information along with medical and epidemiological assistance.

### Increasing plague awareness and knowledge in the health care community

Because of personnel turnover or lack of prior training, it cannot be assumed that health care workers, laboratory personnel and other public health authorities in plague-endemic areas are familiar with plague diagnosis and treatment. It is therefore important that a plague surveillance programme ensure that members of the local health care community are aware of the possibility of cases of plague occurring. This can be accomplished through brief training courses, plague surveillance newsletters, brief notes in other health-related newsletters or periodic contact with other health personnel.

### Active surveillance

Following identification of a suspect case of human plague, surveillance personnel should immediately determine whether other cases exist or have occurred recently in the same vicinity. Hospital and clinical records from areas near where the case occurred should be reviewed and local health care providers should be interviewed to identify other potential cases. If possible, blood and other appropriate samples should be obtained from survivors who are considered to be potential cases to determine whether these persons are infected with or have antibody against *Y. pestis*. If possible, blood samples should be obtained from other family members or likely contacts. Record reviews and interviews with health care personnel should also be done when plague is identified for the first time in a region's animal or flea populations. In such situations, human cases might have occurred recently but may have been misdiagnosed or gone unreported (*1*). While performing the above activities, surveillance personnel should brief local health workers on plague diagnosis, treatment, prevention and control and explain the activities of the plague surveillance programme (*1*).

### Standardized reports

Human case reports should be standardized so that whenever possible the same information is recorded for each case. This will result in a database that can be combined with rodent and vector surveillance data to design better plague prevention and control strategies. The reporting form should include core patient information, clinical observations and treatment, laboratory results and results from epidemiological and environmental investigations.

*Core information*

The following core information should be collected for each patient: age; sex; occupation; residence, including country; place of exposure if known; source of exposure if known; date of onset; clinical presentation (bubonic, septicaemic, pneumonic); treatment; recovered or fatal; possible exposure of others in contact with the patient; and preliminary classification of the case as suspected, presumptive or confirmed.

Case definintion

*Suspect cases* are those cases that lack laboratory confirmation but where the patient has symptoms consistent with plague. Plague should also be suspected when patient specimens contain Gram-negative bacteria that exhibit bipolar staining with Wayson or Wright's Giemsa stains. Cases may be considered *presumptive* when immunofluorescence assays on patient samples are positive, or when a single serum sample is positive. Cases are classified as *confirmed* when *Y. pestis* has been isolated and identified by cultural characteristics, biochemical characterization and specific bacteriophage typing, or when there is a four-fold rise in antibody titres against *Y. pestis* for paired acute phase and convalescent phase serum samples. The upgrade of a case from suspect or presumptive to confirmed should be noted on the report form along with the date of confirmation.

*Clinical observations and treatment*

Whenever possible, additional information on the clinical course and treatment of the disease should be recorded, including: antibiotics administered; dosage given; duration of treatment; elapsed time between the onset of symptoms and initiation of antibiotic therapy; unusual observations or complications (such as the occurrence of skin ulcers, insect bites, disseminated intravascular coagulation, meningitis, other); presence of cough; productivity of cough; intensity and duration of fever; and location and size of buboes.

The last sign (location of buboes) can provide useful information on the likely modes of transmission. For example, the presence of an inguinal bubo is strong evidence that the patient was infected by flea bite.

*Laboratory analyses*

The report should document all relevant laboratory work including: types of samples analysed (blood, sputum, bubo aspirate, serum, other); dates of sample collection; light and fluorescence microscopy results; chest X-ray results; haematological findings; bacteriological results; results of serological tests; and autopsy results for fatal cases.

*Additional epidemiological and environmental information*

An epidemiological investigation should be performed for each human case to determine the source of infection and the risk of additional human cases. Reports of these investigations should include: 1) a complete history of the patients' activities and travel during the incubation period of the infection; 2) results of field studies to determine which animal and flea species are likely sources of infection or pose a continuing threat to humans (surveillance techniques for rodents and fleas can be found in later sections of this chapter); 3) proximity of infected rodents and fleas to human dwellings or workplaces; 4) estimated number of people involved in activities that place them at high risk of plague infection; and 5) information on possible exposure to *Y. pestis* infection of patient contacts (especially important for pneumonic plague cases).

*Epidemiologic follow-up of pneumonic plague cases*

When there is clinical evidence of plague pneumonia, it is important to document the efforts that were made to isolate pneumonic plague patients and protect health care personnel (*2*). The length of time a patient remained in isolation should be recorded, along with the results of periodic sputum tests. These tests are done to determine whether *Y. pestis* is present in the patient=s sputum (patients should remain in isolation until test results are negative). Attempts should be made to identify and treat prophylactically individuals who had contact with the patient during the incubation period of the infection. If possible, throat swabs or serum samples should be collected from known patient contacts. Probable contacts can be ascertained from interviews with the patient, family and friends. A history of the patient=s travel and activities will suggest possible contacts. Even in the absence of plague pneumonia, it should be determined whether other persons with similar exposure histories have contracted plague. The results of tests performed on samples from patient contacts should be recorded.

*Ecological and environmental observations*

A basic understanding of the area's landscape ecology is useful for predicting the future course of epizootics and identifying areas of high risk for humans. Information should be collected on predominant vegetation types and the amount of local land surface covered by each vegetation type, roads, railways, airports, and seaports, land use patterns (agricultural, residential, industrial, other), types of dwellings present and whether these dwellings and associated food storage areas or other man-made sites provide food and harbourage for rodents.

Flea and rodent control programmes implemented as a result of human plague case investigations should be described with an evaluation of their success.

### Surveillance of rodent populations

Rodents are the primary vertebrate reservoirs of plague, and nearly all human cases are associated with rodent epizootics. Surveillance programmes that monitor plague activity in susceptible rodent populations alert public health authorities to increased human plague risks, thus allowing prevention and control programmes to be implemented before human plague cases occur. Identification of plague in rodent populations also serves as a warning that human cases may appear and require treatment and follow-up.

#### Rodent sampling techniques:

The most common techniques for monitoring plague in rodent populations (discussed in detail under vector control) include:

(1) collecting and examining dead rodents;

(2) monitoring activity among plague-susceptible rodents;

(3) trapping rodents for population data, serum, tissue samples and ectoparasite collections; and

(4) conducting serosurveys of carnivore populations that consume rodents.

#### *Recruitment and training of personnel*

The techniques of rodent surveillance are relatively simple, but the quality of samples and data obtained using these methods is likely to be higher if the persons performing them receive adequate training. If there is a shortage of trained personnel, it may be possible to enlist the help of other local health authorities, biologists, game managers, veterinarians, animal damage control personnel, agricultural officials, nature park employees, or other individuals working outdoors in plague-endemic areas. These persons often have some appropriate background training and are likely to be familiar with the area where sampling is to take place (*3*). If local surveillance personnel and volunteer assistants have not received prior training, they should be taught:

(1) rodent and ectoparasite collection techniques;

(2) methods for collecting, preserving and shipping blood, tissues, carcasses and ectoparasite samples;

(3) measures for safely handling rodents and collecting specimens;

(4) how to identify local rodent species; and

(5) methods of preparing voucher specimens to verify field identification of rodents.

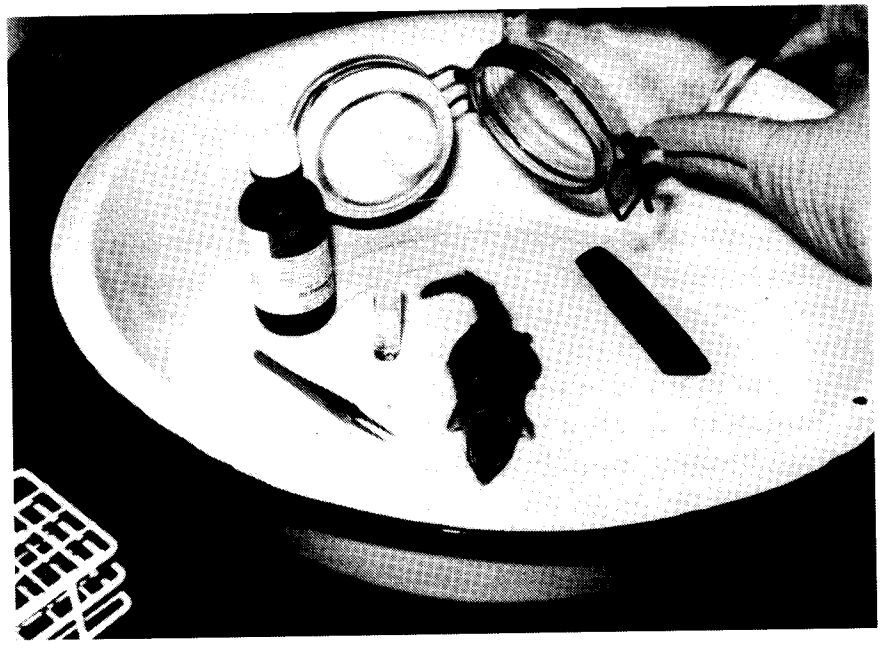
Each of these issues is discussed below or in the flea surveillance section of this chapter.

*Safety concerns and animal handling techniques*

Some collection techniques require surveillance personnel to handle live rodents or rodent carcasses. Personnel must be taught how to protect themselves from infection with plague or other rodent-borne zoonoses. Collectors should always wear gloves when handling animals. Before handling, animals should be anaesthetized, firmly restrained or humanely killed to reduce the danger of pathogen transmission via scratches or bites. Animals can be anaesthetized by placing them in a jar containing an absorbent cotton pad soaked with a suitable anaesthetic, such as halothane or metofane (*Fig. 1*). Ether should not be used for field work because of the danger of accidental explosions. Chloroform also is not recommended because of its presumed carcinogenicity and the possibility that it might interfere with attempts to isolate plague bacteria from sample materials (*4*). Animals also can be anaesthetized by intramuscular injection of a 1:10 mixture of Ketamine and Xylazine, respectively. Dosage will vary with the size and species of animal, but the above Ketamine-to-Xylazine ratio used at a dosage of between 10-150 mg of Ketamine per kilogram of body weight should adequately anaesthetize most small animals (*5*). Animals can also be restrained in a thick cloth bag for bleeding by cardiac puncture; the heart can be located by palpation. The latter technique does not require anaesthesia, but care must be taken to maintain control of the animal. Following bleeding, the animal can be killed by cervical dislocation or other humane means.

It may be appropriate for rodent collectors and animal processors to apply insect repellents or insecticides to clothing as a means of reducing the risk of flea bites. The most commonly used repellents are those containing N,N-diethyl-m-toluamide (DEET) as the active ingredient. Insecticial sprays, such as those containing permethrin, can also be applied directly to clothing and are effective against fleas.

**Figure 1: Rodent anaesthetized in jar containing Metofane. The cotton in the lid is soaked with a small amount of the anaesthetic agent prior to placing the animal in the jar**



Whenever hantaviruses or other rodent-borne haemorrhagic fever viruses are likely to be encountered, workers might be required to take precautions against infection via direct contact and aerosols. Recomendations for plague surveillance workers and others removing rodents from traps in such a situation include wearing rubber or plastic gloves and respirators fitted with filters to prevent aerosol transmission of hantaviral agents. Individuals collecting traps likely to be contaminated with such viruses should wear gloves, but are not required to wear respirators. All traps and processing equipment should be disinfected after use.

Supervisors of collecting teams might also consider recommending vaccination for their employees as an additional protection against plague; however, any protection is likely to be short-lived and frequent booster injections may be necessary to maintain presumedly protective titres. Surveillance personnel can also carry a supply of prophylactic antibiotics which should be taken if the worker is bitten by fleas, exposed to potentially infectious aerosols, or scratched or bitten by potentially plague-infected animals.

### Collection of dead animals after die-offs and ratfalls

One of the simplest techniques for monitoring plague in rodent populations is to collect dead rodents and examine the carcasses for evidence of plague infection. Carcasses of other plague-susceptible animals, such as lagomorphs (hares and rabbits) and domestic cats should also be collected for analysis. Plague surveillance personnel always should be alert for signs of a rodent die-off or ratfall and the public should be encouraged to report sick or dead rodents observed near their homes or work places. Where poisoning can be ruled out, authorities should report rodent die-offs as soon as possible to verify local reports and collect any dead rodents for laboratory analysis.

### Identification of Y. pestis in tissues of dead animals

*Y. pestis* can be detected in tissues of dead animals by direct immunofluorescence assay, agglutination, enzyme-linked immunosorbent assays, or by isolating the organism in pure culture. Direct immunofluorescence assays have many advantages over other methods for routine plague surveillance. When performed by an experienced technician using appropriate controls and plague-specific conjugates, the test has high specificity and sensitivity as well as specimen handling times that are often less than two hours (*6,7*). The rapid specimen handling times of direct immunofluorescence assays make them especially useful for emergency situations because local officials can be notified of positive test results on the same day the specimens are received and use the results to make timely decisions on plague control strategies. Another advantage of immunofluorescence assay is that *Y. pestis* can be detected in carcasses long after an animal has died. Even when animals have been dead for many days to weeks, it is possible to detect plague antigen in moist marrow samples taken from long bones such as the femur. Fraction I-specific fluorescent antibody conjugates can be prepared by hyperimmunizing rabbits with purified Fraction I antigen of *Y. pestis.* The resulting high titre antibody preparation is then conjugated to a fluorescent label by standard methods (*8*).

A definitive diagnosis of plague infection of rodents relies on culturing *Y. pestis* from tissues, but isolation is more time-consuming than direct immunofluorescence and may not be necessary in situations where reliable immunofluorescence assay is available. Samples should be processed for isolation of *Y. pestis* when they are collected from poorly-characterized foci or areas where plague has not been previously identified. Samples from well-characterized areas should also be processed periodically for isolation in order to verify the accuracy of direct

immunofluorescence results and to monitor the variability of plague strains within the foci. Direct isolation of *Y. pestis* from the tissues of decaying carcasses can be complicated by the presence of other microorganisms. For this reason, it is often advisable to first inoculate laboratory mice or guinea pigs subcutaneously with a suspension of tissues from the dead animal. If the sample suspension contains viable *Y. pestis* the animals will become infected and provide a source of *Y.pestis*-infected tissues free from most of the original contaminants. Suspensions for inoculation can be prepared in a mortar and pestle using physiological saline (0.85%) and a small amount of sterile sand to aid the grinding process. Tissue samples (such as liver or spleen) can be aseptically removed from infected laboratory animals and streaked on culture plates for isolation of *Y. pestis*.

### Shipping and labelling specimens

Depending on the materials available and the time required to ship specimens to the laboratory, rodent carcasses or tissues can be shipped on wet ice, dry ice (frozen CO), freezer packs or in special shipping containers filled with liquid nitrogen. If these are not available samples (such as liver or spleen) can be taken from carcasses and sent at ambient temperature in Cary-Blair transport medium (*9,10*). All specimens should be clearly labelled with waterproof labels and indelible inks. Each specimen should be accompanied by a data sheet stating: 1) specimen type; 2) where it was collected; 3) who collected it; 4) what laboratory tests are being requested; and 5) to whom the results should be reported. If an animal has died only recently, it may also be possible to collect fleas from the carcass as described below.

### Observations of rodent colonies and signs of rodent activity

Another useful rodent surveillance technique is to map and periodically check the area for visible signs of activity among plague-susceptible rodents, especially in areas where colonies of diurnal burrowing rodents are abundant. If these animals are normally visible during fair weather, their disappearance following a plague epizootic is usually obvious. The number of animals observed at each site over a set interval of time should be recorded. If it is suspected that a plague epizootic has occurred recently or is still underway in one of these colonies, the area should be inspected for dead animals. Other telltale signs of a rodent die-off include carrion-feeding flies at burrow entrances, bad odours near burrows and poorly-maintained burrows. Potentially infected fleas can also be collected from dead animals or abandoned burrows using techniques described in the vector surveillance section of

this chapter. Other types of rodents also produce visible signs of activity, including droppings, runways, nests, burrows, gnawed objects, or partially-eaten food. Persons familiar with these signs or structures often are able to estimate the age of these signs or structures with reasonable accuracy. This information can be used to determine the level of current rodent activity in an area.

### Trapping rodents

Systematically trapping and examining rodents is important to determine: 1) the potential plague hosts in an area; 2) the number and kinds of fleas infesting these animals; 3) whether new rodent species have entered an area; and 4) whether the abundance of resident rodent species has changed significantly since the previous trapping period.

Trapping is also a source of basic population ecology data, including: 1) population densities (relative or absolute); 2) age structures and reproductive status of rodent populations; 3) rodent habitat preferences; and 4) local distribution. Estimates of absolute densities of rodent populations (number of animals present per unit area) can be made using mark-recapture techniques but these are not practical for most plague surveillance programmes. Percent trap success, a relative density estimate is more easily obtained. This quantity refers to the number of animals caught per unit effort, and equals the number of rodents caught divided by the number of trapping periods, divided by the number of traps set per period, multiplied by 100 {(no. animals caught/no. trapping periods/no. trap sets per period) x 100 = percent trap success}.

### Trap selection and trapping techniques

Many types of traps are available for capturing small mamrnals, but some designs are more suitable than others for collecting certain kinds of samples. Although more expensive, live traps are preferable to snap or dead fall traps for capturing hosts for flea collection because fleas tend to leave a dead host's body as it cools (*11*). Live traps can also be used to capture animals for tissue and blood samples. Live traps are typically rectangular box-shaped devices with hinged doors with spring mechanisms for shutting the door once an animal has entered the trap. Most models have walls made of either wire mesh or sheets of aluminum or light-gauge (usually galvanized) steel (*Figs. 2 and 3*). If large numbers of simple traps are required, they can be constructed locally. Traps can be baited with grains, peanut butter, canned pet food, fish or other bait attractive to a particular rodent species.

Figure 2: Typical wire mesh live trap designed to capture medium-sized mammals. The white material in the trap is upholstery cotton, added to prevent hypothermia during cold weather

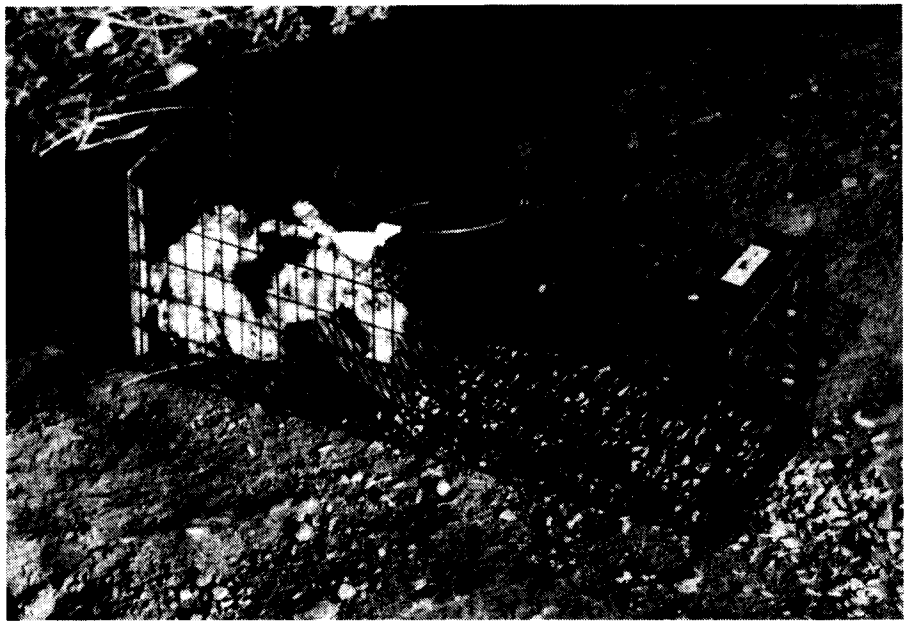
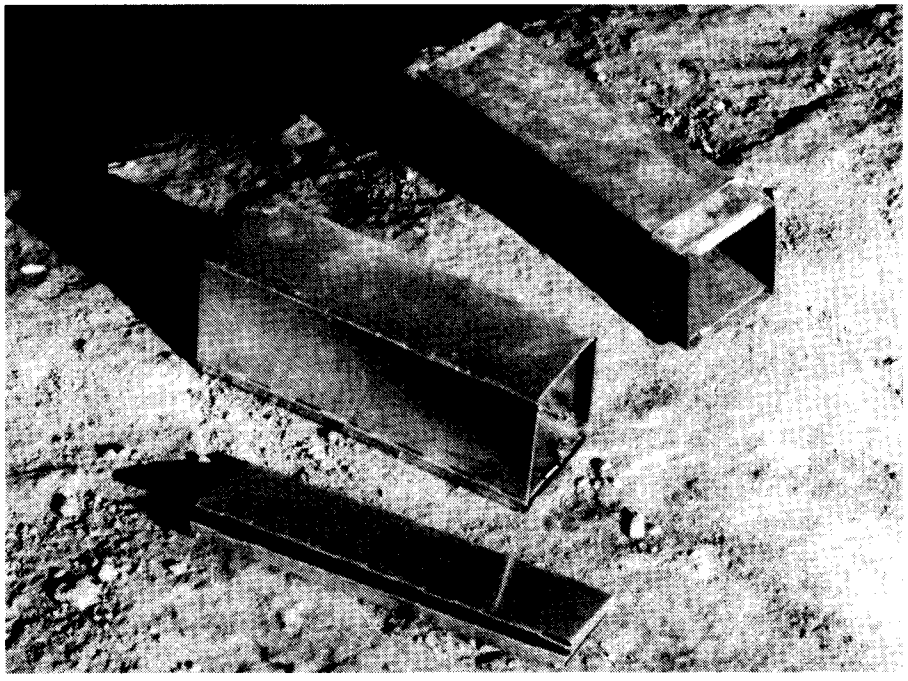
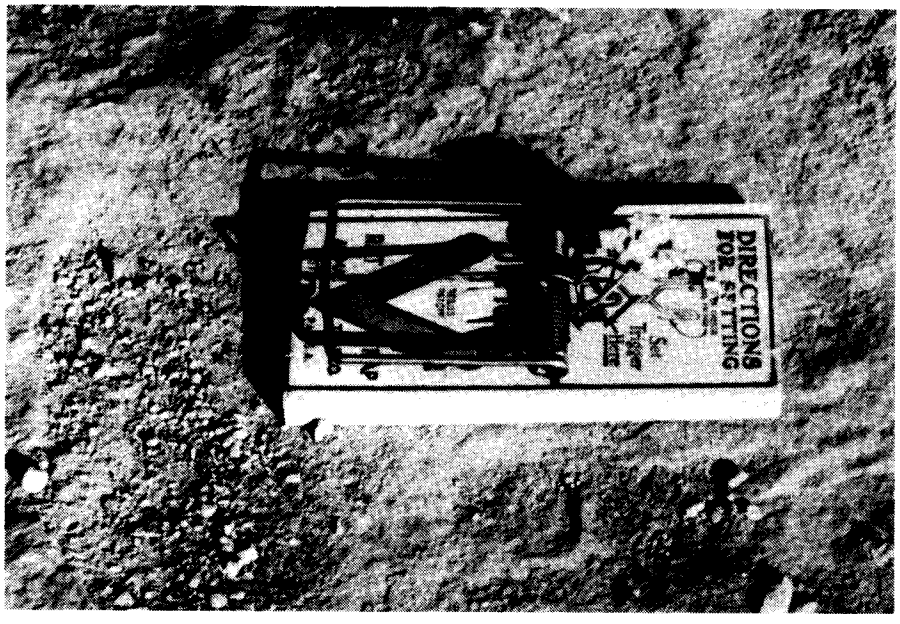


Figure 3: Aluminum live traps used to capture small mammals. The first two traps are collapsible (the trap on the left has been closed for storage). The trap on the far right is a noncollapsible style that is sturdier but less easy to transport and store



Snap traps are less expensive than live traps and are often used to capture animals for collection of tissues and fleas. When these traps are used for flea collection, however, they should be checked every couple of hours or so to reduce the likelihood that fleas will leave the dead host's body as it cools. A common snap trap (*Fig.4*) usually kills captured animals, but occasionally larger rodents are not killed immediately or are only slightly injured. Such animals can drag snap traps a considerable distance, making them difficult to find. For this reason, snap traps should be attached to a wire staked to the ground. Bait preparations similar to those described above for live traps are acceptable; the actual bait selected depends on the species of rodent being trapped.

**Figure 4: A typical snap trap used to capture rodents. These traps are relatively inexpensive and can be used to collect a variety of animals.The trap in this picture has been baited with peanut butter**




## *Placement of traps*

Traps may be set at specific sites where there are burrows, nests, runways, or other evidence of rodent activity, or they can be set along transects with 10-20 traps (or more) spaced at approximately 20m. intervals along each transect. This method allows a variety of habitats to be sampled and gives a good indication of the area's rodent diversity. Trapping grids also can be established, with the intervals for trap spacing based on local conditions.

*Rodent serosurveys*

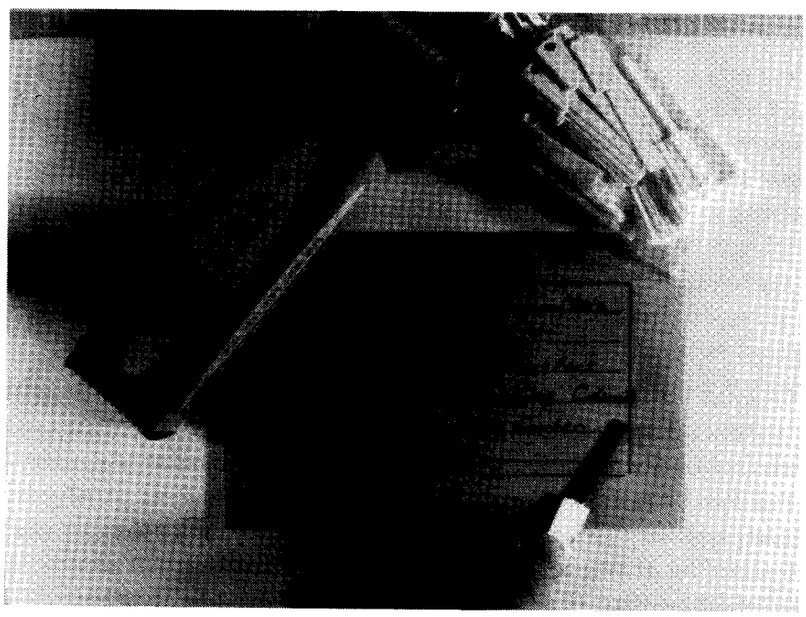
Serosurveys have at least two important advantages over attempts to isolate *Y. pestis* from tissues of captured rodents. First, the likelihood of detecting plague antibodies in rodent sera is many times higher than recovering an isolate of *Y. pestis* from tissues taken from captured animals (*12*). Second, the results of rodent serosurveys are much less likely to be affected by seasonal factors than are attempts to isolate *Y. pestis* from rodent tissues. Rodent serosurveys are most useful when a significant percentage of the affected rodent population survives plague infection and later seroconverts. For example, the percentage of seropositive individuals among resistant populations of California voles (*Microtus californicus*) can exceed 90% during the months following an epizootic (*13,14*). Other rodent species are poor candidates for serosurveys because few individuals survive epizootics and later seroconvert. This is true for the North American sciurid species, *Cynomys gunnisoni*, which may experience greater than 99% mortality during epizootics (*3*).

*Collecting and shipping blood samples for serology and isolation attempts*

Blood for serology can be collected from rodents by a variety of techniques, including cardiac puncture and retro-orbital bleeding from the eye. Blood for isolation attempts can be collected aseptically from animals by cardiac puncture. Blood samples collected for isolation of *Y. pestis* can be shipped directly in sterile, sealed tubes without the addition of transport media or freezing, provided the temperature and time required for shipping do not become excessive. All tubes should be clearly labelled and accompanied by a data sheet containing information similar to that listed in the above section on shipping dead animals.

Rodent sera can be analysed by various techniques, including complement fixation, passive haemagglutination, latex agglutination and enzyme immunoassays *(13,15,16,17,18,19,20,21,22,23,24,25,26,27,28)*. Samples for serological analysis can consist of either whole sera or blood spread onto filter papers or Nobuto strips (*Fig. 5*) (*29*). The latter are especially useful for field studies because there is no need for refrigeration, centrifuges, removal of sera from cell fractions, nor for other special equipment or handling. After the blood-soaked strip has dried it is placed in an envelope with the appropriate data and mailed to a laboratory for testing (*Fig. 5*). The antibodies can then be eluted from the strip into a buffer solution and titrated by passive haemagglutination or other serologic techniques (*29*).

**Figure 5: Nobuto strips and a mailing envelope stamped with blanks for collection data. The long, narrow portion of the Nobuto strip at the bottom of the figure has been saturated with the proper amount of blood. This portion of the strip is removed in the laboratory for further processing**



### *Analysis of tissues and ectoparasites of trapped animals*

If animals are to be killed it is possible to take tissue samples for immunofluresence assay and/or attempts to isolate *Y. pestis*. In most instances, however, analyzing tissues from apparently healthy animals is time-consuming and unlikely to yield a significant number of infected individuals. Greater effort should be placed on obtaining serum and fleas from the trapped animals (see below for flea collection techniques).

## Recording data from trapping studies

Standardized forms should be used to record data from trapping studies. The most important data for each animal are: 1) place of capture; 2) species type; 3) type of samples taken (tissues, serum, ectoparasites, other); 4) age, sex and reproductive status; 5) standard measurements of the animal's weight, total length, hindfoot length, ear and tail length; and 6) a description of the trap site and surrounding habitat. It should be noted whether or not some specimens were kept for confirmation.

### Carnivore serosurveys

One of the most powerful techniques for detecting evidence of plague activity is to collect serum samples from carnivores that consume rodent prey or are likely to scavenge fresh rodent carcasses (*3,20,22,31,32, 33,34,35,36,37*). This technique is much more sensitive than rodent serosurveys or attempts to isolate *Y. pestis* from rodents. Whenever plague-susceptible rodents constitute a major portion of a carnivore's diet, sampling sera from a few of these carnivores is roughly equivalent to sampling hundreds of rodents for plague infection. Carnivore serosurveys are especially recommended when vast areas must be sampled, plague has not previously been detected in local rodent populations, and epizootics have not occurred in local rodent populations for many years and it is suspected that plague may have disappeared from the area.

Although some carnivore species, such as those belonging to the cat family (*Felidae*), often die from *Y. pestis* infection, others apparently suffer little, if any, illness. Wild and domestic dogs and their relatives (family *Canidae*) typically survive plague infection and develop antibodies that can be detected for as long as six months (*3*). Seropositivity has also been reported for members of other carnivore families, including *Mustelidae*, *Procyonidae*, *Ursidae* and *Viverridae* (*3,17,37*).

Typically a small percentage of carnivores will be seropositive in plague-enzootic areas at any given time. A sudden increase in the percentage of seropositive animals indicates that there is ongoing or recent epizootic activity in the area's rodent populations. Such a sudden rise in antibody serves as an early warning of increased human risk of plague infection. For example, canine serosurveys conducted on the Navajo Indian Reservation in the southwestern United States demonstrated that when the percentage of seropositive dogs increased significantly there was heightened epizootic plague activity among local rodent populations and a corresponding increase in the number of human cases reported (*3*). Another advantage of carnivore serosurveys conducted in temperate climates is that sera can be collected early in the year before rodent epizootic activity reaches its peak. A greater-than-normal number of positive carnivore serum titres indicates that the risk of epizootic rodent plague will probably be higher than usual in the months to come and should serve as a warning of potentially-higher plague risk for humans during the upcoming plague season.

*Follow-up investigations for carnivore serosurveys*

Whenever carnivore serosurvey results suggest the presence of plague in a particular area, surveillance personnel should perform site investigations within the suspected home range of these carnivores to determine the location of infected rodent populations and whether the epizootic poses a threat to local human populations. These surveys should include collection of rodent and flea samples for laboratory analysis and visual inspection for dead animals and signs of rodent activity.

*Sources and collectors of carnivore serum samples*

Wild carnivores can be collected by trapping or shooting. Once these animals have been collected, blood samples can be obtained by cardiac puncture of recently killed or anesthetized animals, bleeding from large veins, or opening the body cavity to gain access to blood in this cavity or the heart. Less than 0.2ml of blood are required to coat a Nobuto strip with sufficient blood for serologic testing (*Fig.5*).Valuable samples can also be obtained from domestic dogs that roam freely and consume live rodents or fresh rodent carcasses. Live domestic dogs can be bled from veins in the forelegs or hindlegs without adverse effect. Dogs should be properly restrained and muzzled, or anesthetized prior to bleeding to prevent them from biting handlers.

*Serosurveys using animals other than rodents or carnivores*

Large- to-medium-sized mammals other than carnivores can be used as sentinel hosts under some circumstances (*36,38*). For example, feral swine have proved to be useful sentinel hosts in some areas of California in the United States (*36*).

## Surveillance of vector populations

Fleas are the primary vectors of plague and knowledge of local flea species and their hosts is essential for estimating risks of human plague infection and designing specific control measures appropriate for local situations. The relative importance of local flea species as plague vectors can usually be determined by analysing relevant surveillance data, including the numbers of fleas per host, host preferences and *Y. pestis* infection rates for the species of fleas collected. Future surveillance efforts can then concentrate on important vectors and their hosts, thereby reducing costs while providing the most relevant information for control efforts. Host/flea data also provide indirect clues about which mammalian hosts are involved in local epizootics. For example, mortality among rock squirrels (*Spermophilus variegatus*) is high during plague epizootics, and it is

not unusual at these times to find their usual flea parasite *Oropsylla montana* (*Diamanus montanus*) on other hosts such as other sciurids, rabbits, mice or woodrats. The number of fleas per host also is important. An increase in the average number of fleas per host may be of little concern when the flea species is a poor vector of plague. However, when the numbers of *Xenopylla cheopis* on *Rattus* species increase above a certain level, it may be necessary to initiate control measures to decrease the risk of human cases and plague epizootics (*39*).

*Importance of proper taxonomic identification of fleas*

More than 1500 species of fleas have been described but less than 15% of them have been found to be infected naturally with plague (*40*). Distinguishing important vector species from those of little epizootiological or epidemiological significance often requires the skills of a trained entomologist. However, nonspecialists can learn to recognize common fleas present in their area. The importance of proper taxonomic identification of fleas was demonstrated by studies of the Plague Commission in India during the early 1900s. Initially *X. cheopis* was thought to be the only member of its genus infesting the local *Rattus* examined by the commission. It was eventually discovered, however, that these rats also were infested with *X. astia*, which is a relatively poor vector of plague and presents far less risk to humans than *X. cheopis*. Once it became apparent that two species of flea were present and that these fleas differed in seasonal abundance and in their ability to transmit plague, investigators were able to explain the observed seasonal fluctuations in human cases (*39,41*).

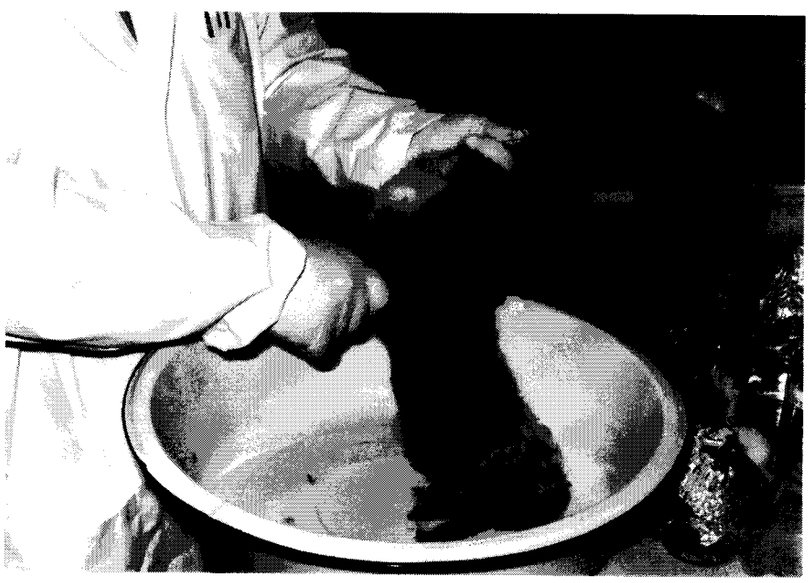
Often, trained entomologists can identify flea species directly from saline or alcohol without having to prepare permanent slide mounts in Canada balsam or other mounting media. Unfortunately, processing fleas for permanent slide mounts destroys any plague bacteria present and thus precludes determination of infection with *Y. pestis*. Nevertheless, at least a few fleas from each surveillance district should be mounted as permanent specimens for future taxonomic reference. Standard techniques for mounting fleas on slides can be found in a number of references (*42,43*).

*Removing fleas from captured animals*

Techniques for collecting fleas are relatively simple and can be carried out simultaneously with existing rodent surveillance programmes. The most common method for collecting fleas is to remove them from captured host animals. If hosts are captured alive, they should be anaesthetized as described in the rodent surveillance section before further processing

(*Fig. 1*). The anaesthetized animals are then placed in a white enamel pan (a depth of 20cm or more is recommended) and brushed vigorously from the tail end forwards with a toothbrush, pocket comb or other similar instrument (*Fig. 6*). This will dislodge fleas from the host; the fleas will fall to the bottom of the pan where they can be removed with forceps or a wetted applicator stick and placed in labelled vials containing either 2% saline or alcohol. Fleas stored in saline can be held for identification, bacterial isolation or other analyses (*15,44,45,46*). Those held in alcohol can be mounted for identification using standard methods (*43*) or analysed for *Y. pestis* infection by polymerase chain reaction (PCR) techniques (*45,46*). Hosts killed by capture in snap traps or other means can be examined directly for fleas, but insecticides or anaesthetic agents should be used to prevent live fleas from escaping onto the investigator or into the laboratory. Any bedding material placed in traps to provide warmth for the host should also be examined for fleas.

**Figure 6: Combing a rabbit for fleas. The animal and fleas have been anaesthetized prior to processing. As the comb passes through the hair it will dislodge fleas into the pan where they can be collected for identification and analysis**



## *Collection of fleas from burrows*

Fleas can be collected from rodent burrows by burrow swabbing or flagging. When fleas are periodically sampled by this method, it is often noted that burrow indices are low during interepizootic periods but



increase dramatically when plague epizootics cause high host mortality. A typical burrow swab consists of a flexible steel cable or hard rubber hose with a piece of white flannel cloth attached to the end (*Fig. 7*) (*47,48*). The cable is used to force the cloth down the burrow entrance; fleas mistake it for their normal hosts and cling to the cloth. The cloth is then removed from the burrow and inspected for fleas or placed in a plastic bag and held for later examination. Fleas in the bags can be killed by freezing, anaesthetization or insecticides.

**Figure 7: Surveillance worker using a burrow swab to collect fleas from burrows**

## *Collection of fleas from nesting material*

Many fleas spend more time in the nests of their hosts than on the host itself. Nest material can be examined for fleas by sorting the contents in an enamel pan such as was described above for brushing fleas from hosts. It may be necessary to kill fleas before sorting to prevent them escaping from the pan. Nest material also can be loaded into a Berlese funnel, a device that uses heat from a lightbulb located at the top of the funnel to drive fleas and other arthropods to the bottom of the funnel. Once the fleas reach the bottom, they fall into a jar containing a saline solution or alcohol (*49*).

*Flea indices*

The most basic information obtained from flea and rodent surveys is the number of fleas of different species found on various species of hosts. This raw data can be used to calculate various indices, including:

- Specific flea index = number of fleas of species *A* collected from host species *Y*, divided by the number of individuals of host species *Y* examined (multiplication of this index by 100 gives the percentage index);
- Total flea index = Total number of fleas collected (regardless of species), divided by the total number of hosts of species *Y* examined;
- Percentage of hosts infested = number of hosts of species *Y* infested with flea species *A*, divided by the total number of hosts of species *Y* examined, multiplied by 100.

Similar indices can be calculated for flea collections taken from burrows, nests or houses:

- Burrow (or nest or house) index = number of fleas of species *A* collected from burrows (or nest or house) of host species *Y*, divided by the total number of burrows (or nest or house) of host species *Y* examined.

The *specific flea index* is the most widely used of the above indices. It can be used in conjunction with other rodent and vector surveillance data to estimate human and epizootic risks. For example, it has been reported that a specific flea index of greater than 1 for *X. cheopis* on rats represents a potentially dangerous situation with respect to increased plague risk for humans (*39*). Many factors affect the reliability of flea indices, including host species, host age, trapping techniques, areas selected for sampling and the natural tendency of fleas to heavily infest several hosts within a population, while many animals have few or no fleas (high variance to mean ratios for sample data). To obtain reliable indices for comparison between different survey sites, all trapping and ectoparasite collection procedures should be standardized as much as possible.

A sequential sampling method for determining how many host animals must be sampled to derive a reliable flea index for a given host/flea relationship has been described by Schwan *(50*). He found that examination of as few as 20 Nile grass rats *(Arvicanthis niloticus*) was sufficient to establish a reliable specific flea index for either *Dinopsyllus lypusus* or *Xenopsylla cheopis bantorum* infestations. Schwan=s method use sequential calculation of the specific flea index to determine how the

inclusion of additional animals and their fleas changes the values for this index. As more and more animals and their fleas are included in the index calculations, the values begin to approach a particular value of the index that remains relatively stable with additional sampling. This can be shown graphically by plotting the number of host animals examined on the *X* axis and the specific flea index (calculated for *X* animals and their fleas) on the *Y* axis. Schwan proposed that the point at which the slope of this graph approaches zero represents the appropriate minimum number of animals that must be sampled to obtain a reliable specific flea index using standardized sampling techniques.

*Identification of Y. pestis in fleas*

Determining which flea species are infected with *Y. pestis* is critical for separating locally-important vectors from those which play only a minor role. Probably the most common method for determining whether fleas are infected with plague is to inoculate susceptible laboratory animals with ground fleas suspended in physiological saline (0.85%) (*44*). Material for flea suspensions may consist of either individual fleas or pools of fleas; fleas should be pooled by species, type of host, and area where collected. At the United States Centers for Disease Control and Prevention, Atlanta, the standard procedure to prepare fleas for inoculation is to grind flea pools (as many as 25 fleas per pool) in a mortar and pestle and then suspend the ground material in approximately 2 ml of physiological saline (0.85%). This suspension is then inoculated subcutaneously into mice (0.5ml suspension per mouse). The mice are monitored over the next 21 days, and those that die are necropsied to obtain tissues for bacterial isolation. Surviving mice can be sacrificed on day 21 postinoculation for sera and tissues. *Y. pestis* has also been detected in fleas using immunologic techniques, and PCR, but these procedures have yet to be widely tested under field conditions (*15, 45, 46*). Recently, PCR has been demonstrated to be more sensitive and reliable in some situations than mouse inoculation (*45*). As with all PCR assays, however, care must be taken to avoid false positives due to contamination with amplicons generated during previous reactions or as a result of contamination from other sources.

*Insecticide sensitivity surveys*

After locally-important flea vectors have been identified, their sensitivity to various insecticides should be determined. Data on insecticide susceptibility for flea populations in plague-endemic areas should be retained in the plague surveillance database and periodically updated. Prior knowledge of insecticide resistance among local flea

populations will enable plague control workers to select an appropriate insecticide and save valuable time in the event of a plague epizootic. Kits for testing fleas for insecticide sensitivity are available through the World Health Organization.

### Evaluation of surveillance data

After each collection period, all data from dead animal collections, colony observations and rodent or carnivore serosurveys should be analysed and mapped to determine the distribution of plague-infected animals in the area under study. Information from human and flea surveillance should be included as well. Mapping this information during epizootics can help determine the extent of the epizootic and whether control efforts have succeeded in preventing its spread to areas where humans would be at high risk of infection. Such mapping helps clarify the risk that plague-infected rodents and their fleas pose to human populations in the surrounding area. The proximity of infected animals to other populations of the same species or those of other susceptible rodent species, as well as habitat availability, should also be noted. This information is useful for estimating the likelihood that plague will spread to new areas and other rodent populations.

Whenever possible surveillance personnel should be aware of human activities that are likely to affect local rodent populations, such as development of new agricultural areas, villages or other development projects. Rodents respond quickly to habitat changes that provide them with new sources of food and harbourage, and existing plague problems will be exacerbated by human activities that create new rodent habitats.

### *Surveillance by health services*

National, regional and local health services should work together to develop a plague surveillance programme with clearly-defined responsibilities for routine surveillance tasks and emergency investigations. Responsibilities should be distributed among the different health services so that human cases and epizootics can be identified and investigated as quickly as possible by individuals trained to assess human plague risks and determine appropriate control measures. Such predictive surveillance and emergency response capabilities require certain personnel, equipment and facilities. The following section describes these basic requirements and suggests how responsibilities for various surveillance tasks can be allocated to local, regional or national services.

Country programmes must have a variety of personnel with training in such diverse fields as medicine, epidemiology, bacteriology, serology,

entomology, mammalogy, health education and environmental sanitation. There should be personnel at the local level trained to inspect areas for evidence of rodent die-offs, collect samples for routine rodent and vector surveillance, and conduct educational programmes to promote plague awareness, prevention and control. If local health services lack adequately-trained staff, experts from the regional or national health services should provide this training. At the local level, health services are also expected to maintain close contacts with the medical community so that human cases are recognized and reported as soon as possible.

Because of time and travel constraints, local agencies are normally responsible for at least the initial stages of human case investigations, including coordinating the collection and shipping of diagnostic specimens, obtaining exposure histories from patients and performing preliminary investigations of likely exposure sites.

Following these initial steps, more extensive epidemiological and environmental investigations (described earlier) should be instigated. Although local workers might be sufficiently trained to perform these investigations, the national (or regional) health services should be prepared to provide additional expert assistance if necessary. For this reason, national health services maintain at least one plague team composed of experts whose combined training includes the disciplines listed above, as well as knowledge of plague prevention and control techniques (*12*,*51*,*52*). A minimum, but adequate, plague team is comprised of an epidemiologist, bacteriologist/serologist and entomologist/zoologist.

At least one of these individuals (usually the epidemiologist) should have medical qualifications (*12*). These experts can participate directly in human case investigations and surveillance activities or serve as consultants for local or regional health officials. They can also train local workers in techniques of plague diagnosis, treatment, surveillance, prevention and control. If a country lacks the resources and personnel to form such a plague team, or if plague has just recently entered a country and a team has yet to be formed, it may be necessary to request the assistance of international consultants working under the direction of the World Health Organization. The national plague team should have sufficient field equipment and supplies to conduct emergency epidemic or epizootic investigations, as well as adequate transportation to move equipment and plague team members to the affected area (*52*). Surveillance programmes should be prepared, if necessary, to hire and

train temporary workers during emergency situations or seasonal peaks in plague activity.

Surveillance programmes must have adequate laboratory facilities for performing bacteriological and serological analyses on plague-suspect specimens. While it is preferable to have several laboratories located near plague foci, at a minimum a central (or national) laboratory that can analyse surveillance and diagnostic samples is essential (*12*). The central laboratory should be able to confirm the presence of *Y. pestis* in samples by culture, biochemical characterization and bacteriophage typing. The laboratory should also be proficient in using standard serological techniques to detect plague antibodies in serum samples. Whenever possible the laboratory should be able to analyse samples by direct immunofluorescence. Personnel at the central laboratory should keep abreast of recent developments in molecular biology and be prepared to adopt new techniques that are cost-effective and useful for surveillance purposes.

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# 7
# NATIONAL HEALTH SERVICES IN PREVENTION & CONTROL

*Dr Kenneth L. Gage*

Although local or regional health departments might have considerable expertise and do an excellent job of managing plague within their districts, the potential for the rapid spread of plague from one region of a country to another requires national prevention and control programmes capable of coordinating and assisting local and regional efforts. Plague's lack of respect for international boundaries also requires that national health services of neighbouring countries cooperate with one another to successfully control this disease. International control activities are best administered by national health services rather than by local or regional agencies. Surveillance and control of plague in port facilities and international airports should also fall under the supervision of the national health services. As was described under plague surveillance, the organization of national, local and regional plague prevention and control programmes may vary considerably from one country to another, but several important features are common to all.

The World Health Organization (*3*) has recommended a four-phased system of plague prevention and control that can be adapted to the requirements and resources of different countries. This section summarizes this system and describes how its implementation will result in a national plague prevention and control programme that is effectively integrated with local and regional programmes.

The first two phases of the WHO system address emergency measures to be implemented whenever a human plague case occurs. Plague prevention and control programmes in each country should have adequate personnel, equipment and laboratory facilities to undertake the phase 1 and phase 2 activities described below. Phases 3 and 4 outline the establishment of a surveillance system and development of long-term prevention and control measures. These activities require a greater commitment of personnel and resources than in phases 1 and 2, but their successful completion will significantly reduce the risk of human plague. It is recommended, therefore, that each country implement phases 3 and 4 to the fullest extent possible.

### Phase 1: Case recognition and medical intervention

National health service officials should verify that local and regional officials are trained and prepared to undertake emergency measures whenever a human case is suspected. After identifying a suspect plague case, local health services should:

(1) notify national and/or regional authorities;

(2) ensure that appropriate specimens are shipped to a qualified laboratory for diagnostic confirmation of *Y. pestis* infection;

(3) verify that patients have been placed on appropriate antibiotic treatment and that local supplies of antibiotics are adequate to handle further cases; and

(4) isolate pneumonic plague patients and cooperate with other health services to identify, monitor and, if necessary, arrange prophylactic treatment for individuals in contact with cases.

In addition to the above measures, a preliminary epidemiological investigation should be initiated. The purpose of this investigation is to obtain an exposure history from the patient in order to make an initial assessment of likely sources of infection and potential risks to others in the area. National and regional health services, including the national plague team described earlier in this manual, may be dispatched to the area if local skills or resources are inadequate. Plague experts with the national health services can also help local and regional authorities determine whether to recommend vaccination for individuals in high-risk areas or occupations. If a vaccination programme is approved and vaccine stocks are not locally available, the national health services should be prepared to provide local and regional authorities with information on where supplies of vaccine can be obtained.

### Phase 2: Epidemiological and epizootical investigation and emergency control

The second phase of the programme should be initiated immediately following phase 1. Phase 2 activities include an intensive environmental investigation of potential exposure sites for the human case(s) and initiation of emergency control measures to prevent additional cases. These investigations require both epidemiologists and persons trained in techniques for surveillance and control of rodents and fleas. The national plague team, whose services may have already been requested during phase 1, can provide this expertise when local or regional personnel lack adequate training. The plague team's central laboratory resources should be made available for the investigation.

The goals of the phase 2 environmental investigation are to:

(1) identify the rodent and flea species most likely to be sources of infection in the area where the human case(s) was exposed;

(2) determine the extent of epidemics and/or epizootics associated with the initial human case; and

(3) identify areas of potential risk to humans.

This information is used to determine emergency control measures to be taken to prevent additional human cases.

### Phase 3: Surveillance and control

The goal of phase 3 is to establish a surveillance and control programme. Because of ecological differences between plague foci in different geographic regions, preliminary research is needed to identify which local rodent and flea species should be targeted for extensive surveillance and control.

The research data can also be used in conjunction with information on local landscape, human activity and host/vector ecology to design prevention and control strategies appropriate for a particular plague focus. Any rodenticidal, insecticidal or environmental control measures developed during this phase should be tested locally to evaluate their effectiveness in reducing the human risk of plague.

Where local or regional health services lack the expertise to perform this research they must be assisted by personnel at the national level. The national health services should also work with local and regional authorities to develop and administer educational programmes to increase awareness and knowledge among health care personnel and the general public.

### Phase 4: Management

The final phase of the plague prevention and control programme stresses long-term management of plague foci. Such management calls for continuous surveillance of the important host and vector species identified during phase 3. Once the surveillance programme identifies a plague epizootic, control measures developed in phase 3 should be implemented as soon as possible. Long-term environmental management of plague foci should also be promoted. Environmental management stresses the elimination or reduction of areas, near homes or workplaces, that are attractive to plague-susceptible rodents. Plague

staff should work with other government officials to regulate and modify activities and practices **B**such as agricultural projects, construction, placement of garbage disposal facilities and so on **B**that are likely to lead to increased food and harbourage for locally-important rodent hosts. Health services should continue the educational programmes developed during phase 3. Finally, research to improve existing surveillance and control techniques should continue, following the procedures outlined for phase 3.

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