Manual for plague surveillance, diagnosis, prevention and control

World Health Organization

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ABBREVIATIONS

ALAT	alanine amino transferase
ASAT	aspartate amino transferase
CIN	cefsulodin–irgasan–novobiocin
CRP	C-reactive protein
DEET	diethyltoluamide
DDT	dichlorodiphenyltrichloroethane
DFA	direct fluorescent assay
DMP	dimethyl phthalate
EDTA	ethylenediaminetetraacetic acid
F1	fraction 1
F1RDT	rapid diagnostic test based on the F1 antigen
FBC	full blood count
GDG	Guideline Development Group
IM	intramuscular
IV	intravenous
PO	per oral
PCR	polymerase chain reaction
qSOFA	quick Sequential Organ Failure Assessment
RDT	rapid diagnostic test
RRT	rapid response team
US CDC	United States Centers for Disease Control and Prevention
WHO	World Health Organization

EXECUTIVE SUMMARY

Plague has killed millions of people in pandemics during the past 25 centuries *(1)*. The disease reappeared in several countries during the 1990s and, consequently, it has been categorized as a re-emerging disease *(2)*. Plague is of particular concern due to its high risk for human outbreaks, which makes it both a medical and a public health emergency *(2–4)*. Plague is an acute bacterial infection caused by the Gram-negative coccobacillus *Yersinia pestis*. Although effective antimicrobials are available, this disease has high mortality because most outbreaks take place in remote places, where proper diagnosis and treatment remain challenging *(2)*. Early identification and prompt management and treatment are crucial for ensuring better outcomes.

The occurrence of an outbreak of severe pneumonic plague in several cities in Madagascar in 2017, highlighted the need for clarifying key technical questions and updating guidelines from the World Health Organization (WHO). This manual was developed to provide comprehensive information about plague epidemiology and recommendations on surveillance, diagnosis, clinical management, and prevention and control. It is also based on the WHO's proposals presented at the Seventy-Fifth World Health Assembly in May 2022 to promote a stronger and more inclusive health emergency preparedness, response, and resilience (HEPR) architecture at global, regional and national levels.

The information in this manual has been compiled from several documents. The background baseline information about the disease and areas of good practice (best practices statement) were drawn from the *Operational guidelines on plague surveillance, diagnosis, prevention and control*, published by the WHO Regional Office for South-East Asia in 2010 *(2)*. However, three key areas needed to be revised: the use of rapid diagnostic tests for plague in different contexts; the choice of antimicrobials for treating the different forms of plague, considering the introduction of fluoroquinolones as a first-line medicine of choice; and the use of personal protective equipment in case of exposure to plague-infected corpses. New evidence-based recommendations on these key areas were developed and published in 2021 in the *WHO guidelines for plague management: revised recommendations for the use of rapid diagnostic tests, fluoroquinolones for case management and personal protective equipment for prevention of post-mortem transmission (5)*. Additionally, the case definitions were updated to reflect these recommendations during an international meeting of experts in September 2020 *(6).*

This manual will be of interest to health policymakers, emergency preparedness and response teams, and health care workers in plague-endemic areas.

1. INTRODUCTION

1.1 Background

Plague has killed millions of people in pandemics during the past 25 centuries *(1)*. While often considered a disease of the past, plague is far from being eradicated, and it remains a current threat in many parts of the world *(7)*. Plague reappeared in several countries during the 1990s and was consequently categorized as a re-emerging disease *(2)*. Plague is of particular concern due to its high risk for causing epidemics and as such Member States at risk for Plague outbreaks (See Chapter 2) should have strong surveillance, prevention and readiness aystems in place. In addition, the plague organism is considered to be a potential biological weapon for bioterrorists. Controlling plague requires an integrated, comprehense, One Health approach that includes prevention (e.g. reservoir and vector control and also infection prevention, preparedness, readiness and control measures for avoiding zoonotic and human-to-human transmission), prompt diagnosis with accurate laboratory confirmation, and prompt management with effective antimicrobials.

1.2 Rationale for this manual

The World Health Organization's (WHO's) guidelines on plague surveillance, diagnosis, prevention and control were published in 2010 by the Regional Office for South-East Asia *(2)*. In 2014, those guidelines were revised. However, the deliberations were general and were not formalized in a published document. The occurrence of a severe outbreak of pneumonic plague that affected several cities in Madagascar in 2017 highlighted the needs to clarify key technical questions and to gather into an easy-to-use document all the information necessary to face endemic and epidemic situations.

This manual compiles in a single document comprehensive background information and operational guidance on the epidemiological, clinical and public health considerations for plague from several documents: the *Operational guidelines on plague surveillance, diagnosis, prevention and control (2)*, *WHO guidelines for plague management: revised recommendations for the use of rapid diagnostic tests, fluoroquinolones for case management and personal protective equipment for prevention of post-mortem transmission (5)* and the revision of the international definition of plague cases *(6)*.

1.3 Target audience

This manual was developed for clinicians and public health professionals who may be tasked with ensuring preparedness or response.

This manual was also developed to inform the policy- and decision-makers responsible for developing national policies and guideline documents as well as for making purchasing arrangements and implementing training programmes.

1.4 Aims

The aim of this manual is to provide up-to-date guidance about the diagnosis, epidemiological surveillance, case management, prevention and control of the different forms of plague, both for sporadic cases and during outbreaks. The manual is also intended to serve as the basis for developing national guidelines, taking into account the available resources and other determinants in each country.

1.5 Methods

The content of the current manual is drawn from different sources.

The background baseline information about the disease, its surveillance and control, and areas of good practice were drawn from the operational guidelines published in 2010 *(2)*. However, this information has been updated as needed, for example, to reflect the current global situation of the disease.

The revised evidence-based recommendations for the use of rapid diagnostic tests (RDTs), and the use of fluoroquinolones for case management and personal protective equipment for prevention of post-mortem transmission were developed in accordance with the *WHO handbook for guideline development (8)*. The guideline proposal was approved by the WHO Guidelines Review Committee in May 2019. The recommendations were formulated by the Guideline Development Group at a meeting in 2019 and were approved by the WHO Guidelines Review Committee prior to their publication in 2021 (Annex 1). The methods used for the revision of the recommendations are detailed in the *WHO guidelines for plague management (5)*.

The plague case definitions were updated to reflect the guidelines during an international meeting of experts in September 2020 *(6)*.

This *Manual for plague surveillance, diagnosis, prevention and control* was peer reviewed by the external reviewers with diverse expertise who are listed in the Acknowledgments. All potential external contributors were asked to complete the standard WHO declaration-of-interests form. A summary of declarations of interests including how any identified conflicts of interest were managed, is presented in Annex 13. No interests were deemed relevant to the work of the group.

The document conforms to the WHO's proposals presented at the Seventy-Fifth World Health Assembly in May 2022 to promote a stronger and more inclusive health emergency preparedness, response, and resilience (HEPR) architecture at global, regional and national levels *(9)*, and presents response actions by Member States accordint to 5 critical areas: Collaborative Surveillance, Community Protection, Clinical Care, Access to Counter Measures and Coordination.

1.6 Dissemination and implementation

The digital version of this manual will be disseminated initially in English on the WHO website. All supporting documents will be also available on the website.

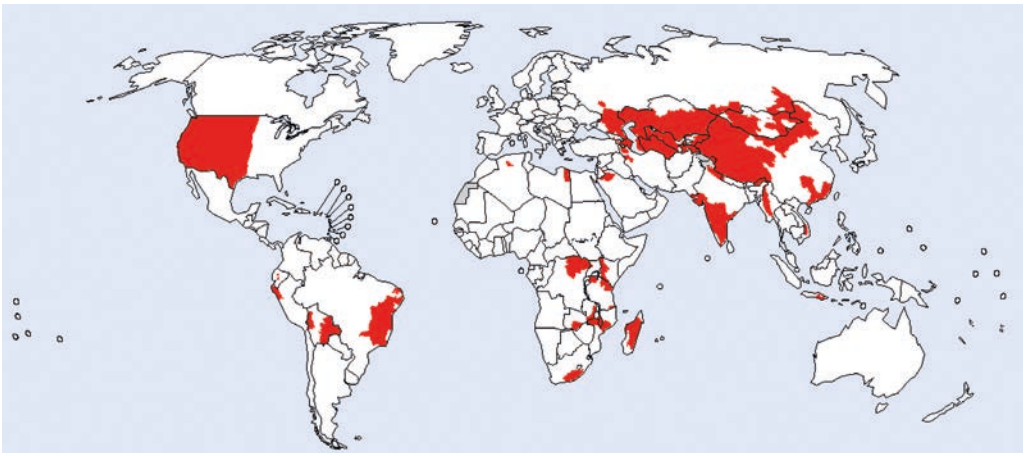
WHO headquarters will work closely with regional and country offices as well as implementing partners to ensure wide dissemination of the manual, which will include translation. The technical team in charge will work with regional offices to make the manual available at the country level. Assistance will be provided to Member States to adapt the manual to their national context.

2. EPIDEMIOLOGY

Plague is an acute bacterial infection caused by the Gram-negative coccobacillus *Yersinia pestis*. *Y. pestis* primarily affects rodents and is transmitted among rodents and other mammals via fleas. Usually, rodents are carriers of the infection, but they do not develop the disease. Humans, however, are susceptible to both infection and disease. *Y. pestis* has the potential to be highly virulent in humans, and the disease should be managed effectively to prevent an epidemic.

A natural focus is the presence of *Y. pestis* along with a compatible animal reservoir (mainly rodents) and a vector (fleas) in a specific geographical area. Although the number of reported human cases has been relatively low since 1945, plague reappeared in several countries during the 1990s and, consequently, was categorized as a re-emerging disease *(2)*. Plague remains a threat because there are vast areas with natural plague foci where plague is endemic. These natural plague foci are correlated with sporadic cases in humans and can cause outbreaks, such as the important one in Madagascar in 2017 *(10)*. The global distribution of natural plague foci in the rodent population as of 2016 is shown in Fig. 1, with no new foci declared since, but with a probable extension of the existing foci throughout surrounding regions or following long-distance transportation of infected reservoirs and vectors, as has happened in the past.

Fig.1 Global distribution of natural plague foci in rodents, 2016



First administrative–level areas with potential natural plague foci, based on historical data and current information

Source: *Weekly Epidemiological Record (4)*; used with permission

Since 2000, more than 95% of the burden of human plague has been concentrated in Africa, with the Democratic Republic of the Congo, Madagascar, Uganda and the United Republic of Tanzania being the countries most affected *(3,4)*. In the Americas, Peru and the United States of America regularly report cases. Central Asia has the largest number of natural foci of the disease, but the reservoir mainly consists of gerbils and marmots, with a limited at-risk population, leading to small and sporadic outbreaks *(3)*. Although plague is predominantly a rural disease, there have been outbreaks in urban populations in Madagascar. Plague outbreaks in urban settings are particularly difficult to control.

Natural plague foci are abundant worldwide. However, the lack of animal surveillance makes it difficult to understand the true prevalence of the foci. Therefore, the disappearance, emergence or re-emergence of human plague does not necessarily mean that there has been a change in the foci, but instead demonstrates the complexity surrounding surveillance. Therefore, during times when no human plague cases are reported, it is difficult to establish whether the bacteria have truly disappeared, whether the bacteria continue to circulate in the animal reservoirs but animal–vector–human transmission has been disrupted, or whether human infections have simply been undiagnosed or misdiagnosed. For these reasons, it is important to understand that risk is not limited to known epidemic or endemic areas. Natural plague foci can remain silent for decades as in Mahajanga, Madagascar, where no human plague cases had occurred for more than 60 years. Additionally, plague foci can suddenly emerge as in Algeria in 2003. Towns are particularly vulnerable due to their high population densities, which often include risk factors such as inadequate sanitation, housing and infrastructure (e.g. slums). In addition, they are transport hubs that are at high risk of importing infected reservoirs and vectors, especially port cities. Displaced and refugee populations in endemic areas are also at high risk. Furthermore, ecological changes, such as natural disasters, deforestation and drought, can affect the occurrence of human plague.

The epidemiology of plague is extremely complex. Infection depends upon the maintenance of a great variety of rodents and vectors, and these differ from country to country and also over time. Ecological studies point to a multiplicity of factors affecting the fluctuating balance that exists between rodents with greater and lesser degrees of susceptibility to the plague bacillus and the degree of risk to which humans are exposed. Understanding the dynamic relationships between animal reservoirs, vectors and human transmission is imperative to the control and management of plague.

2.1 Infectious agent

Y. pestis, the bacteria causing plague, is a pleomorphic non-motile, non-acid-fast, non-spore-forming and Gram-negative coccobacillus measuring 1.5 by 0.75 µm. When stained with aniline dyes, the ends of the bacillus take the stain more intensely; this is described as bipolar staining or looking like closed safety pins. True capsules are seen in living tissues, but less readily in culture. The capsular material is important for antigenicity and protection and is used for preparation of the fraction 1 (F1) antigen for serological testing.

Y. pestis belongs to the group of bacilli with low resistance to environmental factors, which means that sunlight, high temperatures and desiccation have destructive effects on the bacteria, and ordinary disinfectant and preparations containing chlorine (12%) kill the bacteria within 10 minutes.

2.2 Animal reservoirs

Wild rodents are the natural reservoir of plague. Many species of wild and domestic rodents and other small mammals are susceptible to infection but are only occasionally infected and are not necessarily important reservoirs. The two most important commensal rodents involved in *Y. pestis* transmission are *Rattus rattus* (roof rat) and *Rattus norvegicus* (Norway rat). The infection persists in some rodent species and a few other animal hosts. Although wild rodents can be infected and carry the plague bacteria, they are most often resistant to the disease. In contrast, peridomestic rodents (i.e. rats) are usually susceptible to infection and develop the disease: hence the phenomenon of “rat falls”. A rat fall is defined as having more than one dead rat in a house or more than one house with dead rats in cases in which it has been ascertained that the deaths have not been due to poisoning.

2.3 Vector

Fleas are the vectors of plague. Species of *Xenopsylla*, particularly *X. cheopis*, are the most important vectors and commonly infest household rodents in many parts of the world. In some areas, other species have a particular role in the plague cycle.

To act as an efficient plague vector, the flea must be able to ingest the plague organism with its blood meal, live long enough for the pathogen to multiply sufficiently, transfer the pathogen in sufficient concentrations to cause an infection in the local rodent hosts and be present in large enough numbers to maintain infection in the local rodent hosts.

Fleas avoid light and are mostly found among the hairs or feathers of animals, and in people's clothing or their beds. If possible, fleas feed several times during the day or night. Heavy infestations of fleas are recognized by marks on clothing and bedding from undigested blood ejected by the fleas. Most flea species feed on one or two specific host species, but in the absence of their normal host, they feed on humans or other animals.

The life cycle of fleas has four stages: egg, larva, pupa and adult (Fig. 2). Adult fleas are 1–4 mm long and have a flat narrow body. They vary in colour from light to dark brown. The larvae are 4–10 mm long and white in colour; they have no legs, but are very mobile. The pupal stage cocoon is well camouflaged because it is sticky and soon becomes covered with dust, sand and other fine particles. High humidity is required for pupal development. The adult fleas are fully developed within 1–2 weeks, but emerge from the cocoons only after receiving a stimulus, such as vibrations caused by movement of the host. Both female and male adult fleas take blood meals. Fleas breed close to the resting and sleeping places of the host, in dust, dirt, rubbish, cracks in floors or walls, carpets, animal burrows and birds' nests.

Fig.2 Stages of fleas: larva (left), pupa (middle) and adult (right)

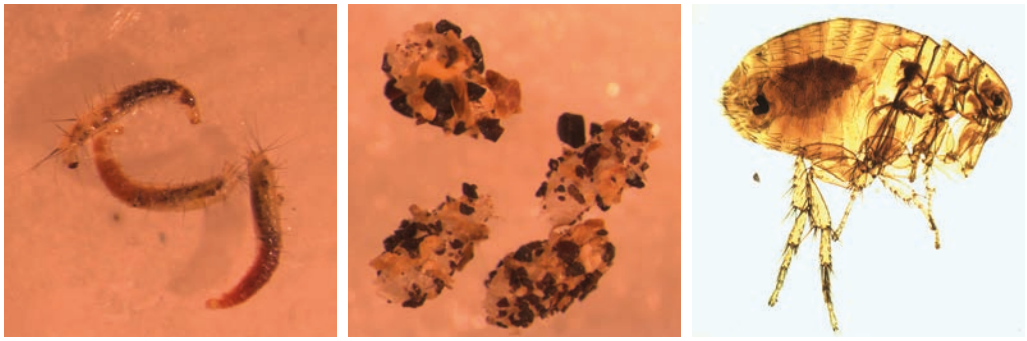


Photo credit: Mireille Harimalala, Institut Pasteur Madagascar; used with permission.

2.4 Seasonality

Geographical, meteorological and climatic factors, as well as the degree of socioeconomic development (e.g. the type of housing, the presence of overcrowding, sewerage, shipping and other forms of transport, and the degree of sanitation), have indirect influences on the qualitative and quantitative distribution of the rodents and fleas that act as potential reservoirs and vectors of *Y. pestis.* Although bubonic plague has occurred in every part of the globe, it is confined mostly to warmer latitudes. Extreme heat and a dry atmosphere are hostile to its spread; thus, in tropical countries it occurs during the colder months of the year, when the mean temperature is between 10 °C and 30 °C and the air has high relative humidity.

2.5 Mode of transmission

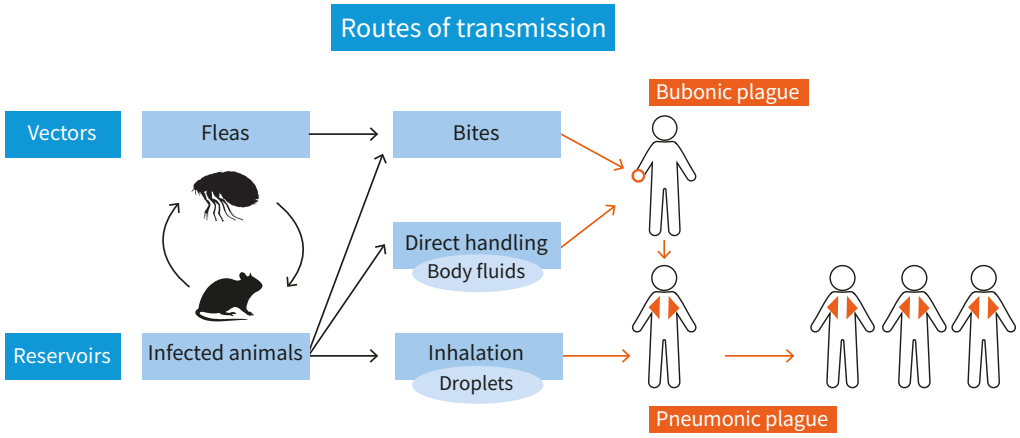
The transmission of plague from animals to humans is usually via the bite of an infected flea, leading to bubonic plague (Fig. 3). When a flea feeds on a rodent host, blood is taken into its midgut. In certain flea species such as the rat flea, *X. cheopis*, if plague bacteria are present in the blood meal, the bacteria multiply and form an obstruction (a biofilm) in the proventriculus, preventing the blood meal from reaching the midgut. Fleas with this blockage are unable to ingest their food, and they increasingly bite their mammalian host, allowing bacteria to evade the biofilm and be regurgitated into the host. The transmission of *Y. pestis* by fleas shortly after infection and in the absence of blockage (termed early-phase transmission) has also been observed across numerous flea species, including *X. cheopis*. The time until flea infectivity, which ranges from hours to days after the infected feed, depends on the flea species, mode of transmission and external temperature and humidity.

When primary bubonic plague progresses to secondary pneumonic plague, droplet transmission of the infective agent may take place via the respiratory route, resulting in primary pneumonic plague among close contacts. All persons with primary or secondary pneumonic plague can spread the disease via respiratory droplets, causing primary pneumonic plague in others.

Other modes of transmission include:

- direct, unprotected handling of infected animal tissues, for example, from a plague-infected rodent (live or dead) or other animal while skinning it or cutting meat;
- inhalation of contaminated respiratory droplets from animals, most commonly domestic cats;
- consumption of infective materials (e.g. infected meat that has not been cooked sufficiently to kill the *Y. pestis* bacteria).

Fig.3 Plague tranmission (simplified cycle)



Plague can also be nosocomially transmitted in the following circumstances: through direct contact with objects contaminated with purulent discharge or sputum from patients with pneumonic plague; laboratory-acquired infection; transmission to health care workers or other patients in the case of pneumonic plague; direct handling of the bodies of patients who died of plague (through contact with body fluids); and infected fleas on the clothing of a patient with bubonic plague.

2.6 Risk factors

A combination of conditions (e.g. environmental, climatic, hygienic) and relationships between the host reservoir, the flea vector, the microorganism and humans is necessary for a human case of plague to occur. There is an increased risk of exposure in:

- occupations and lifestyles associated with animal handling (e.g. hunting, trapping) and meal preparation (e.g. skinning, cutting of meat);
- unsanitary living conditions (e.g. poor waste management, overcrowding, residing in a home with heavy flea infestation or rats, or both);
- traditional death rituals, including during body preparation and population gathering.

Ecological changes created by natural disasters – such as earthquakes, volcano eruptions, flooding, drought – or human activities – such as encroachment, deforestation and mining – disturb the equilibrium density of rodents and their fleas. As a result, human populations can be exposed to vectors of plague.

3. CLINICAL MANIFESTATIONS

Human plague presents in several forms, with the two most common being bubonic and pneumonic, both of which can evolve to septicaemic plague. Less common forms include meningeal and pharyngeal plague. Plague is treatable, but a high index of suspicion is required to recognize the disease. The clinical presentations detailed below describe the classical presentations of the disease. The use of antibiotics before medical examination could lead to less obvious and milder forms of plague. Case fatality rates depend strongly on the clinical form of the disease, whether treatment is started early and health care workers' awareness of the disease, as well as the technical capacities of the health care system. Diagnosis can be aided by carefully collecting the epidemiological and clinical histories, such as exposure history, information about the recent use of antibiotics, the specific characteristics of the plague bubo, the presence of cough with blood-stained sputum, high fever and quick onset of symptoms.

Patients typically experience a sudden onset of illness characterized by malaise, fever, chills and headache, sometimes with gastrointestinal complaints (i.e. abdominal pain, nausea, vomiting, diarrhoea).

3.1 Bubonic plague

Bubonic plague is the most common presentation. The incubation period typically lasts 2 to 6 days and the case fatality rate is estimated at around 17% to 26% for both treated and untreated patients, based on data from confirmed cases of bubonic plague in Madagascar and Uganda (2017–2018) *(11, 12)*.

Usually, the causative agent infects humans through an infected flea bite. Following inoculation, local cutaneous proliferation ensues, normally not clinically evident. In some cases, a vesicle, pustule or ulcer (known as a plague carbuncle) develops at the inoculation site (Annex 2). The plague bacteria travel in the lymphatic system to the nearest lymph nodes, resulting in an inflammatory lymph node, called a bubo. Buboes may occur at the site of any regional lymph node, including inguinal (most common), axillary, supraclavicular, cervical, post-auricular, epitrochlear, popliteal or pharyngeal (Annex 2). Deeper nodes (such as intra-abdominal or intrathoracic) may also be involved through lymphatic or haematogenous spread.

Buboes are usually solitary but may be grouped in an irregular cluster with the overlying skin being warm and erythematous. The location of the primary bubo often suggests the site of infection. In circumstances in which patients are exposed to flea bites while sleeping, such as when plague-infected rats and rat fleas have invaded residences, localization to the upper or lower torso is typical. Inguinal buboes suggest that infection occurred in the lower extremities, for instance during field activities. Axillary buboes suggest upper extremity inoculation through the handling of infected animal tissues.

The progression of symptoms is usually rapid, with regional lymphadenitis becoming excruciatingly tender and painful. Small-to-moderately enlarged buboes may be masked by extensive perinodal inflammation and oedema. In advanced states of infection, buboes may suppurate and present as open sores and progress to septicaemia.

The differential diagnosis includes many infectious diseases, such as streptococcal or staphylococcal lymphadenitis, infectious mononucleosis (Epstein–Barr virus), cat-scratch disease (*Bartonella* infection), lymphatic filariasis, scrub typhus (*Orentia tsutsugamushi* infection), tularaemia (*Francisella tularensis* infection), syphilis and other causes of acute lymphadenopathy, for instance, developing after having an infected wound. Due to the

high prevalence of tuberculosis in low-income countries, tuberculous lymphadenitis, especially at the cervical area, should also be considered in the differential diagnosis, but the onset is usually more gradual. The involvement of intra-abdominal lymph nodes may mimic appendicitis, acute cholecystitis, enterocolitis or other intra-abdominal surgical emergencies.

3.2 Pneumonic plague

Pneumonic plague is one of the most fulminating diseases known to humankind. The incubation period is 1 to 3 days, although rapid onset and death can occur in less than 24 hours. Death usually ensues if effective antibiotic therapy is not initiated within 24 to 36 hours from disease onset. The case fatality rate was estimated at between 22% and 71% for treated and untreated patients in pneumonic plague outbreaks in Madagascar during the past decade *(13)*.

Primary pneumonic plague is transmitted through the inhalation of droplets from infected humans or animals. The onset of disease is typically manifested by the sudden start of chills, fever, headache, body pains, weakness and chest discomfort. Secondary pneumonic plague results from the haematogenous spread of *Y. pestis* to the lungs, usually due to an untreated or advanced infection of the initial bubonic form. Usually, a patient has been acutely ill for several days prior to lung invasion. Both primary and secondary pneumonic plague progress rapidly, and cough, sputum production, increasing chest pain, difficulty in breathing, hypoxia and often haemoptysis become prominent (Annex 2).

Pneumonic plague is contagious from the onset of symptoms. Pneumonic plague requires strict adherence to appropriate infection prevention and control measures, which include respiratory droplet precautions in addition to standard precautions. Patients maintaining enough physical strength to generate an intense cough reflex produce thin serosanguineous sputum containing the bacteria and are most likely to spread the infection. Many patients with secondary pneumonic plague die before they develop well-advanced pneumonia. When treated with effective antibiotic therapy, patients with pneumonic plague usually are not contagious after 48 hours.

The differential diagnosis is wide as clinical presentation does not differ from severe acute pneumonia caused by other pathogens – such as *Streptococcus pneumoniae*, *Haemophilus influenzae, Legionella pneumophila,* coronaviruses, influenza viruses – or conditions – such as anthrax (*Bacillus anthracis* infection), tularaemia (*Francisella tularensis* infection) and hantavirus pulmonary syndrome. However, bloody sputum, rapid deterioration and high case fatality rate are suggestive of pneumonic plague.

3.3 Other forms of plague

Other forms of plague are unusual, such as meningeal or pharyngeal. Plague meningitis presents similarly to meningitis caused by other bacteria. Pharyngeal plague presents with local erythema and painful and tender anterior cervical nodes. The cervical buboes may precede pharyngitis or develop secondary to pharyngeal involvement.

All forms of plague may evolve to septicaemic plague. Cases are always fatal if not treated. The presence of rapidly replicating *Y. pestis* in the bloodstream initiates a self-perpetuating immunological cascade typically linked to the host response to severe injury. The host response may result in a wide spectrum of pathological events, including disseminated intravascular coagulation, multiple organ failure and acute respiratory distress syndrome. Disseminated intravascular coagulation can lead to haemorrhage into the skin, and it sometimes results in cyanosis and tissue necrosis of distant extremities.

4. LABORATORIES IN SURVEILLANCE AND DIAGNOSIS

The laboratory plays a vital role in surveillance for and diagnosis of plague since laboratory examination of specimens from clinically and epidemiologically suspected cases is crucial to establish the diagnosis of plague and to support appropriate preventive and control measures.

4.1 Collecting, storing and transporting human samples

The collection, storage and transport of human specimens are detailed in this section. For information about animal specimens, see Annex 3.

When plague is suspected, clinical specimens should be collected urgently, but specific antimicrobial treatment must begin without waiting for laboratory confirmation of the disease. Specimens should be transported to the reference laboratory in a timely manner, and the laboratory must be informed of the shipment in advance. Efficient utilization of laboratory services involves collecting the right specimen, in the right quantity, in the right container, at the right temperature and analysing it in the right laboratory.

4.1.1 Collecting samples

Different specimens need to be collected, depending on the clinical form of plague suspected, as indicated in Table 1. As far as possible, specimens should be collected before antimicrobial therapy is initiated.

TABLE 1. Biological specimens required to diagnose plague

Clinical presentation	Specimens to collect
Bubonic	• Bubo aspirate • Venous blood (serum separator tube, EDTA tube and blood culture bottle)
Pneumonic	• Sputum, or bronchial or tracheal washing • Venous blood (serum separator tube, EDTA tube and blood culture bottle)
Septicaemic	• Venous blood (serum separator tube, EDTA tube and blood culture bottle)
Meningitis	• Cerebrospinal fluid • Venous blood (serum separator tube, EDTA tube and blood culture bottle)
Post-mortem	• Tissues from lymph nodes, lungs, bone marrow, spleen or liver

EDTA: ethylenediaminetetraacetic acid.

The preferred specimen for microscopic examination and isolation from a bubonic case is aspirated fluid (pus) from the bubo, which is expected to contain numerous organisms. Methods for collecting bubo pus are described in Annex 4.

Methods for collecting sputum from a suspected case of pneumonic plague are described in Annex 5.

The following points should be considered when collecting blood specimens.

- A large volume of blood should be taken for culture of *Y. pestis* whenever possible.
- Organisms may be seen in blood smears if the patient is bacteraemic.
- Bacteria may be intermittently released from affected lymph nodes into the bloodstream; therefore, a series of blood specimens taken 10–30 minutes apart may be helpful for isolating *Y. pestis*.

If specimens are taken post-mortem, lymphoid tissues, spleen, liver, lung and bone marrow samples may yield evidence of plague infection by culture on selective agar (e.g. cefsulodin-irgasan-novobiocin, or CIN, agar), microscopy, RDT or polymerase chain reaction (PCR).

Some specimens may require special handling, so specific instructions from the testing or receiving laboratory should always be sought prior to collection.

The following precautions should be applied when taking specimens (Annexes 4 and 5).

- Wash hands before and after collecting specimens.
- Follow strict aseptic technique and wear appropriate personal protective equipment (i.e. gown, goggles, face mask and gloves).
- Label and date the container (include the patient's information).
- Place the specimen aseptically into an appropriate sterile container.
- Tightly close the container.

4.1.2 Storing and transporting samples

In any outbreak investigation, before setting out into the field, it is essential to consult the receiving laboratory about the handling of the most relevant specimen types.

For bacterial culture other than blood culture, specimens should be collected on transport swabs and then kept in Cary–Blair transport medium to ensure *Y. pestis* viability while minimizing the overgrowth of other microorganisms. Specimens and blood culture bottles should be kept at ambient temperature if they will be processed within 24 hours. If using a medium other than Cary–Blair or if storage for longer than 24 hours is needed, specimens and blood culture bottles should be stored at 4–8 °C. Longer storage is not advisable as the yield of bacteria may fall significantly.

For antigen or antibody detection, serum should be stored at 4–8 °C or at –20 °C if it will be stored for longer than 48 hours. It is important to avoid unnecessary freeze–thaw cycles.

The above conditions must be preserved throughout transport to the laboratory and will vary according to transportation time. For clarifications, check with the laboratory that will be receiving the specimens.

Extensive vibration of samples, such as that encountered during transport for long periods over rough roads, can cause haemolysis of blood samples, rendering them useless for serology. If possible and if the necessary biosafety measures can be applied, serum should be separated from clotted blood before transport.

Adequate packaging and labelling of specimens for air and ground transport are required. Using standardized packaging methods and materials will ensure the safety of personnel and specimen integrity. If international transport is necessary, authorization to import the specimens should be organized by the reference laboratory, which should also inform the sender of receipt or non-receipt of the specimens. Specific guidance on the regulations for transporting infectious substances in 2021–2022 are available from WHO *(14)*.

4.2 Biosafety in the laboratory

Biosafety measures are required when handling material suspected to harbour *Y. pestis*. The biosafety risk control measures used depend on the outcome of the local risk assessment. Heightened control measures should be implemented when performing aerosol-generating procedures or when working with a high concentration or a high volume of *Y. pestis*. Enhanced control measures should be used when handling *Y. pestis,* including respiratory protection, a biosafety cabinet (with vertical laminar airflow) and strict decontamination and emergency response protocols. Only trained and competent personnel, working in a restricted area, should undertake plague diagnostic work. Further details can be found in the *Laboratory biosafety manual (15)*.

Personal safety. All laboratory staff must adhere to the standard precautions relevant to the enhanced biosafety control measures, including using appropriate personal protective equipment. It is mandatory for staff to wash their hands before leaving the laboratory.

Laboratory surfaces. A solution of 0.5% (5000 mg/L) sodium hypochlorite should be used to decontaminate laboratory surfaces. To decontaminate nonporous surfaces, a contact time of ≥ 10 minutes is recommended.

Managing accidental spills. Absorbent material should be placed over any spill to prevent aerosols from forming. A solution of freshly made 0.5% sodium hypochlorite should be sprayed or poured on the spill, working from the outside in, and left for 15–20 minutes; the spill should then be wiped up and the surface washed with a 70% alcohol solution. The material used to clean up the spill should be discarded with biohazardous waste. Germicidal solutions containing phenol or quaternary ammonium compounds should be used in instances in which hypochlorite is considered corrosive.

Managing materials. All contaminated material must be placed in biohazard bags. The biohazard bags can be filled to two thirds capacity and should be sealed with sterility indicator autoclave tape. The bags should be autoclaved at 121 °C for 20 minutes before disposal. Sharps must be disposed of in rigid puncture-proof containers that are labelled with a biohazard sign and an indicator that the container has sharps, and then decontaminated by autoclaving. Waste disposal must also conform to all other national and institutional guidelines.

4.3 Biological investigations

4.3.1 Rapid diagnostic tests

RDTs detect pathogen-specific antigens in a small quantity of different body fluids through lateral flow immunochromatography. In the case of plague, the RDT detects the F1 capsular antigen of *Y. pestis* (F1RDT), which is present in large amounts in buboes, blood and sputum from patients infected with plague.

Within 15 minutes, the F1RDT gives a semiquantitative result that is interpreted according to the intensity of the line (from 1+ to 4+), although it is most commonly used as a qualitative test (positive or negative result) in which positivity is interpreted as soon as the line is visible.

The test is performed on sputum from patients with suspected pneumonic plague, on bubo aspirate from patients with suspected bubonic plague, and on post-mortem specimens. Although the test is easy to use and interpret, it must be performed only by trained health care workers.

F1RDTs are commercially available and can be purchased by countries. Prior to use, it is the role of the laboratory that is utilizing the commercial tests to verify the accuracy

of the test for diagnosing plague in human samples. The Pasteur Institute of Madagascar can also provide a test through WHO. Instructions for the use of the RDT from the Pasteur Institute of Madagascar are described in Annex 6. RDTs should be stored according to the manufacturer's recommendations.

RDTs are first-line diagnostic tests in many countries. In patients with suspected plague, the use of an F1RDT can provide rapid diagnosis at the point of care to ensure appropriate case management and an immediate public health response. Additional testing is required in all cases to confirm or reject the diagnosis of plague (see case definitions in Section 5 and case management information in Tables 5–7).

The following points need to be considered when using RDTs.

- Tests must be performed by properly trained and competent health care or laboratory workers, according to the manufacturer's technical specifications.
- The F1RDT is not a screening test for the general population: it must be used on patients with clinical presentation of pneumonic or bubonic plague.
- Because the F1RDT has limited specificity, if the test is positive and while the initial public health response is being implemented, it is recommended to wait for the results of a confirmatory test (or tests), such as culture or molecular testing, before declaring a confirmed plague outbreak.[1]
- During an outbreak, in patients for whom there is clinical suspicion of any form of plague, treatment should be started independently of F1RDT findings and does not rely on a positive test. The likelihood of diagnosing bubonic plague in a person with a painful bubo during an already declared outbreak is high, and antibiotics are likely to be prescribed based on clinical diagnosis. Therefore, the main interest in using the F1RDT is to consider other diagnoses in patients with a negative RDT.[1] Similarly, during a pneumonic plague outbreak, a negative RDT should encourage consideration of an alternative diagnosis.

4.3.2 Laboratory tests for plague

Additional testing is required to confirm or reject the diagnosis of plague. These tests are performed in clinical (i.e. hospital) or reference laboratories and often require specimens to be shipped from the field. The main laboratory techniques are described in this section. See Annex 7 for a summary of laboratory tests for plague.

4.3.2.1 Microscopy after Gram or Wayson stain

Direct microscopic examination of specimens and cultures by Gram and Giemsa or Wayson staining can provide a rapid presumptive diagnosis (Fig. 4). However, the presence of Gram-negative, bipolar-staining bacilli alone is not enough to confirm the diagnosis of plague because other bacteria also have this morphology.

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1. For detailed recommendations, see *WHO guidelines for plague management: revised recommendations for the use of rapid diagnostic tests, fluoroquinolones for case management and personal protective equipment for prevention of post-mortem transmission (5)*. These recommendations were developed based on the evidence, mainly from the performance of the F1RDT developed at the Pasteur Institute of Madagascar. Therefore, policymakers need to assess the applicability of the findings if other F1RDTs are used to diagnose plague, depending on their performance.

Fig.4 Gram (left) and Wayson-stained (right) *Yersinia pestis* in mouse spleen showing the typical bipolar or safety pin appearance.

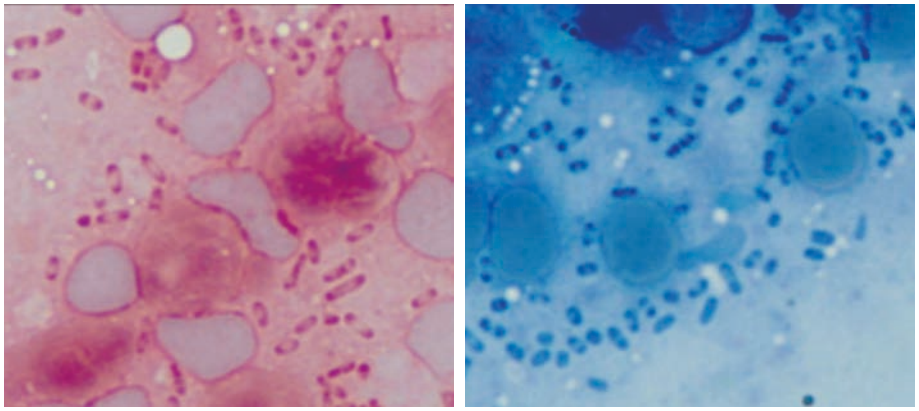


Photo credit: Jenny Rossouw, National Institute for Communicable Diseases, South Africa; used with permission

4.3.2.2 Yersinia pestis *culture and characterization*

Culture. The gold standard for confirming plague remains isolation of *Y. pestis* in culture. This method is highly specific and allows for antibiotic sensitivity testing. However, the technique is not often available in the field. In addition, it is a lengthy process that is sensitive to the presence of contaminants and prior antibiotic treatment. Culture requires a minimum of 4 days plus specimen transport time.

Characterization by bacteriophage lysis. Cultures can be identified as *Y. pestis* by using specific bacteriophage lysis. *Y. pestis* is sensitive to lysis with a temperature-dependent bacteriophage and can be differentiated from *Y. pseudotuberculosis* because the latter is sensitive to lysis only at incubation temperatures > 28 °C.

Characterization by biochemical testing. Biochemical testing with standard test tubes or commercial detection kits can be used to identify *Y. pestis*. Biochemical tests should be considered supplementary to confirmative identification that uses colony morphology, direct fluorescent antibody testing, bacteriophage lysis or PCR (Section 4.3.2.3). Automated bacteriological systems can be used to assist in identifying culture isolates, but these systems are prone to misidentify *Y. pestis*.

Characterization by mass spectrometry. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry may be used. However, a specialized database is required, and this technique cannot reliably differentiate between *Y. pestis* and the closely related *Y. pseudotuberculosis*. Cultures suspected to be *Y. pestis* should be inactivated with a validated method before spotting on the test plate.

Characterization by F1 detection. A rapid, specific staining method for detecting *Y. pestis* includes using a fluorescent-labelled antibody that binds to the capsular F1 antigen. Cultures may be stained and then analysed by fluorescence microscopy. As the F1 antigen is expressed only at temperatures > 33 °C, this staining procedure cannot be performed on cultures that have been maintained at < 33 °C.

Typing of *Y. pestis*. Molecular tools are used in advanced laboratories to type *Y. pestis*. These techniques aim at defining the limits of various plague foci, at identifying the source of an outbreak or at tracing the spread of the bacillus.

Antibiotic-susceptibility testing. Although a multidrug-resistant isolate from Madagascar has been recovered from a bubonic plague case, drug resistance has not been a problem in treating patients with plague. Naturally occurring strains of resistant *Y. pestis* isolates are rare. The potential for the spread and public health implication of multidrug-resistant *Y. pestis* needs to be monitored by research and through heightened surveillance.

For antibiotic-susceptibility testing, laboratories may choose from the simple disk diffusion method or more sophisticated automated formats, depending on the availability, flexibility of the test, the growth requirement of the bacterial agents and the laboratory's capability.

4.3.2.3 Detecting Yersinia pestis *DNA by species-specific polymerase chain reaction*

PCR requires specific equipment. Real-time PCR amplification with *Y. pestis*-specific probes has been shown to be useful for detecting *Y. pestis* in biological fluids. Compared with conventional PCR amplification, the hands-on time and detection limit are decreased, and the risk of cross-contamination between amplification products is also reduced because postamplification procedures are not required. However, real-time PCR equipment is expensive and needs to be operated by skilled personnel.

The performance of molecular diagnostic techniques is influenced by biological and technical variability. Therefore, these techniques for the diagnostic laboratory should be validated before application.

Species-specific PCR detection of *Y. pestis* requires the following.

- A combination of at least two different target genes must be used for *Y. pestis* species-specific PCR.
- PCR assays detecting two different *Y. pestis* target genes must both be positive to fulfil confirmatory criteria.
- PCR detection can be applied either to clinical samples or cultures.
- In order to confirm the first case in a suspected outbreak, PCR testing should be repeated on a newly extracted DNA sample.
- Both real-time and conventional PCR can be used to detect *Y. pestis* DNA.

Y. pestis genes targeted by PCR:

- are located on plasmids or the chromosome. The detection of these genes differs in sensitivity (gene copy number) and specificity (whether a nucleotide sequence is unique to *Y. pestis*);
- are summarized in Table 2.

TABLE 2. *Yersinia pestis* gene targets used for diagnostic testing with polymerase chain reaction

Gene	Location	Desccription of gene	Gene sequence unique to *Y. pestis*	Gene copy number
caf1	pFra or pMT1 plasmid	Encodes the F1 antigen	Yes; no gene homologs in other organisms	Low copy number
pla	pPla or pPCP1 plasmid	Encodes the plasminogen activator	No; gene homologs present in other Enterobacterales	High copy number
ypo2088	Chromosome	Encodes a methyltransferase	No; a gene homolog is present in *Y. hibernica*	Single
ypo2486	Chromosome	Encodes a hypothetical protein	No; partial gene homologs present in other *Yersinia* species; 3′ end of gene is unique to *Y. pestis*	Single
inv pseudo-gene with insertion of IS200-like element	Chromosome	The *inv* gene encodes invasin in *Y. eudotuberculosis*; it is a pseudogene in *Y. pestis* due to an IS element insertion	No; there is a homolog of the *inv* gene in *Y. pseudotuberculosis.* The presence of the IS200-like element within the *inv* pseudogene is unique to *Y. pestis.* Differing amplicon sizes based on the presence of an IS200-like element (1100 bp for *Y. pestis*; 400 bp for *Y. pseudotuberculosis*) can be used to provide specificity.	Single

4.3.2.4 Serology

Immunological methods (enzyme-linked immunosorbent and haemagglutination assays) may be used for plague confirmation (serodiagnosis) when the causative agent cannot be isolated. Confirmation is provided by evidence of seroconversion, that is at least a four-fold increase in the anti-F1 titre in paired serum samples or negative serology that turns positive in a minimum of 7 days. A patient with a single positive serology test is a presumptive case because it cannot be ruled out that positivity indicates previous infection with *Y. pestis* or a vaccination.

5. CASE DEFINITIONS

The recommended plague case definitions are based on epidemiology and laboratory findings, in accordance with new diagnostic techniques (Table 3) *(6)*. These case definitions can be used as a reference for notification under the International Health Regulations framework, and they should be used for epidemiological investigation when possible and appropriate, but they can be adapted for local purposes depending on local or national conditions and means.

TABLE 3. WHO plague case definitions

Plague case	Suspected	Probable	Confirmed	Not a case
Clinical and contextual	Clinical presentation suggestive of plague AND Epidemiological context suggesting possible exposure to plague (exposure to infected humans or animals, or residence in or travel to a known endemic focus within 10 days prior to onset of the disease)			
Tests	AND NO TEST PERFORMED	AND ONE of the following: • F1 antigen-positive in bubo aspirate, sputum, blood or post-mortem tissues by F1RDT or DFA • single positive anti-F1 serology test without evidence of previous *Y. pestis* infection or vaccination • direct microscopy in a clinical sample positive for Gram-negative coccobacilli that display bipolar staining with Wayson or Giemsa stain	AND AT LEAST ONE of the following three criteria: • isolation of *Y. pestis* from a clinical sample – must have appropriate colony morphology and be identified as *Y. pestis* based on at least two of the following: – bacteriophage lysis at 20–25 °C – biochemical profile – F1 antigen detection • seroconversion or a four-fold difference in anti-F1 antibody titre in paired serum samples drawn at least 2 weeks apart • *Y. pestis* DNA-positive by species-specific PCR on either clinical sample or culture, according to standard practice[a]	AND EITHER: • AT LEAST TWO of the following laboratory tests are conducted AND they are negative: F1RDT, DFA against F1 antigen, direct microscopy, convalescent serology, culture, PCR **OR** • when no confirmatory tests can be performed, TWO negative F1RDTs on two clinical specimens collected within a 24-hour interval

DFA: direct fluorescent assay; F1RDT: rapid diagnostic test based on F1 antigen; PCR: polymerase chain reaction.
[a] See Section 4.3.2.3 for positivity criteria for species-specific PCR detection of *Y. pestis*.

6. CLINICAL MANAGEMENT

- Bubonic plague is a **medical emergency** due to the high mortality if untreated.
- Pneumonic plague is a **medical and public health emergency** due to the high mortality and danger of person-to-person spread.
- Patients suspected of having bubonic plague should be managed under **standard precautions**, which include a point-of-care risk assessment.
- Patients suspected of having pneumonic plague must be placed in isolation and managed under **respiratory droplet precautions** in addition to **standard precautions**.
- Appropriate specimens for diagnosis should be obtained immediately.
- Patients should be started on specific antimicrobial therapy immediately after the specimens are collected without waiting for a confirmed diagnosis.

Rapid diagnosis and treatment are essential to reduce complications and death. If effective treatments are given in time, including antibiotics and supportive measures, plague patients can be cured. A patient with pneumonic plague represents a danger to everyone with whom they come into close contact. Health care workers and patients should protect themselves, other patients and other health care workers, including by wearing surgical masks.

Referrals of patients to other hospitals or clinics must be limited in the case of pneumonic plague. Cases should be treated at the site of diagnosis to reduce the risk of spreading the infection during transportation from site to site. The larger the number of contacts the patient has, the more complex it is to provide chemoprophylaxis for all contacts.

6.1 Clinical management according to epidemiological context and clinical presentation

The clinical management of individual cases depends on the epidemiological context and the clinical presentation of plague and should not be based on the plague case definitions (Table 3), which serve epidemiological purposes. Although the final decision about diagnosis relies on clinical expertise, the clinician should always keep a differential diagnosis workup in mind. The suggested steps to follow for a suspected case of plague are:

1. assess the risk of plague according to the epidemiological context, including the geographical area, the context of an epidemic and the presence of a contact with *Y. pestis* (Table 4);

2. conduct clinical assessment, based on:
   - presenting signs and symptoms suggestive of bubonic, pneumonic or septicaemic plague (Section 3);
   - signs of severity, including hypoxia, increased respiratory rate, signs of sepsis, shock, high score on the quick Sequential Organ Failure Assessment (qSOFA)[2] (or other recommended scores for sepsis);

2. At least two of the following clinical criteria that together constitute a new bedside clinical score termed quick SOFA (qSOFA): respiratory rate of ≥ 22 breaths/minute, altered mental status or systolic blood pressure ≤ 100 mm Hg.

3. investigate and begin clinical management according to the epidemiological and clinical assessments, **while awaiting microbiological confirmation** (Tables 5–7);
4. ensure close follow-up and consider adjusting the antibiotic regimen depending on the microbiology results and clinical evolution.

TABLE 4. Risk that a patient has plague, according to the epidemiological context

High risk	Intermediate risk	Low risk
Within 10 days[a] prior to onset of the disease, any of the following occurred: • close and unprotected contact (no personal protective equipment) with a confirmed or probable[b] case of pneumonic plague; • unprotected contact with an animal infected with *Y. pestis* (e.g. a bite, contact with body fluids when skinning an animal or conducting a necropsy, eating raw meat); • accidental exposure to *Y. pestis* by puncture (e.g. in a research lab).	Any of the following occurring within 10 days[a] prior to onset of the disease: • close and unprotected contact with a suspected case of pneumonic plague; • residing in or travelling to an area where a human case of plague has recently been reported or *Y. pestis* circulation within reservoir species has been documented; • bitten by a flea in a known endemic area.	• Residing in or recent travel (within 10 days[a] prior to onset of the disease) to a known endemic area

[a] The incubation period is usually 1–6 days, depending on transmission pathways. However, a longer duration is considered here for risk assessment purposes.

[b] See the plague case definitions (Table 3).

TABLE 5. Investigations and clinical management in the context of a high epidemiological risk for plague

HIGH EPIDEMIOLOGICAL RISK				
Clinical classification	**Clinical presentation suggestive of pneumonic or septicaemic plague WITH signs of severity[a]**	**Clinical presentation suggestive of PNEUMONIC plague with NO signs of severity[a]**	**Clinical presentation suggestive of BUBONIC plague with NO signs of severity[a]**	**Plague clinically unlikely**
Investigations	Perform all the following when possible: • chest X-ray[b] • blood analysis (FBC,[c] CRP,[c] lactate,[c], biochemistry[d] and coagulation[d]) • F1RDT on sputum[e] • on blood and sputum, molecular testing, culture and characterization of *Y. pestis*.		Perform all the following when possible: • collect bubo aspirate for F1RDT, culture and molecular testing for *Y. pestis* • blood analysis (FBC,[c] CRP,[c] biochemistry[d] and coagulation[d]) • blood for molecular testing, culture and characterization of *Y. pestis*.	• No testing required for investigation of plague. • Complementary testing should be performed as required for alternative diagnoses, depending on clinical presentation.
Management (while awaiting microbiological confirmation)	• Initiate hospital care and isolation. • Start IV plague treatment as soon as possible with combination therapy that includes a fluoroquinolone.	• Consider hospital care initially and isolation. • Start plague treatment as soon as possible with a single oral antibiotic, such as a fluoroquinolone (consider parenteral administration, depending on the context). • If in outpatient care, closely follow up within 24–48 hours or immediately if illness worsens.	• Start plague treatment as soon as possible with a single oral antibiotic, such as a fluoroquinolone or doxycycline. • Follow up within 48–72 hours or immediately if illness worsens.	• Start postexposure prophylaxis (Section 6.5.1). • Conduct clinical follow up as per the alternative diagnosis; ask the patient to return to the health care centre if symptoms suggestive of plague develop or as soon as they worsen.

CRP: C-reactive protein; F1RDT: rapid diagnostic test based on the F1 antigen; FBC: full blood count; IV: intravenous.

[a] Signs of severity include hypoxia, an increased respiratory rate, signs of sepsis, shock, high score on the quick Sequential Organ Failure Assessment (qSOFA) or other score for sepsis.

[b] The chest X-ray is looking for alveolar or alveolar–interstitial infiltrates. Extensive or bilateral infiltrates favour the diagnosis of pneumonic plague, whereas the absence of infiltrates suggests alternative diagnoses (except for patients seen < 24 hours after the onset of symptoms or those who have already been treated with an effective antibiotic regimen).

[c] Abnormal white blood cell counts (< 4 000/L or > 12 000/L) and high CRP suggest a systemic inflammatory process; lactate > 2 mmol/L is a sign of severity that requires close follow up.

[d] Biochemistry (i.e. Na, K, Cl, HCO3-, creatinine, aspartate amino transferase [ASAT], alanine amino transferase [ALAT], bilirubin, alkaline phosphatase) and coagulation are investigated to examine whether organ dysfunction is present, such as kidney or liver failure.

[e] Additional testing is required in all cases to confirm or reject the diagnosis of plague (Section 4.3.1).

TABLE 6. Investigations and clinical management in the context of an intermediate epidemiological risk for plague

INTERMEDIATE EPIDEMIOLOGICAL RISK				
Clinical classification	**Clinical presentation suggestive of pneumonic or septicaemic plague WITH signs of severity[a]**	**Clinical presentation suggestive of PNEUMONIC plague with NO signs of severity[a]**	**Clinical presentation suggestive of BUBONIC plague with NO signs of severity[a]**	**Plague clinically unlikely**
Investigations	See Table 5	See Table 5	See Table 5	See Table 5
Management (while awaiting microbiological confirmation)	• Initiate hospital care and isolation. • If F1RDT is positive or not done, start plague treatment as soon as possible with combination therapy that includes a fluoroquinolone. • If F1RDT is negative, consider starting postexposure prophylaxis depending on exposure (Section 6.5.1), with antibiotics that cover alternative diagnoses of pneumonic or septicaemic plague (follow national reference guidelines).	• Consider hospital care initially and isolation. • If F1RDT is positive, start plague treatment as soon as possible with a single oral antibiotic, such as a fluoroquinolone (consider parenteral administration, depending on the context). • If F1RDT is negative, start treatment for non-severe community-acquired pneumonia with an oral antibiotic such as amoxicillin (depending on local antibiotic-resistance patterns, and follow national reference guidelines), and consider starting postexposure prophylaxis. • If F1RDT is not done, treat for non-severe community-acquired pneumonia while considering that it could be plague; for example, treat with an oral fluoroquinolone. • If patient in outpatient care, closely follow up within 24–48 hours or immediately if illness worsens.	• Start plague treatment as soon as possible with a single oral antibiotic, such as a fluoroquinolone or doxycycline. • If F1RDT is negative, consider alternative diagnoses. • Follow up within 48–72 hours or immediately if illness worsens.	• Conduct clinical follow up as per the alternative diagnosis; ask the patient to return to the health care centre if symptoms suggestive of plague develop or immediately if their illness worsens.

F1RDT: rapid diagnostic test based on the F1 antigen.

[a] Signs of severity include hypoxia, an increased respiratory rate, signs of sepsis, shock, high score on the quick Sequential Organ Failure Assessment (qSOFA) or other score for sepsis.

TABLE 7. Investigations and clinical management in the context of a low epidemiological risk for plague

LOW EPIDEMIOLOGICAL RISK				
Clinical classification	**Clinical presentation suggestive of pneumonic or septicaemic plague WITH signs of severity[a]**	**Clinical presentation suggestive of PNEUMONIC plague with NO signs of severity[a]**	**Clinical presentation suggestive of BUBONIC plague with NO signs of severity[a]**	**Plague clinically unlikely**
Investigations	See Table 5	See Table 5	See Table 5	See Table 5
Management (while awaiting microbiological confirmation)	• Initiate hospital care and isolation. • If F1RDT is positive or not done, start plague treatment as soon as possible with combination therapy that includes a fluoroquinolone. • If F1RDT is negative, treat with antibiotics for the alternative diagnoses of pneumonic or septicaemic plague (follow national reference guidelines).	• Initiate outpatient care and isolation. • Start treatment for non-severe community-acquired pneumonia with an oral antibiotic such as amoxicillin (follow national reference guidelines). • Follow up within 24–48 hours or immediately if illness worsens.	• Start plague treatment as soon as possible with a single oral antibiotic, such as a fluoroquinolone or doxycycline. • If F1RDT is negative, consider alternative diagnoses. • Follow up within 48–72 hours or immediately if illness worsens.	• No pharmacological treatment required for plague. • Follow up as per the alternative clinical diagnosis.

F1RDT: rapid diagnostic test based on the F1 antigen.

[a] Signs of severity include hypoxia, an increased respiratory rate, signs of sepsis, shock, high score on the quick Sequential Organ Failure Assessment (qSOFA) or other score for sepsis.

6.2. Infection prevention and control

Infection prevention and control measures are aimed at preventing the transmission of plague bacteria to health care workers and spread among other patients. They include using:

- standard precautions in addition to droplet precautions. Standard precautions should be used to manage all patients with suspected plague. Droplet precautions should be adhered to for all patients with suspected pneumonic plague in addition to isolation;
- isolation arrangements for patients with suspected or confirmed pneumonic plague (Annex 8);
- safe handling and burial practices for bodies (Section 8.3.1.1);
- carefully cleaning and disinfecting instruments. Instructions on preparing solutions for disinfection are given in Annex 9;
- source control for patients with primary or secondary pneumonic plague, including applying a medical mask to the patient when possible and tolerated.

6.3 Antibiotic treatment

When human plague is suspected on epidemiological and clinical grounds, patients should be started on specific antimicrobial therapy immediately after specimens are obtained without waiting for a confirmed diagnosis of the disease (Tables 5–7). Therapy should not be withheld even if disease onset was more than 24–36 hours before the patient was seen by health care workers. Antimicrobial therapy should be started as recommended by national guidelines. If national guidelines are not available, the treatment should be chosen from the options listed below. Re-evaluation and de-escalation of antibiotics should occur once the results of antibiotic-sensitivity tests are available and based on the clinical evolution of illness.

6.3.1 Antibiotics recommended for treating plague

6.3.1.1 Aminoglycosides: streptomycin and gentamicin

Streptomycin is an effective antibiotic against *Y. pestis* that has been used since 1948 to treat pneumonic, septicaemic and bubonic plague. Gentamicin is also effective and offers the advantage of once daily administration. Other aminoglycosides that can be used for treating plague include amikacin, tobramycin and plazomicin; these should be given in once daily to reduce the risk of side effects. While aminoglycosides are effective in curing the disease, they have some disadvantages: they need to be given parenterally; are not widely available in primary care; and have important adverse effects, such as hearing loss and nephrotoxicity; additionally, injection site abscesses are especially associated with streptomycin because its use requires numerous intramuscular injections. Therapeutic monitoring is advised when using aminoglycosides.

6.3.1.2 Chloramphenicol

Chloramphenicol is another effective antibiotic used for many years to treat plague, and it is the medicine of choice for plague meningitis. Rare but notable adverse effects include “grey baby” syndrome (i.e. vomiting, diarrhoea, hypothermia, respiratory distress and shock) and bone marrow suppression.

6.3.1.3 Doxycycline and other tetracyclines

Tetracyclines are broad-spectrum bacteriostatic agents. The main tetracycline used for treating plague is doxycycline. Doxycycline is effective in the primary treatment of bubonic plague, but it has reduced efficacy in pneumonic plague. Tetracycline is also used and effective for treating bubonic plague. Other tetracyclines that are likely to be effective for bubonic plague include omadacycline, minocycline and eravacycline. Tetracyclines may be used as an adjunct to other antibiotics and are effective in patients for whom aminoglycosides are contraindicated. The adverse effects of tetracyclines include photosensitivity, oesophagitis and secondary fungal infection.

6.3.1.4 Fluoroquinolones

Fluoroquinolones have been used recently for treating people with plague. They have proved to be effective in animal studies and in a small number of human cases. Fluoroquinolones have several advantages over other antibiotics, including their safety and the possibility of oral administration. They are or can be made easily available and accessible in all settings (including remote areas), do not present any particular concerns in terms of storage and administration, and are usually well accepted by patients. Fluoroquinolones have been associated with several side effects (e.g. tendinopathy). The benefits of using fluoroquinolones to treat plague outweigh the risks, given the patient’s risk of mortality. The main fluoroquinolones used for treating plague include ciprofloxacin, levofloxacin and moxifloxacin.

6.3.1.5 Sulfonamides

Sulfonamides have been used extensively to prevent and treat plague and can be administered orally or intravenously. However, if used alone, some studies have shown higher mortality, increased complications and longer duration of fever as compared with the use of streptomycin, chloramphenicol or tetracyclines. Their indication is now limited to prevention or an alternative option for treatment, but they must always be used in the combination sulfamethoxazole + trimethoprim.

6.3.1.6 Other antibiotics

Penicillins, cephalosporins and macrolides have poor activity against *Y. pestis* and are not recommended.

6.3.2 Treating plague according to its clinical form and in specific populations

The treatment of plague differs according to the clinical form of presentation, the severity of the disease and the variation of penetration of antibiotics in different tissues.

Route of administration. Patients should be started on antibiotics via the enteral or parenteral route, depending on the severity of their disease and on the expected tolerance of the enteral route, as per the clinical judgment of the treating physicians. Parenteral antibiotics can be switched to oral after clinical improvement, as per the clinical judgment of the treating physicians.

Duration of treatment. Antibiotics should be given for a total of 10 to 14 days for all forms of plague, depending on the clinical evolution of the illness, or longer if symptoms continue.

Antibiotic resistance. Resistant strains of *Y. pestis* have been isolated, but they remain rare worldwide. When the results of antibiotic-susceptibility testing are available, the choice of antibiotics should be adapted accordingly.

6.3.2.1 Pneumonic and septicaemic plague

First-line and alternative antibiotic options for treating patients with pneumonic or septicaemic plague are summarized in Table 8. When possible, it is recommended to start with combination therapy that includes antibiotics from two distinct classes. Narrowing therapy to a single antibiotic can be considered after there is clinical improvement.

For suspected cases of pneumonic plague and while awaiting diagnostic confirmation, antimicrobial coverage should also consider the usual causative pathogens of pneumonia in addition to *Y. pestis*. Fluoroquinolones such as levofloxacin or moxifloxacin are good options for these cases.

TABLE 8. Antibiotics for treating pneumonic or septicaemic plague

Antibiotic	Age group	Dosing (per dose)	Interval (hours)	Route of administration
First-line options				
Ciprofloxacin	Adults	400 mg 750 mg[a]	8 12[a]	IV PO
	Children	10 mg/kg (max. 400 mg/dose) 15 mg/kg (max. 750 mg/dose)	8 or 12 12	IV PO
Levofloxacin	Adults	750 mg	24	PO or IV
	Children ≥ 6 months	< 50 kg: 8 mg/kg (max. 250 mg/dose) ≥ 50 kg: 500–750 mg	12 24	PO or IV PO or IV
Moxifloxacin[b]	Adults	400 mg	24	PO or IV
Gentamicin	Adults	5 mg/kg	24	PO or IV
	Children	4.5–7.5 mg/kg	24	IM or IV
Streptomycin	Adults	1 g	12	IM or IV
	Children	15 mg/kg (max. 1 g/dose)	12	IM or IV
Alternative options				
Moxifloxacin[b]	3–23 months	6 mg/kg (max. 200 mg/dose)	12	PO or IV
	2–5 years	5 mg/kg (max. 200 mg/dose)	12	PO or IV
	6–17 years and < 45 kg	4 mg/kg (max. 200 mg/dose)	12	PO or IV
	12–17 years and ≥ 45 kg	400 mg	24	PO or IV
Doxycycline[c]	Adults and children ≥ 45 kg	200 mg loading dose followed by 100 mg	12	PO or IV
	Children < 45 kg[d]	4.4 mg/kg (max. 200 mg) loading dose followed by 2.2 mg/kg (max. 100 mg)	12	PO or IV

IM: intramuscular; IV: intravenous; max.: maximum; PO: per oral.

[a] The recommended per oral dose during pregnancy is 500 mg every 8 hours.

[b] Moxifloxacin is not recommended as a first-line choice but as an alternative in children and adolescents aged < 17 years due to having a higher risk of side effects compared with other fluoroquinolones.

[c] The Guideline Development Group of the *WHO guidelines for plague management* (*5*) noted that doxycycline was an option for treating pneumonic and septicaemic plague, although some clinicians consider that it is less effective than other alternatives. With limited supporting evidence, the Guidelines Development Group recognized that doxycycline is used as first-line treatment in some settings for milder forms of pneumonic or septicaemic plague, but clinicians would probably not use doxycycline to treat more severe forms.

[d] Doxycycline has been contraindicated in children aged < 8 years for a long time due to concerns about the adverse effects of dental staining and enamel hypoplasia. However, there is no evidence supporting this association for short-term courses (≤ 21 days). Doxycycline can be used to treat plague in children aged < 8 years.

6.3.2.2 Bubonic plague

First-line and alternative antibiotic options for treating patients with bubonic plague are summarized in Table 9. Tetracycline, chloramphenicol and sulfamethoxazole + trimethoprim remain alternative options (*2*). A regimen with a single antibiotic can be started in patients with bubonic plague if they do not have signs of pneumonic or septicaemic plague. Patients with primary bubonic plague progressing to secondary pneumonic or septicaemic plague need to be treated according to Table 8.

TABLE 9. Antibiotics for treating bubonic plague

Antibiotic	Age group	Dosing (per dose)	Interval (hours)	Route of administration
First-line options				
Ciprofloxacin	Adults	400 mg 750 mg[a]	8 12[a]	IV PO
	Children	10 mg/kg (max. 400 mg/dose) 15 mg/kg (max. 750 mg/dose)	8 or 12 12	IV PO
Levofloxacin	Adults	750 mg	24	PO or IV
	Children ≥ 6 months	< 50 kg: 8 mg/kg (max. 250 mg/dose) ≥ 50 kg: 500–750 mg	12 24	PO or IV PO or IV
Moxifloxacin[b]	Adults	400 mg	24	PO or IV
Doxycycline	Adults and children ≥ 45 kg	200 mg loading dose followed by 100 mg	12	PO or IV
	Children < 45 kg[c]	4.4 mg/kg (max. 200 mg) loading dose followed by 2.2 mg/kg (max. 100 mg)	12	PO or IV
Gentamicin	Adults	5 mg/kg	24	IM or IV
	Children	4.5–7.5 mg/kg	24	IM or IV
Streptomycin	Adults	1 g	12	IM or IV
	Children	15 mg/kg (max. 1 g/dose)	12	IM or IV
Alternative options				
Moxifloxacin[b]	3–23 months	6 mg/kg (max. 200 mg/dose)	12	PO or IV
	2–5 years	5 mg/kg (max. 200 mg/dose)	12	PO or IV
	6–17 years and < 45 kg	4 mg/kg (max. 200 mg/dose)	12	PO or IV
	12–17 years and ≥ 45 kg	400 mg	24	PO or IV
Tetracycline	Adults	500 mg	6	PO
	Children > 9 years	10 mg/kg (max. 500 mg/dose)	6	PO
Chloramphenicol	Adults	12.5–25 mg/kg (max. 1 g/dose)	6	PO or IV
	Children > 1 year	12.5–25 mg/kg (max. 1 g/dose)	6	PO or IV
Sulfamethoxazole + trimethoprim	Adults	5 mg/kg (trimethoprim)	8	PO or IV
	Children > 2 months	5 mg/kg (trimethoprim)	8	PO or IV

IM: intramuscular; IV: intravenous; max.: maximum; PO: per oral.

[a] The recommended per oral dose during pregnancy is 500 mg every 8 hours.

[b] Moxifloxacin is not recommended as a first-line choice but as an alternative option in children and adolescents aged < 17 years due to having a higher risk of side effects compared with other fluoroquinolones.

[c] Doxycycline has been contraindicated in children aged < 8 years for a long time due to concerns about the adverse effects of dental staining and enamel hypoplasia. However, there is no evidence supporting this association for short-term courses (≤ 21 days). Doxycycline can be used to treat plague in children aged < 8 years.

6.3.2.3 Plague meningitis

Chloramphenicol remains the antibiotic of choice for treating plague meningitis. It has been used effectively for many years. Fluoroquinolones with good cerebrospinal fluid penetration, such as moxifloxacin, ofloxacin or levofloxacin, have shown promising results for treating plague meningitis, but there are no human data on the effectiveness of these antibiotics for plague meningitis at the time of writing this manual.

All patients with plague meningitis need combination treatment with two antibiotics from different classes. Patients receiving antibiotics for plague but who develop secondary plague meningitis should have chloramphenicol added to the already established antibiotic regimen for 10 additional days.

6.3.2.4 Treating plague during pregnancy

Complications of plague in pregnancy can be minimized with correct and early therapy. The choice of antibiotics during pregnancy is confounded by the potential adverse effects of some of the most effective antibiotics. Streptomycin may be ototoxic and nephrotoxic to the fetus, while tetracyclines have an adverse effect on the developing teeth and bones of the fetus. Therefore, these antibiotics should be avoided.

The first-line antibiotic options for treating pneumonic, septicaemic and bubonic plague during pregnancy are gentamicin and fluoroquinolones (ciprofloxacin or levofloxacin) at the dosing shown in Tables 8 and 9. Alternative antibiotics include other fluoroquinolones (moxifloxacin), other aminoglycosides (streptomycin, amikacin, tobramycin or plazomicin), doxycycline and sulfamethoxazole + trimethoprim.

Chloramphenicol should be reserved to treat plague meningitis during pregnancy. Chloramphenicol carries a potential risk of grey baby syndrome or bone marrow suppression, but there is no evidence of such adverse effects resulting from treatment during pregnancy.

6.3.2.5 Treating plague in neonates

Only a few cases of neonatal plague have been reported worldwide. However, there are important considerations for treating plague in neonates (defined as infants at ≤ 28 days of life). For this age group, the intravenous route is recommended irrespective of the antibiotic regimen used. The selection of antibiotics must take into consideration the specific adverse effects that may occur in this population. Lastly, dosing may need to be adjusted according to gestational age and postnatal age.

The first-line options for treating pneumonic, septicaemic and bubonic plague in neonates include gentamicin, streptomycin, ciprofloxacin and levofloxacin. Chloramphenicol is contraindicated in this age group due to the potential for grey baby syndrome and bone marrow suppression, but it is still considered to be an appropriate option for plague meningitis, given that the benefits outweigh the risks.

6.3.2.6 Breastfeeding

The risk of plague transmission from an infected mother to her child through breast milk has neither been assessed nor reported, but it is believed to be low. However, in cases of pneumonic plague, the close contact that occurs with breastfeeding represents a significant risk of interhuman transmission.

Practically, mothers with bubonic or septicaemic plague and mothers under postexposure prophylaxis can breastfeed their child. A mother with pneumonic plague can breastfeed her child if she has received appropriate antimicrobial treatment for at least 48 hours and the child is being treated with an antimicrobial for postexposure prophylaxis. The mother should wear a medical mask. If breastfeeding needs to be interrupted for a few days, expression of breast milk is recommended, and the expressed breast milk can be used to feed the child.

Although children might be receiving antibiotics via breast milk in addition to their own treatment or prophylaxis, the level of antibiotics in breast milk is low, and the consequent potential drug toxicity or drug interaction is limited.

The antibiotics recommended for plague treatment (Tables 8, 9 and 10) are considered safe for breastfeeding mothers, with the exception of chloramphenicol. However, if chloramphenicol has to be used, the child should be closely monitored for lethargy, gastrointestinal symptoms and aplastic anaemia. Tetracyclines are considered safe unless they are used long term or for repeated courses.

6.4 Supportive therapy

Clinicians must prepare to provide intense supportive management of plague complications while considering the latest developments for dealing with Gram-negative sepsis. Oxygen, intravenous fluids and respiratory support are often required for people with pneumonic or septicaemic plague or plague meningitis. Aggressive monitoring and management of possible septic shock, multiple organ failure, adult respiratory distress syndrome and disseminated intravascular coagulation should be instituted.

6.5 Prophylaxis

6.5.1 Postexposure prophylaxis

Postexposure prophylaxis should be considered in the following circumstances.

- Persons who are likely to have been exposed to *Y. pestis*-infected fleas (e.g. members of the household of a patient with bubonic plague), to *Y. pestis* bacteria (e.g. during a laboratory accident) or to a *Y. pestis*-infected mammal (e.g. directly or through contact with its body fluids or tissues) should be offered antimicrobial postexposure prophylaxis if the exposure has occurred within the previous 10 days.
- Persons who have come into close contact (less than 2 m) within the previous 10 days with a patient who has suspected, probable or confirmed pneumonic plague. Such a patient is considered contagious from the onset of the first symptom (e.g. cough) until the patient has completed 48 hours of antimicrobial treatment.

In these scenarios, the exposed person should be presumed to be infected with plague, although they may be asymptomatic. Therefore, postexposure prophylaxis aims to avoid the possible evolution from infection to disease. Exposed persons do not need to be isolated as long as they are asymptomatic.

The preferred antimicrobials for treatment are a fluoroquinolone or tetracycline for a duration of 7 days (Table 10).
Due to the operational constraints in field conditions, particularly during outbreaks, a single oral dose daily will be preferred.

TABLE 10. Antibiotics for plague prophylaxis

Antibiotic	Age group	Dosing (per dose)	Interval (hours)
First-line options			
Ciprofloxacin	Children > 2 months	5 mg/kg (trimethoprim)	12
	Children	15 mg/kg (max. 750 mg/dose)	12
Doxycycline	Adults and children ≥ 45 kg	100 mg	12
	Children < 45 kg[a]	2.2 mg/kg (max. 100 mg/dose)	12
Sulfamethoxazole + trimethoprim	Adults	5 mg/kg (trimethoprim)	8
	Children > 2 months	5 mg/kg (trimethoprim)	12
Alternative options			
Tetracycline	Adults	500 mg	6
	Children > 9 years	10 mg/kg (max. 500 mg/dose)	6
Chloramphenicol	Adults	12.5–25 mg/kg (max. 1 g/dose)	6
	Children > 1 year	12.5–25 mg/kg (max. 1 g/dose)	6

[a] Doxycycline has been contraindicated in children aged < 8 years for a long time due to concerns about the adverse effects of dental staining and enamel hypoplasia. However, there is no evidence supporting this association for short-term courses (≤ 21 days). Doxycycline can be used for postexposure prophylaxis of plague in children aged < 8 years.

6.5.2 Pre-exposure prophylaxis

The administration of antimicrobial chemoprophylaxis prior to potential exposure may be indicated when persons such as a vector control team must be present for short periods in areas where plague is active and under circumstances in which exposure to plague sources is difficult or impossible to prevent.

The antibiotic options for chemoprophylaxis prior to exposure are the same as for postexposure prophylaxis (Table 10). Chemoprophylaxis should begin 1 day prior to entering the area where there is a risk of plague and continue for the duration of exposure in the risk area and until 48 hours after the end of the exposure.

6.5.3 Vaccination

The available plague vaccines have not been proven to prevent plague effectively. They do not protect against primary pneumonic plague. In addition, they may have major side effects. Vaccination is not recommended for communities in endemic regions.

7. MANAGING PLAGUE EVENTS: EMERGENCY PREPAREDNESS, RESPONSE AND RESILIENCE

Plague events can be considered:

- in an endemic area, as an occurrence that was unexpected due to the type of plague, the magnitude of the outbreak or the specific local conditions (e.g. among displaced persons, in an urban context). The occurrence of a human case of bubonic plague in an endemic area is considered an expected incident, and local health care workers must be able to notify the appropriate authorities and manage it on a routine basis;
- in a non-endemic area, as the occurrence of a human case of bubonic or pneumonic plague that constitutes an event of public health importance requiring a specific investigation and the implementation of control measures without delay.

Usually, a plague event will be reported to WHO in accordance with the International Health Regulations (Box 1).

Box 1. International Health Regulations

- The International Health Regulations, which came into effect in 2007, require the notification of any event of potential international public health concern within 24 hours of assessment.
- The decision instrument of the Regulations provides an algorithm that can assist in assessing whether an event should be reported. The criteria include the following questions.
  - Is the public health impact of the event serious?
  - Is the event unusual or unexpected?
  - Is there a significant risk of international spread?
  - Is there a significant need for international restrictions on travel and trade?
- Plague cases should be notified only if the assessment done by the country shows that the public health impact can be considered serious and the event has at least one of the following characteristics: it is an unusual or unexpected event; there is a risk of international spread; there is a significant risk to international travel; or there is a significant need to restrict trade.
- **The algorithm must always be used when there are cases of suspected pneumonic plague due to its potential to have serious public health impacts and to spread rapidly.**
- It is not always necessary to wait for laboratory confirmation of plague; a suspected case of plague that occurs in an area not known to be endemic should be reported as an event to WHO.
- If the outcome of the decision tool is a requirement for notification, the national government should inform WHO about the plague event and provide the following details: the region affected, number of cases, number of deaths, control measures taken and current situation.
- Refer to the *International Health Regulations (16).*

In endemic areas, it is important that all cases of plague are properly managed, whether sporadic or occurring during an outbreak. Plague has re-emerged in several countries where it had been silent for decades and has also re-emerged in new or reactivated foci within endemic countries.

The ability to prepare for, prevent, detect and respond effectively to the plague events at national level depends on the operational readiness and capacities in four core subsystems: i) collaborative surveillance and public health intelligence, ii) community protection, iii) clinical care and iv) emergency coordination.

7.1 Collaborative surveillance and public health intelligence

Collaborative surveillance includes an integrated disease, threat and vulnerability surveillance, laboratory capacity for pathogen and genomic surveillance when possible and collaborative approaches for risk assessment, event detection and response monitoring.

Effective plague prevention and control programmes require up-to-date information about the incidence and distribution of the disease. The information should identify human cases and epizootics as quickly as possible so that steps can be taken to control the spread of the disease. Surveillance in plague-endemic countries should be conducted continually.

7.1.1 Integrated disease threat and vulnerability surveillance

The objectives of surveillance programmes are to detect early warning signals of an outbreak, to assess the impact of intervention measures, to identify local ecological factors or human activities that may increase the risks of plague exposure among humans and to detect trends in the epidemiology and epizoology of plague in a given region.

Different ministries, such as the ministry in charge of agriculture and other relevant governmental bodies, should be involved in the surveillance activities, following a One Health approach *(17)*. By definition, a comprehensive surveillance system for plague is multisectoral and should monitor the occurrence and spread of Y. pestis infection in humans, the flea vectors, the rodent reservoirs and the non-reservoir mammalian species that seroconvert following infection (carnivore surveillance). The collaboration between the various surveillance actors is essential for a full understanding of risks, vulnerabilities, event detection and response monitoring. In areas known to be endemic for plague, all health care workers and community members should be on the alert for patients with symptoms suggestive of plague. Information must be disseminated to peripheral health care workers as well as to the community in local languages to strengthen lay reporting. Health facilities must immediately report any case of suspected plague.
A suspected human case of plague is a medical emergency and should trigger immediate epidemiological investigations and follow-up actions.

7.1.2 Laboratory

Laboratory is an essential component of the surveillance. In endemic countries, a national plague reference laboratory should be designated, and its staff trained and equipped to perform confirmatory diagnoses at least by rapid testing and molecular analysis and to perform biochemical characterization from a culture. It should be closely linked to the national public health system.The geographical area for surveillance may be defined by using one or more of the following criteria: known focus of plague, detection of plague (Y. pestis) activity, or report of a suspected human case (or cases) of plague. If limited resources do not permit active surveillance to be undertaken in a wider area, villages closest to the known foci should be prioritized. When ecological changes occur, such as earthquakes, flooding or heavy rainfall, or when populations are displaced in endemic

areas, surveillance for plague and other rodent-associated zoonoses needs to be initiated or intensified.

7.1.3 Environmental surveillance within natural foci

Natural foci of plague require constant surveillance. Plague is primarily a disease of rodents; therefore, a natural decline in the incidence of human plague would not justify the conclusion that plague has disappeared from an area. Plague is not static, but shifts from place to place through the contiguity of colony infection among wild rodents, which eventually transfers the infection to the commensal rodents in their path.

Many factors influence the persistence of the disease in wild populations and the occurrence of epizootic plague. Some plague outbreaks in humans occur when plague causes sudden large-scale mortality among local rodent reservoirs. Although most cases of human plague occur in areas that have been occupied for decades or longer, exposures also can occur when humans invade natural plague foci, especially if their activities increase the likelihood that they will come into contact with potentially infected rodents and fleas. There is growing evidence that cyclical epizootic events are influenced by climatic factors – notably increased or prolonged rainfall, or both – that increase the vegetation on which rodents feed, leading to increased rodent population density, which can then lead to a higher transmission risk, particularly when these events are followed by mild temperatures that facilitate flea survival.

Because the risks of human plague are greatest during epizootics, it is important to know which regional conditions lead to these epizootics and whether any localized warning signs – for example changes in rainfall, rodent or flea density, or both, or higher rodent mortality – exist that might help public health programmes effectively target limited prevention and control efforts towards those areas most likely to be at risk. Consequently, surveillance programmes are essential parts of epidemic preparedness and early warning systems. These systems should include a data management component for reporting common sources of infection, plans for analysing the data and appropriate actions that should occur following the identification of plague activity.

7.1.3.1 Role of animal-bassed surveillance in preventing human plague

The primary goal of most long-term surveillance programmes is to quickly recognize epizootic activity among rats and other animals so that appropriate prevention and control measures, such as heightened human surveillance and vector control, can be undertaken as quickly as possible in affected areas to limit the spread of plague to local human populations. The surveillance system should be effective at detecting early warning signals of epizootic activity and increased human plague risk (Table 11). Several methods are used for detecting the signals described in Table 11, with different spatial or temporal precision and accuracy in predicting epizootics or human cases.

TABLE 11. Early warning signals of increased risk of human plague

Early warning signal	Surveillance mechanism for detection
Sudden decreases in rodent density or identification of greater than normal numbers of dead rats (i.e. rat fall)[a]	Rodent surveillance
Total flea index or specific flea index > 1[b]	Flea surveillance
Increase in positive serum samples from canines[c]	Carnivore surveillance
Identification of *Yersinia pestis* in rodent or other host carcasses	Carcass surveillance (including rat fall surveillance)

[a] Rat fall has been operationally defined by some plague programmes as the identification of more than one dead rat in a house or more than one house with dead rats in cases in which deaths among the rats have not been due to poisoning. If adequate baseline data are available for rates of rat death during non-epizootic periods, a rat fall also can be defined as any significant increase in rat deaths due to causes other than poisoning.

[b] The total flea index is defined as the total number of fleas collected (regardless of species) divided by the total number of hosts being examined. The specific flea index is defined as the number of fleas of a specific species from host species divided by the number of individuals of hosts being examined. Although a total or specific flea index > 1 has been associated with plague epizootics and outbreaks, these are labour-intensive metrics to obtain, have limited spatial coverage and are often poor predictors of plague activity.

[c] The increase is compared with baseline serological values observed during non-epizootic periods.

The secondary roles of animal-based plague surveillance include:

- searching for evidence of plague infection in animals and fleas living in areas where plague has not been previously detected but is suspected to occur. Canine serology is likely the most effective means of tracking this because canines have a large home range, are likely to consume large numbers of rodents and have a high likelihood of surviving infection and seroconverting;
- assisting with human case investigations in an attempt to identify likely sources of human infection and clarify the extent of epizootic spread near suspected sites of case exposure. In this case, the identification of unusual flea–host associations might be as useful as a flea index. A plague-positive animal may not be found as part of an investigation, but finding ground squirrel fleas on mice (especially when the abundance of ground squirrels is lower than expected) might be an indication of a recent epizootic in ground squirrels. Such findings enable the assessment of ongoing risks and identification of areas that need to be targeted for flea control and other preventive measures;
- tracking changes in key environmental variables associated with past epizootics or unexpected increases in rodent populations. If variations in environmental data are linked to the appearance of epizootics, they should serve as early warning signals that surveillance activities should be increased and preventive and control measures, including flea control, should be strengthened;
- identifying plague-maintaining reservoirs during interepidemic periods to improve preventive surveillance and control strategies;
- monitoring on a regular basis levels of antibiotic resistance in *Y. pestis* in the environment and insecticide resistance in fleas in order to adapt clinical treatment and vector control strategies.

7.1.3.2 Strategies for animal-based plague surveillance

Many strategies exist for monitoring plague activity in rodents (reservoirs) and fleas (vectors). These strategies include:

- conducting visual surveillance and reporting rat falls (commensal rat die-offs) or die-offs of field (sylvatic) rodents and collection of host carcasses for laboratory testing;
- trapping suspected rodent reservoirs to obtain samples that can be tested for evidence of current plague infection (e.g. molecular analysis, direct fluorescent assay, culture) or prior infection (e.g. serology);
- collecting and testing suspected flea vectors from captured animals (on-host populations) or from human homes, rodent burrows and nests, or other likely sites (off-host populations);
- collecting and testing serum samples from carnivores (usually dogs) for evidence of Y. pestis antibodies;
- monitoring meteorological data (particularly rainfall and temperature), harvest information and other types of environmental data that can provide early indications of changing conditions that are likely to lead to increases in local populations of rodents and fleas.

Each of the surveillance methods discussed above, along with the specific techniques, equipment and supplies associated with them, are summarized in Annex 10.

7.1.3.3 Selecting a regionally appropriate plague surveillance strategy

To determine which of the above strategies (or combination of strategies) is best for a particular area, a number of factors must be evaluated, including:

- local environmental conditions, agricultural practices and house construction;
- behavioural and cultural aspects of the local human population, with special emphasis placed on local perceptions of reservoir and vector species, disease (and, if known, plague) and sick persons, as well as mortuary practices;
- the types of rodent reservoirs and flea vectors present and, if feasible, their fine-scale distribution (preferred habitats) and seasonal dynamics;
- knowledge of past plague activity, rodents and fleas in the area being investigated;
- information about previous plague surveillance, prevention and control activities in the region that may suggest which strategies have worked or not worked;
- the presence of other animals, such as dogs, that might be useful sentinel species for plague activity;
- the availability of trained surveillance personnel, sufficient equipment, transportation and financial resources to conduct animal-based plague surveillance on a sustained basis or for a sufficient length of time to gather useful data;
- the availability of a local or regional microbiological laboratory to test animals or fleas for evidence of *Y. pestis* infection or the opportunity to have samples transferred to a national laboratory under good transport and storage conditions;
- additional risk factors, for instance, an unexpected occurrence of a type of plague or the magnitude of the outbreak or the specific local conditions (e.g. displaced persons, urban context). The occurrence of a human case of bubonic plague in an endemic area is considered an expected incident, and local health staff must be able to alert the appropriate people and manage it on a routine basis.

7.1.3.4 Evaluating programme needs and performance

Each of the animal-based methods of plague surveillance has unique requirements, needs and necessary resources. In some instances, funding, personnel and other resources might be insufficient to maintain ongoing programmes involving frequent rodent trapping and flea collection. In such cases it might be best to implement a visual surveillance and village- or urban neighbourhood-level monitoring programme that relies heavily on local health officials (and, if pertinent, local communities) to recognize rat falls or wild rodent die-offs, unusual increases in rodent activity or other signs that an epizootic might be likely in the near future. Such a programme could include protocols for collecting rodent carcasses and sending these to regional or national microbiological laboratories for testing.

Alternatively, rapid plague diagnostics can be used in the field to test tissues from dead animals for *Y. pestis* infection, thereby allowing on-the-spot decisions to be made regarding emergency flea control and other steps that need to be taken immediately to reduce the risk of human plague. It is also possible to have a mixed surveillance strategy in which part of the programme might rely on visual surveillance for long-term monitoring but employ rodent trapping and flea collection techniques during human case investigations or the transmission season or while searching for new plague foci, or some combination of these.

7.1.3.5 Vector control

Flea control measures should be undertaken when:

- any locality reports a rat fall attributable to plague (i.e. with laboratory confirmation of *Y. pestis* in a rodent carcass);
- an increase in the population of fleas (i.e. an increase in the total flea index, particularly when associated with laboratory confirmation of *Y. pestis* in rodents or fleas; see Table 11) or an increase in flea nuisance is reported; or
- when the specific flea index (see definition in Table 11) is found to be > 1 through active surveillance in areas of known foci of natural plague.

Because the flea index is an imprecise predictor of plague activity (e.g. the flea index may be > 1 in the absence of plague activity), secondary verification of plague activity is recommended following the observation of a flea index > 1 (e.g. through direct detection of *Y. pestis* in fleas, rodents or rodent carcasses, or through verification of elevated rodent mortality).

Recommended strategies for plague vector control include using personal prophylactic measures, dusting with insecticide and using residual insecticidal spray; these strategies are described in Annex 11. Thought must be given to the possibility of resistance evolving due to the overuse of insecticides. As such, it is reasonable to consider limiting indoor residual spraying or dusting to periods when there is evidence of local transmission of *Y. pestis* and spatially limiting application of insecticides to places where persons are at risk for exposure to infected fleas.

7.1.3.6 Rodent control

Rodent control requires intensive effort, often using multiple strategies; unfortunately, results are often short-lived after control measures end. It is virtually impossible to completely eliminate a wild rodent population and equally impossible to fully control their fleas. Prevention should serve as the first layer of protection, and the principles are based on controlling rodent populations in rural and urban settings as much as possible. This includes reducing food and harbourage for rodents in and around homes, rodent-proofing human dwellings to the extent possible and using rodent-targeted insecticides to reduce fleas on rodents in the home environment. Rodent control activities (i.e. trapping or poisoning) should be undertaken primarily during interepidemic periods as a means

of preventing plague. Killing rodents during epidemic situations may result in large numbers of fleas leaving the dead rodents and biting humans, thus transmitting the infection. Therefore, rodent control measures should be undertaken during outbreaks only after rodents are adequately treated with insecticides to control fleas, per label instructions. Generally, in plague-endemic regions, rodent control should never be implemented without preliminary or concomitant vector control.

The methods employed for rodent control are described in Annex 12 and include environmental sanitation and physical, chemical and biological methods. Modifying the home environment to the extent feasible to reduce rodents in the home is one of the main actions and includes strategies such as rodent-proofing food storage areas or containers, storing food away from the home and sealing access points to prevent rodent incursion.

Surveillance in ships and seaports

Seaports were major points of entry for food, other goods and people before the development of air transport. But even today, major trade in food, goods and other materials is carried out in seaports. Ships are frequently infested with rodents, and the activities undertaken at ports, such as the handling of foodstuffs, attract many species of vermin. Similarly, ports are exposed to the risk of vectors being introduced from any other part of their country or any other port in the world.

State parties need to carry out surveillance activities on ships and in seaports to ensure effective vector surveillance and control. The WHO handbook on Vector surveillance and control at ports, airports and ground crossings provides technical guidance on the optimal use of resources, planning, monitoring and decision-making to assist state parties in managing vector surveillance and control programmes at points of entry *(18)*.

For plague, rodent surveillance and control activities include inspecting ships and issuing exemption certificates, systematically checking recent (issued < 6 months ago) deratting certificates for each ship (as imposed by the International Health Regulations), eliminating rats in and around port areas and controlling rodents on ships.

Disposing of dead rodents during a rat fall

Persons disposing of rats must cover their face with a mask. Dead rats should not be touched with bare hands. Disposable gloves should be worn or polythene sheeting should be wrapped around the hands before handling dead rats.

The dead rats may be disposed of by:

- spraying insecticidal dust or powder over the dead rat (Annex 11) and subsequently burying it in a 1-metre-deep pit;
- picking up the rat using long forceps, long tongs or similar tools and then putting it into a container containing cotton wool soaked in insecticide for final disposal;
- burning or burying the disposable items used and disinfecting any other items.

7.2 Community protection

Community protection includes risk communication and infodemic management, community engagement to help design and implement public health and social measures, and multisectoral action to address community concerns.

People living in endemic areas should be provided with health education regarding the significance of a sudden increase in the number of dead rats as an early indicator of plague activity, the risks of handling rodent carcasses and of the lethal trapping of rats in the

absence of associated vector control, the modes of transmission, the presenting symptoms of plague, the importance of reporting immediately to the nearest health facility if a case is suspected and steps to prevent and control plague. The community should be educated about the common myths and misconceptions about plague.

Basic health education should contain the following messages.

- Never handle a wild animal that is found dead.
- During a plague epizootic, do not implement lethal rodent control without first treating rodents with insecticides to kill fleas.
- Avoid flea bites by wearing clothes that cover the body, specifically trousers and shoes, and using insect repellents (Annex 11).
- Report to the authorities any suspect death of a domestic animal or an unusually high number of dead rats (a rat fall). Do not handle any dead animal.
- Do not kill a sick animal or prepare or eat it: a sick animal could be infected by plague.
- Seek medical care immediately in case of fever and bubo (a painful swelling of a lymph node).

7.3 Clinical care

Clinical care includes lifesaving and scalable clinical care, protection of healthcare workers and patients, and health systems that can maintained essential health services. See chapter 6.

The following actions must be taken.

- Treat cases promptly with the recommended antibiotics (Section 6).
- Prevent exposure:
  - prevent nosocomial infection, isolate patients with pneumonic plague, dust clothing with insecticidal powder and use insect repellents;
  - safely dispose of bodies and ensure appropriate disposal of dead rodents, as necessary;
  - disinfecting the houses of people with plague is useless because *Y. pestis* is a fragile bacteria that does not persist on inert surfaces.
- Prevent infection and progression from infection to disease (e.g. provide postexposure prophylaxis for close contacts or chemoprophylaxis, increase public awareness).
- Implement vector- and rodent-control measures (Annexes 11 and 12).

7.3.1 Safe disposal of bodies of victims of plague

The level and duration of infectiousness of the body of someone who was infected with plague has practical consequences when managing a plague event. However, the length of time that Y. pestis can survive in body fluids and for how long the remains are contagious are unknown.

Due to the risk of plague transmission from the bodies of people who were infected with plague and based on safety precautions and indirect evidence, those who handle and dispose of the body of a victim of plague must strictly adhere to the following precautions.

- Funeral ceremonies should be discouraged from taking place in the houses of plague victims if they involve a gathering of people.
- The bodies of plague victims should not be handled or put into coffins by relatives

or friends of the deceased. These procedures should be done by professional undertakers who are well-versed in safety procedures.

- The undertakers should use personal protective equipment to handle the body of someone infected by plague. The minimum required equipment includes a gown, goggles, an N95 or FFP2 mask, and gloves (see the section on Implementation considerations below) *(5)*.
- Professionals handling the body should receive chemoprophylaxis at recommended doses, as per the advice of a doctor. Chemoprophylaxis should NOT replace personal protective equipment when handling the body of plague victims.
- The body should be packed in an impervious body bag for transport from the place of death and should not be extracted from the bag. The body should not be bathed before cremation or burial.
- A layer of lime, acting as an absorbent material, should be placed in a coffin before the body is placed in it.
- The garments and other belongings of a person who died of bubonic plague should be dusted with a 5% formulation of malathion.
- The contaminated belongings of pneumonic plague victims should be packed into a bag and then incinerated or autoclaved.

In addition, special adaptations may be considered by national authorities that take into account local culture, religion, customs or beliefs.

The availability and adequate quality of personal protective equipment should be ensured in all settings, so that people have access to the appropriate standard of personal protective equipment and adequate information about its use.

There are some circumstances and settings in which the preparation of the body and funeral rites are culturally very important and during which personal protective equipment might interfere with rituals, consequently decreasing the acceptability of these measures. It is recommended that risk communication strategies and approaches address a community's concerns in relation to preparing the body at home and funeral rites *(19)*.

7.4 Emergency coordination

Emergency coordination should draw on health emergency alert and response teams that are interoperable and rapidly deployable; coherent national action plans for preparedness, prevention, risk reduction and operational readiness; and scalable health emergency response coordination through a standardized and commonly applied emergency response framework.

7.4.1 National action plan

It is essential that preparedness plans be created and implemented in all areas that have plague foci. Important items to consider when developing the plan include:

- for protocol development and implementation:
  - clinical management and infection prevention and control strategies;
  - public health measures (including vector and rodent surveillance and control and infection prevention and control protocols);
  - the diagnostic capabilities of laboratories that comply with the requirements for quality management systems;
  - organizational aspects, such as a crisis committee and a rapid response team (RRT);

- the availability of trained biologists for investigations in reservoir and vector species;
- the availability of trained health care workers;
- appropriate logistics services and trained workers to manage the transportation of infected or potentially infected patients and samples;
- improvements to the capacity of health facilities to treat patients;
- stockpiles of essential treatment supplies, laboratory kits, appropriate antibiotics and personal protective equipment;
- communication strategies for the population.

7.4.2 Emergency response coordination

The Ministry of Health is essential to efficiently and effectively manage plague events. It should work closely with other concerned ministries. When events fall under the International Health Regulations, the Ministry of Health should report directly to WHO. The Ministry of Health's actions are supported by a crisis committee and a technical committee.

A crisis management committee should be constituted to provide overall guidance and make prompt decisions about administrative, financial and technical issues during an outbreak. The committee should include representatives from decision-making authorities, relevant governmental bodies, transport, communications, the police, agriculture, education, financial institutions and a representative from the technical committee. The crisis committee is essential to eliminate duplication of efforts and to coordinate the appropriate distribution of personnel and resources. It is crucial that the committee has the authority to implement emergency measures.

The roles of the crisis committee include:

- supervising and coordinating the implementation and achievement of control measures;
- establishing procedures for accessing funding;
- developing policies and sustaining executive structures to ensure that responsibilities for the emergency health response are clear
- coordinating communication with and education for the health care community and the general public about symptoms and precautions;
- establishing working links among the Ministry of Health, national relief organizations, nongovernmental organizations, and bilateral and intergovernmental agencies;
- communicating with local and international mass media;
- reporting to higher-level authorities daily.

The technical committee should know about plague prevention and control techniques. One person should be appointed to communicate daily with the crisis committee.

Committee members should include national health staff (e.g. logistics personnel, epidemiologist, clinicians, biologists) and staff from nongovernmental organizations involved in the field and international experts, if any are present in the country.

The roles of the technical committee include:

- defining at-risk populations;
- assigning specific responsibilities to individuals or units for plague outbreak detection and response activities;
- identifying the resources needed for the rapid response and updating information about these resources at the local and national levels;

- estimating the requirements for adequate sampling of clinical or animal specimens, or both, and for controlling the plague event (e.g. medicines, human resources, insecticides, transport, financial resources);
- informing health care workers within the region about the extent of the outbreak, appropriate transportation for samples to the reference laboratory and clinical management;
- preparing reports with relevant information for the crisis committee and requesting needed support.

7.4.3 Health emergency and response team

Health emergency and response teams (often called “Rapid Response Team, RRT”) should comprise well-trained, equipped and multi-sectoral profesionnals. The team deployment will be facilitated by standardized protocols, operating guidelines and activation procedures and mechanisms. The deployment will be based on alert definitions and its support draw on contingency planning. Impact and effectiveness of the deployment will be monitored and evaluated in order to later refine and adapt the process.

Proper management of a plague event includes investigation, control activities and a final evaluation.

The steps to be taken to properly manage a plague event include:

- the RRT identifying and investigating the outbreak;
- activating the crisis management committee and technical committee;
- implementing infection prevention and control strategies and appropriate clinical management;
- communicating with communities;
- conducting a final evaluation of the response.

To effectively manage a plague event, it is important to manage cases from both the clinical and public health perspectives. Outbreaks can be contained if there is rapid diagnosis and treatment, along with notification to authorities, which ultimately reduce mortality.

Once an outbreak is suspected, a field investigation should be implemented as soon as possible. It should include epidemiological and environmental investigations whenever they are feasible. Timeliness will not only assist in controlling the current outbreak but may also allow for programme development to reduce the likelihood of future outbreaks. RRTs from existing surveillance systems may be briefed about plague epidemiology and relevant response mechanisms. If there are no RRTs at either the state or district level, then team members should be identified for both levels. Ideally, the composition of the RRT should include a leader (i.e. an epidemiologist or public health officer), a microbiologist, a clinician and an entomologist or biologist, or both.

The following actions must be taken to control a plague event.

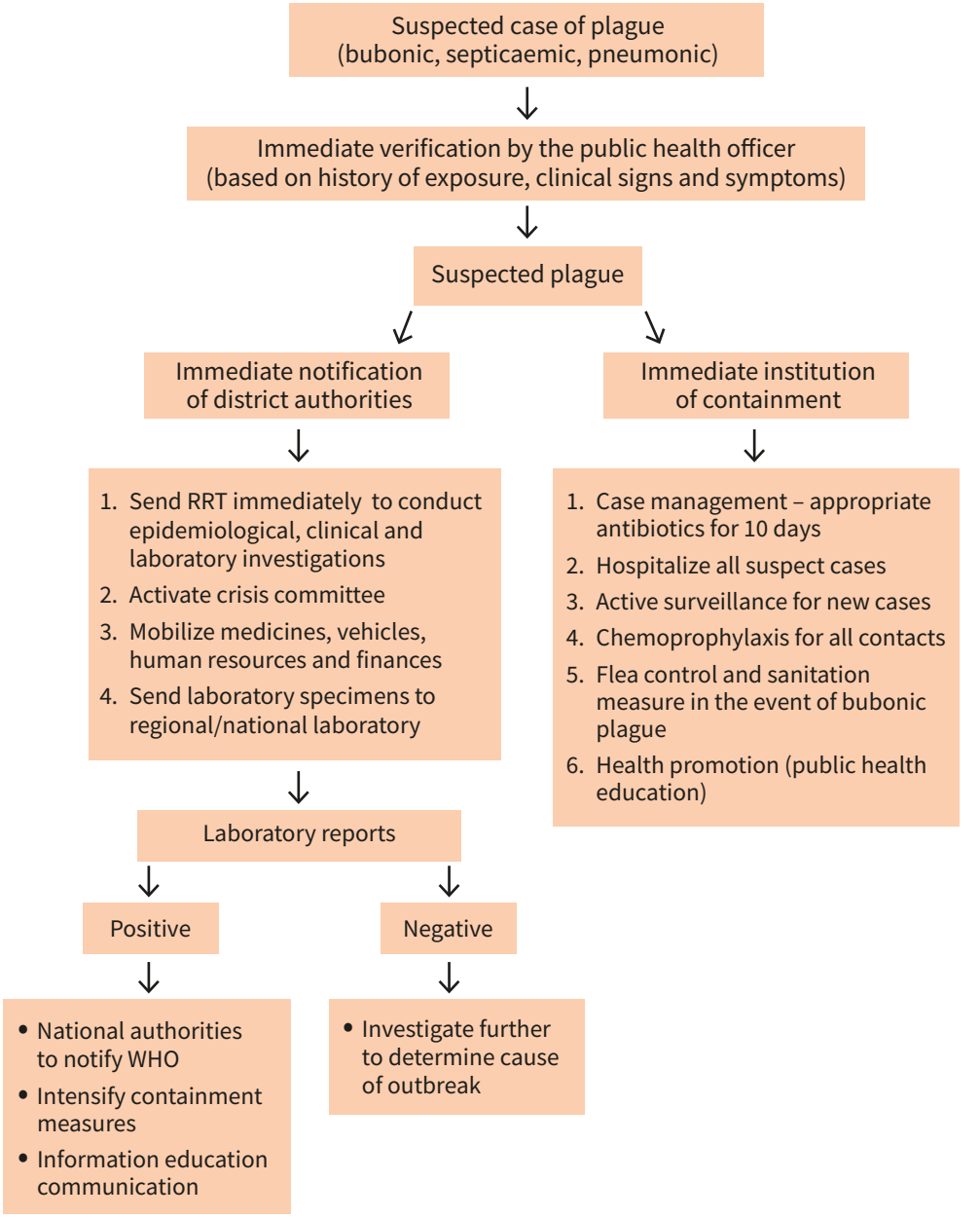
- Treat cases according to national guidelines or if these are not available, according to this manual.
- Prevent additional exposures, for example, by isolating patients who have pneumonic plague, dusting clothing with insecticidal powder and using insect repellents daily.
- Prevent additional infections, for example, by providing chemoprophylaxis for close contacts, increasing public awareness and enhancing surveillance.
- Implement environmental control measures for fleas and rodents.

- Mobilize the community and conduct health education sessions.
- Communicate with the media.

The structure of the investigation of a plague event and implementation of control measures are given in Fig. 5.

A final evaluation must be conducted to assess the timeliness and efficacy of detection and response activities and to determine whether changes to public health policy are indicated (e.g. in terms of preparedness).

Fig 5. Investigating and controlling a plague event



RRT: rapid response team; WHO: World Health Organization.

REFERENCES

1. Rasmussen S, Allentoft ME, Nielsen K, Nielsen R, Kristiansen K, Willerslev E. Early divergent strains of *Yersinia pestis* in Eurasia 5,000 Years Ago. Cell. 2015;163:571-82.
2. Operational guidelines on plague surveillance, diagnosis, prevention and control. New Delhi: World Health Organization, Regional Office for South-East Asia; 2010 (https://apps.who.int/iris/handle/10665/205593).
3. Plague around the world, 2010–2015. *Wkly Epidemiol Rec.* 2016;91:89-93 (https://apps.who.int/iris/bitstream/handle/10665/254296/WER9108_89-93.pdf).
4. Plague [Internet]. Geneva: World Health Organization; 2019 (https://www.who.int/csr/disease/plague/en/, accessed 1 February 2019).
5. WHO guidelines for plague management: revised recommendations for the use of rapid diagnostic tests, fluoroquinolones for case management and personal protective equipment for prevention of post-mortem transmission. Geneva: World Health Organization; 2021 (https://apps.who.int/iris/handle/10665/341505).
6. Bertherat EG, Jullien S. Revision of the international definition of plague cases. Wkly Epidemiol Rec. 2021;96:238-40 (https://apps.who.int/iris/handle/10665/341853).
7. Interregional meeting on prevention and control of plague. Geneva: World Health Organization; 2008 (https://www.who.int/publications/m/item/WHO-HSE-EPR-2008-3, accessed 4 April 2022).
8. WHO handbook for guideline development, second edition. Geneva: World Health Organization; 2014 (https://apps.who.int/iris/handle/10665/145714).
9. 10 proposals to build a safer world together – Strengthening the Global Architecture for Health Emergency Preparedness, Response and Resilience. Geneva: World Health Organization; 2022 (https://www.who.int/publications/m/item/10-proposals-to-build-a-safer-world-together---strengthening-the-global-architecture-for-health-emergency-preparedness--response-andresilience--white-paper-for-consultation--june-2022).
10. Global distribution of natural plague foci as of March 2016 [Internet]. Geneva: World Health Organization; 2016 (https://www.who.int/health-topics/plague#tab=tab_1, accessed 21 May 2022).
11. Randremanana R, Andrianaivoarimanana V, Nikolay B, Ramasindrazana B, Paireau J, ten Bosch QA, et al. Epidemiological characteristics of an urban plague epidemic in Madagascar, August–November, 2017: an outbreak report. Lancet Infect Dis. 2019;19:537-45. doi:10.1016/S1473-3099(18)30730-8.
12. Forrester J, Apangu T, Griffith K, Acayo S, Yockey B, Kaggwa J, et al. Patterns of human plague in Uganda, 2008–2016. Emerg Infect Dis. 2017;23(9):1524-8. doi:10.3201/eid2309.170789.
13. Bertherat E. Plague around the world in 2019. Wkly Epidemiol Rec. 2019;94:289-92 (https://apps.who.int/iris/handle/10665/325482).
14. Guidance on regulations for the transport of infectious substances 2021–2022. Geneva: World Health Organization; 2021 (https://apps.who.int/iris/handle/10665/339825).
15. Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/handle/10665/337956).
16. International Health Regulations (2005), third edition. Geneva: World Health Organization; 2016 (https://apps.who.int/iris/handle/10665/246107).

17. One Health [Internet]. Geneva: World Health Organization; 2017 (https://www.who.int/news-room/q-a-detail/one-health, accessed 25 September 2020).

18. Vector surveillance and control at ports, airports, and ground crossings. Geneva: World Health Organization; 2016 (https://www.who.int/publications/i/item/vector-surveillance-and-control-at-ports-airports-and-ground-crossings).

19. Risk communication strategies: module B3 [Internet]. Geneva: World Health Organization; 2014 (https://www.who.int/emergencies/risk-communications/emergency-risk-communication-training, accessed 11 December 2019).

ANNEXES

Annex 1. Summary of the WHO recommendations on using rapid diagnostic tests and fluoroquinolones for early diagnosis and treatment of plague and on the appropriate use of personal protective equipment, 2019

See the *WHO guidelines for plague management: revised recommendations for the use of rapid diagnostic tests, fluoroquinolones for case management and personal protective equipment for prevention of post-mortem transmission*[1] for details of the methods used to develop the recommendations (Table A1.1).

TABLE A1.1. Summary of recommendations for rapid diagnostic tests and for using fluoroquinolones and personal protective equipment for plague

Recommendations	Strength of the recommendation	Quality of the evidence
Use of F1RDT for plague		
In areas where plague is known to occur, the GDG suggests using F1RDT in people with suspected pneumonic plague to rapidly detect the disease and implement an immediate public health response (alert tool). While the initial public health response is being implemented, a confirmatory test (such as culture or molecular testing) should be carried out before declaring a confirmed plague outbreak because F1RDT has limited specificity.	Conditional	Very low
During an outbreak, the GDG suggests using F1RDT in people with suspected pneumonic plague to provide rapid diagnosis at the point of care. A negative result helps rule out the disease and encourages consideration of an alternative diagnosis. A confirmatory test (such as culture or molecular testing) should be carried out at the same time.	Conditional	Very low
In areas where plague is known to occur, the GDG recommends using F1RDT in people with suspected bubonic plague to rapidly detect plague and implement an immediate public health response (alert tool). While the initial public health response is being implemented, a confirmatory test (such as culture or molecular testing) should be carried out before declaring a confirmed plague outbreak because F1RDT has limited specificity.	Strong	Very low
During an outbreak, the GDG suggests using F1RDT in people with suspected bubonic plague to provide rapid diagnosis at the point of care. A confirmatory test (such as culture or molecular testing) should be carried out at the same time.	Conditional	Very low

Antibiotics for treating plague		
The GDG suggests adding fluoroquinolones (ciprofloxacin, levofloxacin and moxifloxacin) to the first-line medicines recommended for treating pneumonic or septicaemic plague (streptomycin and gentamicin).	Conditional	Very low
The GDG suggests adding fluoroquinolones (ciprofloxacin, levofloxacin and moxifloxacin) to the first-line medicines recommended for treating bubonic plague (streptomycin, doxycycline and gentamicin).	Conditional	Very low
The GDG suggests adding fluoroquinolones (moxifloxacin and ofloxacin) to the first-line medicine recommended for treating plague meningitis (chloramphenicol).	Conditional	Very low
The GDG suggests adding fluoroquinolones (ciprofloxacin) to the first-line medicines recommended for postexposure prophylaxis (doxycycline and sulfamethoxazole + trimethoprim).	Conditional	Very low
Use of personal protective equipment		
The GDG suggests using personal protective equipment when handling the dead body of a person who was infected with plague. The minimum required equipment includes a gown, goggles, an N95 or FFP2 mask and gloves.	Conditional	Very low

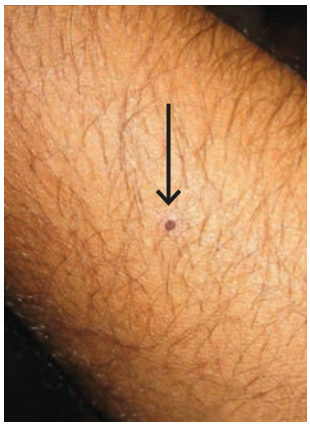
GDG: Guideline Development Group; F1RDT: rapid diagnostic test based on the F1 antigen.

1 WHO guidelines for plague management: revised recommendations for the use of rapid diagnostic tests, fluoroquinolones for case management and personal protective equipment for prevention of post-mortem transmission. Geneva: World Health Organization; 2021 (https://apps.who.int/iris/handle/10665/341505).

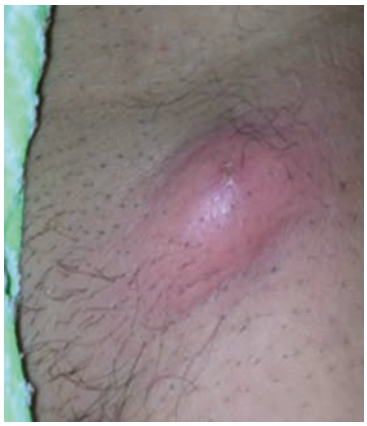
Annex 2. Clinical presentation: atlas

Highlights some of the more common presentations of plague

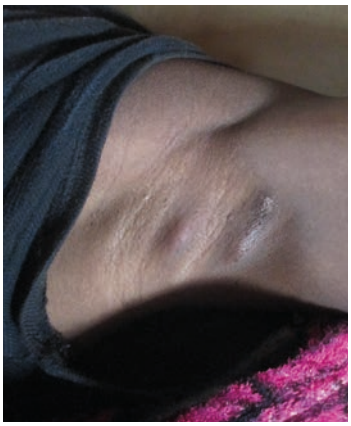
Fig A2.1. Clinical presentation of plague



(a): plague carbuncle

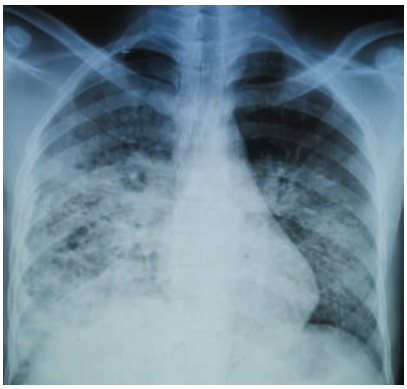


(b): inguinal bubo

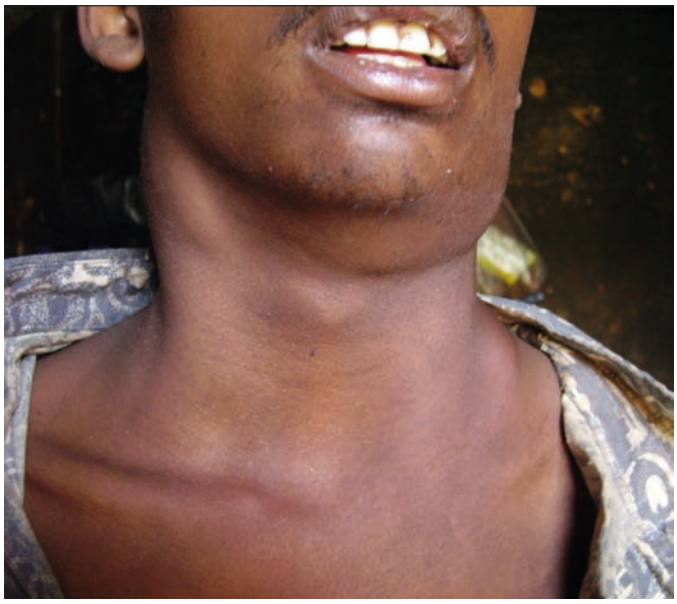


(c): axillary buboes

(d): cervical bubo



(e): chest radiography showing consolidation due to pneumonic plague



(f): cervical buboes

Photo credits:
(a, b, d, f),
Dr Eric Bertherat, WHO;

(c and e), Pasteur Institute Madagascar, Plague Unit.

Annex 3. Collecting and transporting animal specimens

Table A3.1 summarizes techniques used to collect and transport specimens from animals suspected of being infected with plague.

TABLE A3.1. Techniques for collecting and transporting animal specimens

Specimen from	Collection and transport
Rodents	**Dead rodents** • Rodent carcasses or tissues can be transported on wet ice, dry ice, freezer packs or in containers filled with liquid nitrogen. • If these are not available, blood and other samples (such as from the liver or spleen) can be taken from carcasses. Tissue samples can be sent at ambient temperatures in Cary–Blair transport medium; alternatively, samples from the spleen or liver can be preserved in 96% ethanol for subsequent PCR-based screening for *Yersinia pestis*. Blood samples should be refrigerated at 2 °C to 8 °C and sent via the cold chain. **Trapping rodents** • Using multiple-catch live traps is preferable to using snap or dead fall traps because fleas tend to leave a dead host's body as it cools. • Live traps are useful for capturing hosts for flea collection and for taking tissue and blood samples. • Traps must be set for at least 24 hours (including an overnight period) at sites where there are burrows, nests, runways or other evidence of rodent activity. **Rodent serum** • Blood for serology can be collected from rodents through a variety of techniques, including cardiac puncture. • Samples can be transported directly in sterile, sealed tubes at 2 °C to 8 °C via the cold chain (e.g. a cool box with ice packs).
Vectors (fleas)	• If hosts are captured alive, they should be anaesthetized, placed in a white enamel pan and then brushed or combed vigorously from the tail towards the head. This will dislodge fleas from the host. • Fleas will fall to the bottom of the pan and can be collected using thin tweezers or tubes to aspirate them from the rodent's fur and placed in ethanol-filled vials for morphological identification and molecular analysis. • These may then be transported to the nearest laboratory capable of identification and further processing. • Fleas can also be collected from rodent burrows by burrow swabbing.
Carnivore reservoirs	**Carnivore serum** • Blood can be obtained from dogs who have been properly restrained and muzzled from the large veins in the forelegs or hind legs. • Samples can be transported directly in sterile, sealed tubes under cold conditions.

PCR: polymerase chain reaction.

Annex 4. How to safely collect pus samples from buboes of patients suspected to be infected with bubonic plague

Step 1: Before entering patient room, assemble all equipment

This document will focus only on the collection of pus from buboes. Note, that ALL suspected plague patients should have the following samples collected:

- ❑ **Pus from buboes**
- ❑ Venous blood (serum separator tube, EDTA tube, blood culture)

Step 1a: Assemble equipment for collecting pus from buboes

- ❑ Collection kit (if available)

If a collection kit is not available:

- ❑ Laboratory sampling tube to be used for RDT (plastic or glass tube)

- ❑ Single-use sampling device (18 gauge needle and syringe)

- ❑ Cary-Blair transport medium tube or transport swab

- ❑ Sterile swab

- ❑ Skin antiseptic solution: 70% isopropyl alcohol

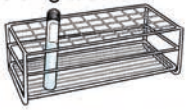
- ❑ Plague Rapid Diagnostic Test (RDT) (if available)

- ❑ Gauze pads

- ❑ Adhesive bandage

- ❑ Tray for assembling buboes pus collection tools

- ❑ Rack for holding tubes



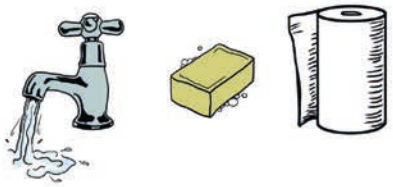
- ❑ Saline solution or PBS solution (if available)

- ❑ Durable marker for writing on laboratory samples

Step 1b: Assemble equipment for preventing infections

For hand hygiene use

- ❑ Alcohol-based handrub **OR**
- ❑ Clean running water, soap and disposable (paper) towel



Personal Protective Equipment (PPE)

- ❑ Several pairs of disposable gloves (non-sterile, ambidextrous, single layer)
  - One pair for pus collection
  - Additional pairs as a replacement if they become damaged or contaminated

- ❑ Footwear: closed-toe shoes with puncture resistant soles
- ❑ Face protection: face mask + [face shield **OR** goggles]

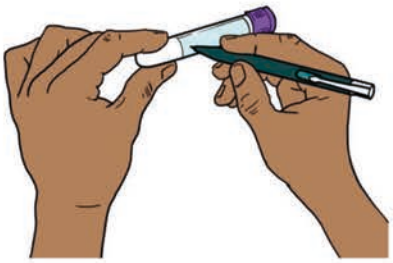
- ❑ Long-sleeved, cuffed gowns

Waste management

- ❑ Leak-proof and puncture resistant sharps container
- ❑ Two leak-proof infectious waste bags
  - one for disposable material (destruction)
  - one for reusable materials (disinfection)

Step 1: Before entering patient room, assemble all equipment

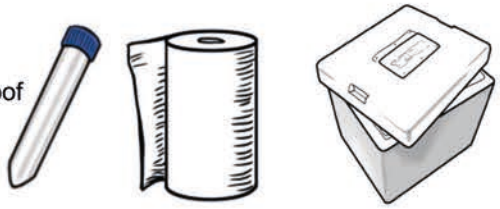
Step 1c: Fill out patient documentation



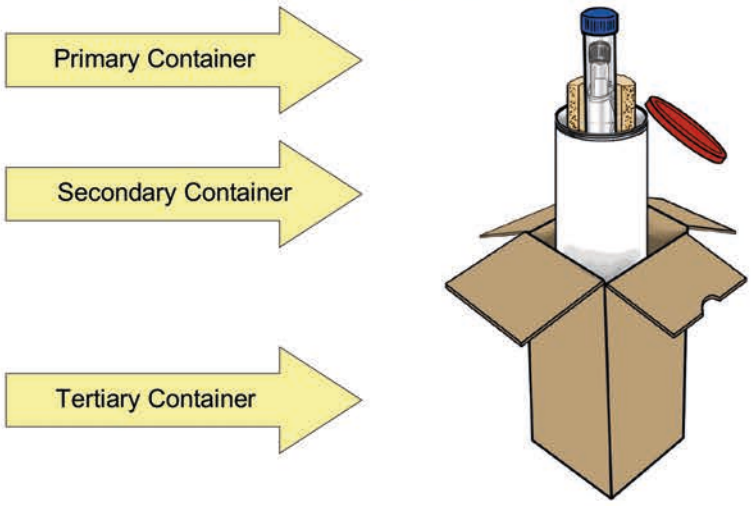
- ❑ **Label Cary-Blair transport medium tubes** with date of collection, patient name, and his/her identifier number.
- ❑ **Complete the necessary laboratory form and epidemiological questionnaire.**
- ❑ **If several patients have to be sampled in the same place or during the same investigation, create a line list.** One patient per line. The list should include: patient name, identifier number, sex, age (birth date), clinical information: symptoms, date of onset, date sample was collected, type of sample taken.

Step 1d: Assemble equipment for packaging of samples

- ❑ Plastic leak-proof packaging container
- ❑ Disposable (paper) towels
- ❑ 0.5% Chlorine solution for disinfecting plastic leak-proof packaging container
- ❑ Cooler or cold box, if sample requires refrigeration



For the shipment of samples to the National Reference Laboratory follow sample shipment packaging requirements (Follow WHO documents about how to safely ship human samples from patients suspected to be infected with highly infectious pathogens)



Important: A designated assistant wearing gloves should be available to help you. This person should stand outside the patient room. He/She will help you prepare the sample for transport and will provide any additional equipment you may need. He/She will monitor you while you remove the personal protective equipment.

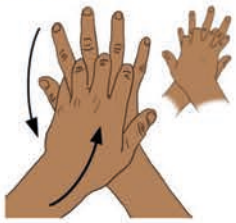
Step 2: Put on all personal protective equipment (PPE)

DO NOT ENTER THE PATIENT AREA IF YOU DO NOT HAVE ALL PROTECTIVE GEAR ON
REMOVE ALL PERSONAL ITEMS (jewellery, watches, mobile phones, pens, etc.)

Step 2a: Perform hand hygiene. Duration of the entire procedure: **40-60 sec** if handwashing with soap and water; **20-30 sec** if handrubbing with an alcohol-based solution.

Wet hands with water and enough soap to cover all hand surfaces

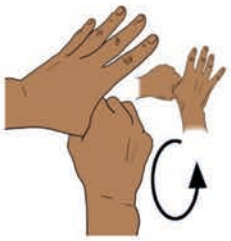
Rub hands, palm to palm



Right palm over left dorsum with interlaced fingers and vice versa

Palm to palm with fingers interlaced

Back of fingers to opposing palms with fingers interlocked



Rotational rubbing of left thumb clasped in right palm and vice versa

Rinse hands with water

Dry hands thoroughly with single use towel

Step 2b: Put on a gown

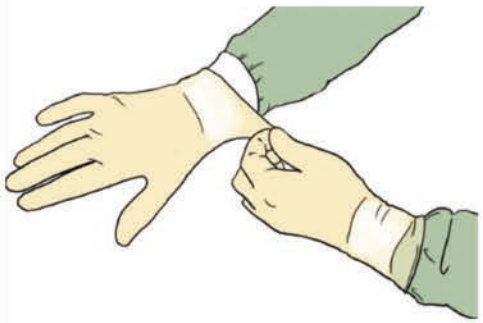
Step 2c: Put on face protection

Put on a medical mask

Put on eye protection (goggles or face shield)

OR

Step 2d: Put on gloves (over gown cuffs)



Step 2e: You are ready to enter the patient room

Step 3: Collect the pus sample from the buboes of the patient

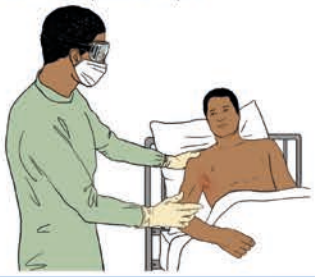
Step 3a: Prepare room

- ✓ Bring all equipment for pus collection and waste management into the patient room as you enter
- ✓ Set up infectious waste bags and leak-proof and puncture resistant sharps container for use
- ✓ Set up pus collection equipment in a place that is easy to access

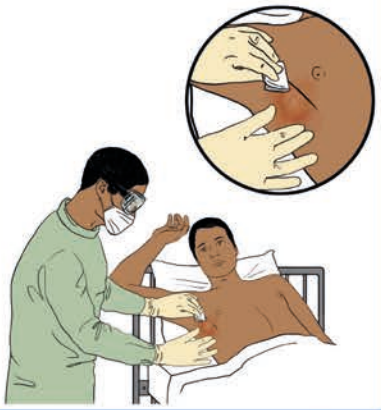
Destruction Disinfection

Step 3b: Identify and prepare the patient

- ✓ Introduce yourself to the patient and explain what you will do, and why the pus collection is necessary
- ✓ Make sure that this is the correct patient from whom you wish to take the pus sample



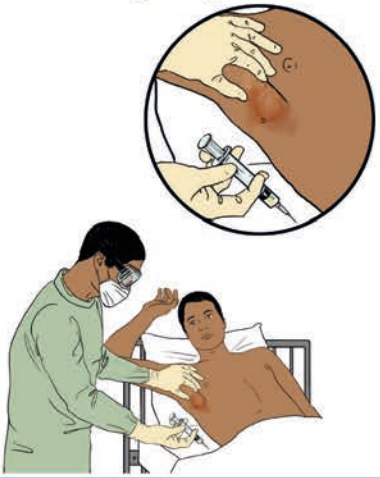
Step 3c: Locate the bubo and disinfect the skin over and around the bubo with the alcohol pad



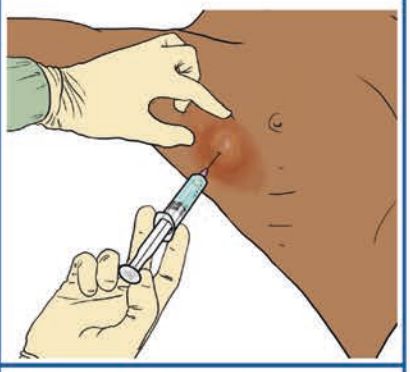
Step 3d: Aspirate the PBS solution (from collection kit) or saline solution into syringe

- ✓ This will help make aspiration of the bubo pus easier.

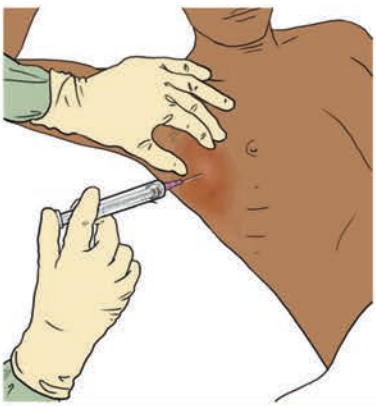
Step 3e: Immobilize the bubo with your gloved hand



Step 3f: Put the needle into the bubo at a perpendicular angle

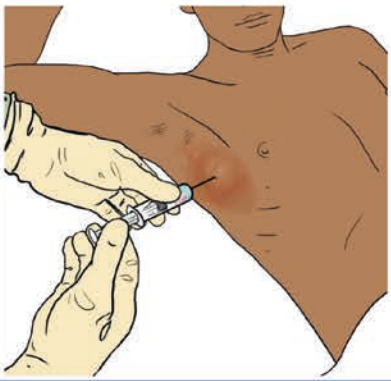


Step 3g: Inject a few millilitres of saline solution or PBS solution into bubo

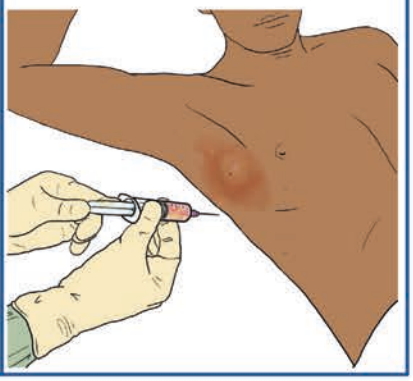


Step 3h: Aspirate the pus

- ✓ Collect a minimum of 2 millilitres of bubo pus.



Step 3i: Withdraw the needle gently

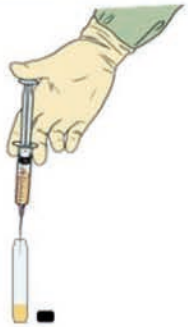


Step 4: Prepare sample for Rapid Diagnostic Test Kit (RDT) analysis or transportation to the National Reference Laboratory

Step 4a: If plague RDT kit is available, perform diagnostic analysis as per RDT manufacturer's instructions and proceed to step 4d

✓ If no RDT kit is available, continue to step 4b and prepare sample for shipment.

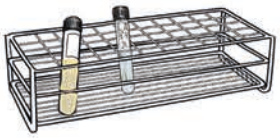
Step 4b: Place needle of syringe into transport tube and slowly push to transfer pus from the syringe to the transport tube. Do not make aerosols. DO NOT remove the needle from syringe.



Step 4c: Push swab into the pus to saturate and transfer to Cary-Blair transport medium/carrier

✓ Tightly close the top of the Cary-Blair transport medium tube/carrier

Step 4d: Place Cary-Blair transport medium tube with swab and remainder of pus in transport tube on rack



Step 4e: Put needle into leak-proof and puncture resistant sharps container

If the sharps container DOES NOT HAVE a needle remover:

- ✓ Put the needle and holder into a sharps container
- ✓ Do not remove the needle from the holder
- ✓ **Do not reuse the needle**
- ✓ **Do not recap the needle**

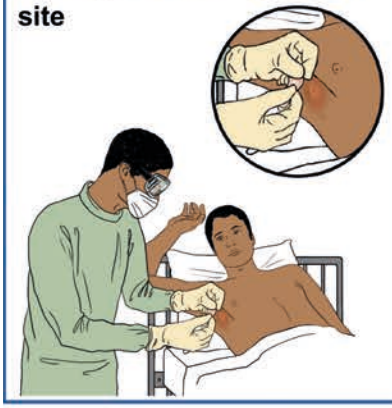
If the sharps container DOES HAVE a needle remover:

- ✓ Remove the needle following instructions on the sharps container
- ✓ Put the holder into the infectious waste bag for disinfection

*** Follow health facility procedures on waste destruction**

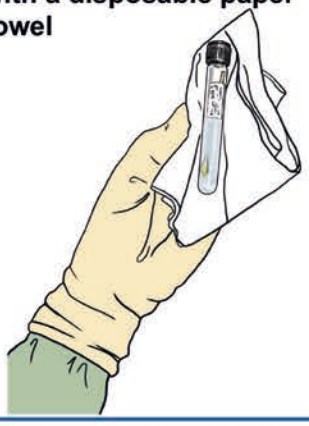
Step 5: Return to patient to bandage puncture site

Step 5a: Put adhesive bandage over the puncture site



Step 6: Prepare sample for transportation to the National Reference Laboratory

Step 6a: Take the Cary-Blair transport medium tube from the tray and wipe the tube with a disposable paper towel



Step 6b: Place all items that came into contact with patient and bubo pus into the infectious waste bag for destruction

Step 6c: Protect the sample from breaking or leaking during transport by wrapping the Cary-Blair transport medium tube in a paper towel

Step 6d: Ask the designated assistant to approach the patient room, without entering

- ✓ This person should have gloves on
- ✓ This person should come close to you holding the open plastic leak-proof packaging container.
- ✓ This person should **not** enter the patient room

Step 6e: The person who has collected the sputum sample should put the wrapped tube of sputum into the plastic leak-proof packaging container

- ✓ Be careful not to touch the leak-proof plastic tube with your gloves while transferring the wrapped tube of pus to the leak-proof plastic tube

Step 6f: Have the gloved assistant tightly close the top of the plastic leak-proof packaging container

- ✓ Disinfect the outer side of the plastic leak-proof packaging container 0.5% chlorine solution

Step 6g: The assistant removes gloves and performs hand hygiene

Note: The sample is now ready for shipment to the National Reference Laboratory. Follow sample shipment packaging requirements for infectious substances.

- ❑ Store samples at room temperature for up to 24 hours. If you need to store the sample for longer periods before shipping, store between 4-8° Celsius.
- ❑ Do not freeze pus samples.
- ❑ Pus samples and serum samples can be shipped in the same package.

Quick Tip: Ensure that patient is undergoing prompt treatment without waiting for lab results and that clinical management is appropriate according to plague guidelines.

Step 7: Remove Personal Protective Equipment (PPE)

Step 7a: Remove the gloves

1. Hold the outer edge of the first glove and peel it off

2. Hold the first glove in the gloved hand and drag a bare finger under the second glove

3. Remove second glove from the inside, creating a "bag" for both gloves and put in the infectious waste bag for disposal

Step 7b: Perform hand hygiene

- ✓ Alcohol-based handrub solution (20-30 sec) **OR**
- ✓ Soap and water (40-60 sec)

Step 7c: Remove the gown

1. Untie the gown
2. Remove the gown from behind starting at the neck and shoulders
3. Pull gown away from you, turning it inside out, removing hands last

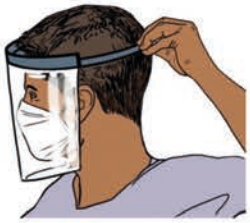
4. Fold or roll the gown into a ball
5. Put the gown in the infectious waste bag for destruction

Step 7d: Perform hand hygiene

- ✓ Alcohol-based handrub solution (20-30 sec) **OR**
- ✓ Soap and water (40-60 sec)

Step 7e: Remove face protection

When wearing a face shield



- ✓ Remove face shield from behind
- ✓ If face shield is reusable, place it in an infectious waste bag for disinfection
- ✓ If face shield is disposable, place it in an infectious waste bag for destruction

OR

When wearing goggles

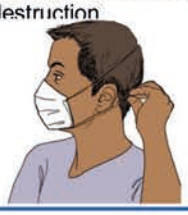
- ✓ Remove goggles from behind
- ✓ If reusable goggles, place it in an infectious waste bag for disinfection
- ✓ If disposable goggles, place it in an infectious waste bag for destruction

Step 7f: Perform hand hygiene

- ✓ Alcohol-based handrub solution (20-30 sec) **OR**
- ✓ Soap and water (40-60 sec)

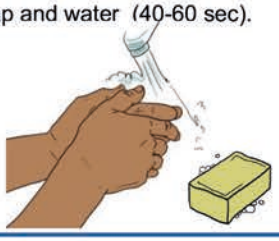
Step 7g: Remove medical mask

- ✓ Remove the mask from behind, starting with the bottom strap
- ✓ Place it in an infectious waste bag for destruction



Step 7h: Perform hand hygiene

- ✓ Alcohol-based handrub solution (20-30 sec) **OR**
- ✓ Soap and water (40-60 sec).



Quick Tips

- Place all reusable equipment into a separate infectious waste bag for disinfection
- Dispose of hazardous waste according to facility protocols

When collecting bubo pus samples from multiple patients

- Change gloves between each patient
- Wash hands between each patient
- DO NOT WASH GLOVED HANDS
- DO NOT REUSE GLOVES

Annex 5. How to safely collect sputum samples from patients suspected to be infected with pneumonic plague

Step 1: Before entering patient room, assemble all equipment

This document will focus only on the collection of sputum. Note, that ALL suspected plague patients should have the following samples collected:

- ❑ **Sputum**
- ❑ Venous blood (serum separator tube, EDTA tube, blood culture bottle)

Step 1a: Assemble equipment for sputum sample

- ❑ Plastic container with screw top lid 
- ❑ Sterile swab 
- ❑ Cary-Blair transport medium tube or transport swab 
- ❑ Durable marker for writing on laboratory samples 
- ❑ Tray for assembling sputum collection tools 
- ❑ Clean water for oral rinse 
- ❑ Plague Rapid Diagnostic Test (if available) 

Step 1b: Assemble equipment for preventing infections

For hand hygiene use

- ❑ Alcohol-based handrub **OR**
- ❑ Clean running water, soap and disposable (paper) towel



Personal Protective Equipment (PPE)

- ❑ Several pairs of disposable gloves (non-sterile, ambidextrous, single layer)
  - One pair for sputum collection
  - Additional pairs as a replacement if they become damaged or contaminated

- ❑ Long-sleeved, cuffed gowns

- ❑ Footwear: closed-toe shoes with puncture resistant soles
- ❑ Face protection: face mask + [face shield **OR** goggles]

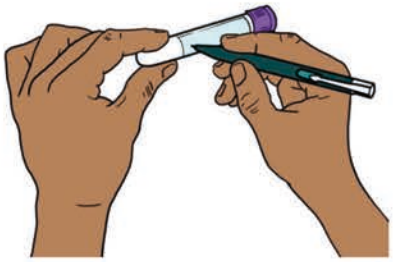
Waste management

- ❑ Two leak-proof infectious waste bags
  - one for disposable material (destruction)
  - one for reusable materials (disinfection)

NOTE: In case of aerosol generating procedure, use a fit-tested N95 face mask or equivalent face protection. Countries with high TB prevalence, always use N95 face mask or equivalent face protection

Step 1: Before entering patient room, assemble all equipment

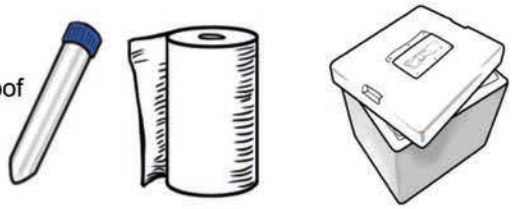
Step 1c: Fill out patient documentation



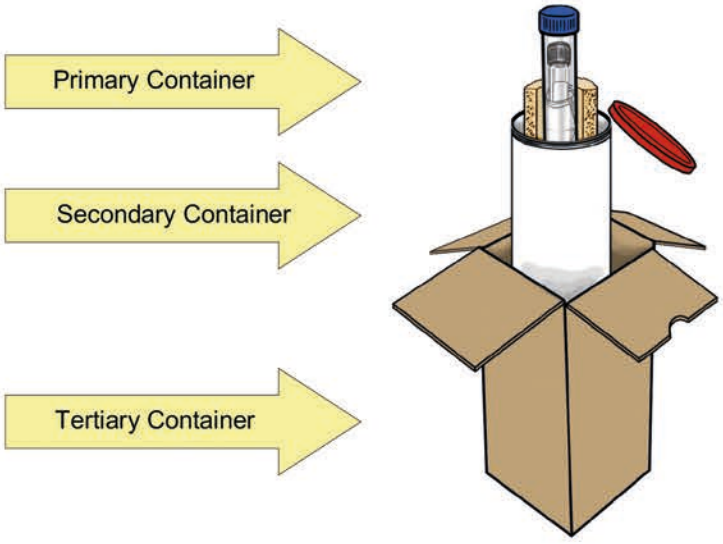
- ❑ **Label Cary-Blair transport medium tubes and the plastic container with screw top lid** with date of collection, patient name, and his/her identifier number.
- ❑ **Complete the necessary laboratory form and epidemiological questionnaire.**
- ❑ **If several patients have to be sampled in the same place or during the same investigation, create a line list.** One patient per line. The list should include: patient name, identifier number, sex, age (birth date), clinical information: symptoms, date of onset, date specimen was collected, type of sample taken.

Step 1d: Assemble equipment for packaging of samples

- ❑ Plastic leak-proof packaging container
- ❑ Disposable (paper) towels
- ❑ 0.5% Chlorine solution for disinfecting plastic leak-proof packaging container
- ❑ Cooler or cold box, if sample requires refrigeration



For the shipment of samples to the National Reference Laboratory follow sample shipment packaging requirements (Follow WHO documents about how to safely ship human samples from patients suspected to be infected with highly infectious pathogens)



Important: A designated assistant wearing gloves should be available to help you. This person should stand outside the patient room. He/She will help you prepare the sample for transport. He/She will assist you with putting on the personal protective equipment. He/She will provide any additional equipment you may need. He/She will monitor you while you remove the personal protective equipment.

Step 2: Put on all personal protective equipment (PPE)

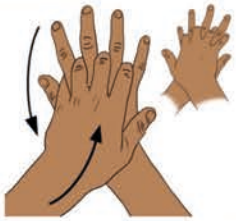
DO NOT ENTER THE PATIENT AREA IF YOU DO NOT HAVE ALL PROTECTIVE GEAR ON

REMOVE ALL PERSONAL ITEMS (jewellery, watches, mobile phones, pens, etc.)

Step 2a: Perform hand hygiene. Duration of the entire procedure: **40-60 sec** if handwashing with soap and water; **20-30 sec** if handrubbing with an alcohol-based solution.

Wet hands with water and enough soap to cover all hand surfaces

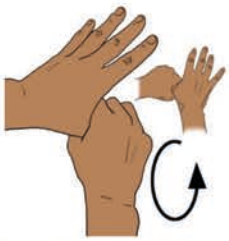
Rub hands, palm to palm



Right palm over left dorsum with interlaced fingers and vice versa

Palm to palm with fingers interlaced

Back of fingers to opposing palms with fingers interlocked



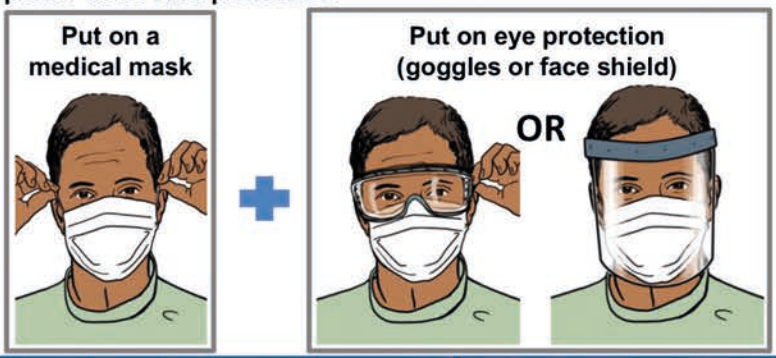
Rotational rubbing of left thumb clasped in right palm and vice versa

Rinse hands with water

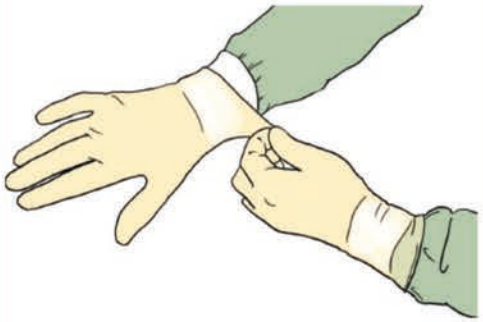
Dry hands thoroughly with single use towel

Step 2b: Put on a gown

Step 2c: Put on face protection



Step 2d: Put on gloves (over gown cuffs)



Step 2e: You are ready to enter the patient room

Step 3: Set up station to collect sputum from patient

(at least 2 metres away from all people in an isolated area)

Step 3a: Prepare room

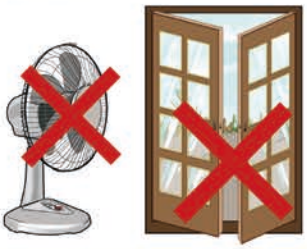
- ✓ Bring all equipment for sputum collection and waste management into the patient room as you enter
- ✓ Set up infectious waste bags for use
- ✓ Set up sputum collection equipment in a place that is easy to access

Destruction

Disinfection

Step 3b: Determine whether there is wind or air blowing in the room

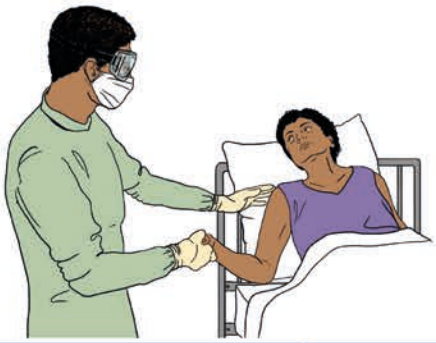
- ✓ If there is a wind, position sputum collection area so patient has the wind blowing on his/her back.
- ✓ If there is a fan, be sure to stop it.
- ✓ You will be standing behind the patient when collecting the sample.



Step 4: Collect sputum sample from patient

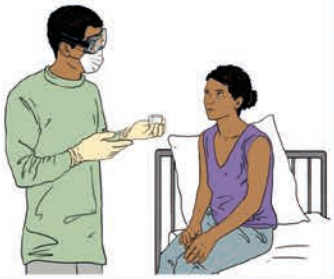
Step 4a: Identify and prepare the patient

- ✓ Introduce yourself to the patient
- ✓ Make sure that this is the correct patient from whom you wish to take the sputum sample
- ✓ explain what you will do, and why the sputum sample is necessary



Step 4b: Show patient the plastic container and describe what sputum is and what it looks like:

- ✓ Explain to patient that they should aim to produce enough sputum to fill container to one spoon full.
- ✓ It is okay if patient does not produce enough sputum as long as the sputum description.
- ✓ Sputum is thick and mucoid and comes from the lungs.
- ✓ The colour is white to green or bloody.
- ✓ Sputum is NOT saliva or nasal secretions which are runny and clear.



Step 4c: Ask patient to rinse mouth out with clean water

- ✓ This will reduce food/bacteria in the mouth.

Step 4d: Give the labelled container to patient

- ✓ Tell patient not to touch inside of the container at any time.
- ✓ Maintain sterility of container and lid

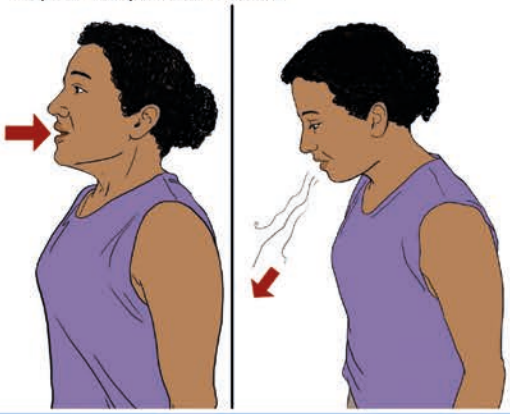
Step 4e: Position patient at sputum station and stand behind the patient

- ✓ Make sure the air stream (fan, air conditioner) is behind your back so you do not become exposed when the patient coughs.

Step 4: Collect sputum sample from patient

Step 4f: Tell patient to take a deep breath and hold for a few seconds then breathe out slowly

✓ Repeat the process 3 times



Step 4g: Tell patient to blow out hard during their third blow

✓ It may be helpful to count the blows for the patient.

Step 4h: Ask patient to lift container close to his/her mouth and blow out hard once more

✓ The container should not touch the mouth.

✓ This motion will bring sputum from the lungs.

Step 4i: Tell patient to cough directly into plastic container

Step 4j: Compare sputum sample to description in 4b

✓ If the sputum sample looks similar to description in 4b, tell patient to return to resting position.

✓ If the sputum sample does not match description in 4b, obtain a new container from designated assistant outside of patient room, repeat steps 4c to 4i.

Step 4k: Put items that have body fluid on them into the infectious waste bag

✓ Place fluid from mouth rinsing station in infectious waste bag

Quick tips:

✓ **Ensure that the patient is undergoing prompt treatment without waiting for lab results and that clinical management is appropriate according to plague guidelines.**

✓ **Isolate patient with suspected pneumonic plague from other sick patients.**

✓ **Verify that all healthcare workers are wearing appropriate PPE when coming within two metres of a suspected pneumonic plague patient (refer to Step 2)**

Step 5: Prepare sample for Rapid Diagnostic Test Kit (RDT) analysis or transportation to the National Reference Laboratory

Step 5a: If plague RDT kit is available, perform diagnostic analysis as per RDT manufacturer's instructions

- ✓ If no RDT kit is available, continue to step 5b and prepare sample for shipment.

Step 5b: Transfer sputum from plastic container to the Cary-Blair transport medium tube using swab

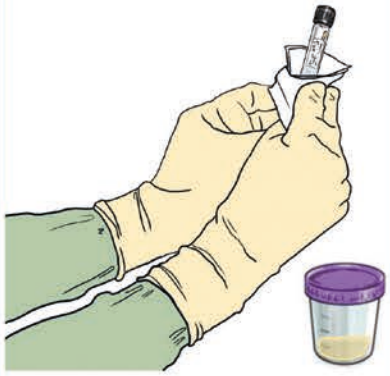
Step 5c: Once sputum is on the swab, push the swab into the Cary-Blair transport medium tube

- ✓ Tightly close the top of Cary-Blair medium tube/carrier

Step 5d: Take the Cary-Blair transport medium tube and container with remainder of sputum from the tray and wipe the tube and container with disposable paper towel

Step 5e: Place all items that came into contact with the patient into the infectious waste bag for destruction

Step 5f: Protect the two samples from breaking or leaking during transport by wrapping the tube and container in a paper towel or absorbent material



Step 5: Prepare sample for Rapid Diagnostic Test Kit (RDT) analysis or transportation to the National Reference Laboratory

Step 5g: Ask the designated assistant to approach the patient room, without entering

- ✓ This person should have gloves on
- ✓ This person should come close to you holding the open plastic leak-proof packaging container.
- ✓ This person should **not** enter the patient room

Step 5h: The person who has collected the sputum sample should put the wrapped Cary Blair tube of sputum into the plastic leak-proof packaging container

- ✓ Be careful not to touch the leak-proof plastic tube with your gloves while transferring the wrapped tube of sputum to the leak-proof plastic tube
- ✓ Place container with remaining sputum into a sealable bag with absorbent material

Step 5i: Have the gloved assistant tightly close the top of the plastic leak-proof packaging container

- ✓ Disinfect the outer side of the plastic leak-proof packaging container and outer side of bag containing remaining sputum with 0.5% chlorine solution

Step 5j: The assistant removes gloves and performs hand hygiene

Note: The sample is now ready for shipment to the National Reference Laboratory. Follow sample shipment packaging requirements for infectious substances.

- ❑ Store at room temperature for up to 24 hours. If you need to store the sample for longer periods before shipping, store between 4-8 Celsius.
- ❑ Do not freeze bacteria samples.
- ❑ Blood (Haemoculture) and sputum samples can be packaged and shipped together.

Step 6: Remove Personal Protective Equipment (PPE)

Step 6a: Remove the gloves

1. Hold the outer edge of the first glove and peel it off

2. Hold the first glove in the gloved hand and drag a bare finger under the second glove

3. Remove second glove from the inside, creating a "bag" for both gloves and put in the infectious waste bag for disposal

Step 6b: Perform hand hygiene

- ✓ Alcohol-based handrub solution (20-30 sec) **OR**
- ✓ Soap and water (40-60 sec)

Step 6c: Remove the gown

1. Untie the gown
2. Remove the gown from behind starting at the neck and shoulders
3. Pull gown away from you, turning it inside out, removing hands last

4. Fold or roll the gown into a ball

5. Put the gown in the infectious waste bag for destruction

Step 6d: Perform hand hygiene

- ✓ Alcohol-based handrub solution (20-30 sec) **OR**
- ✓ Soap and water (40-60 sec)

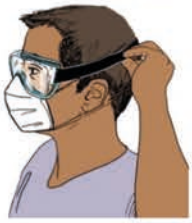
Step 6e: Remove face protection

When wearing a face shield

- ✓ Remove face shield from behind
- ✓ If face shield is reusable, place it in an infectious waste bag for disinfection
- ✓ If face shield is disposable, place it in an infectious waste bag for destruction

OR

When wearing goggles



- ✓ Remove goggles from behind
- ✓ If reusable goggles, place it in an infectious waste bag for disinfection
- ✓ If disposable goggles, place it in an infectious waste bag for destruction

Step 6f: Perform hand hygiene

- ✓ Alcohol-based handrub solution (20-30 sec) **OR**
- ✓ Soap and water (40-60 sec)

Step 6g: Remove medical mask

- ✓ Remove the mask from behind, starting with the bottom strap
- ✓ Place it in an infectious waste bag for destruction

Step 6h: Perform hand hygiene

- ✓ Alcohol-based handrub solution (20-30 sec) **OR**
- ✓ Soap and water (40-60 sec).

Quick Tips

- Place all reusable equipment into a separate infectious waste bag for disinfection
- Dispose of hazardous waste according to facility protocols

When collecting sputum samples from multiple patients

- Change gloves between each patient
- Wash hands between each patient
- DO NOT WASH GLOVED HANDS
- DO NOT REUSE GLOVES

Annex 6. Instructions for using the rapid diagnostic test developed by the Pasteur Institute of Madagascar

SAMPLE COLLECTION & INSTRUCTION FOR USE OF RAPID DIAGNOSTIC TEST FOR PLAGUE

Materials : dipstick in an aluminium foil bag, PBS (Phosphate Buffer Saline) in eppendorf tube, transport medium (carry blair), swab, syringe, needle, alchool pad, distilled water

Step 1 : Identification

Label the test tube **(sample reference)**

Step 2 : Preparation of the sample

F1 Antigen detection

Bubonic plague: Inject half of PBS in the affected bubo, aspire pus; homogenate in eppendorf tube. Test 150 µl of this suspension. If migration incomplete (viscous pus) dilute 1/5 or 1/10 with PBS and test with a new dipstick.

Pneumonic plague: Collect sputum in a plastic container. Aspirate 0.5 ml of sputum and all of the PBS; homogenate in eppendorf tube. Test 150 µl of this suspension. If migration incomplete (sputum too thick), dilute 1/3 with PBS and test with new dipstick.

Rodent spleen: Aspire all the PBS, inject half in rodent spleen, aspire spleen homogenate. Homogenate in eppendorf tube. Test 150 µl of this suspension.

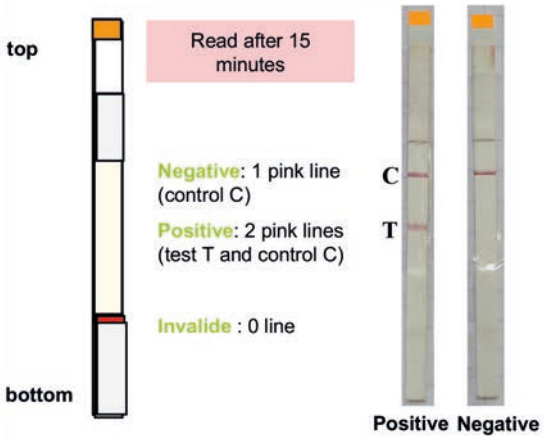
Step 3: Test procedure

- Put 150 µl of diluted sample in the test tube (5 ml)
- Introduce the dipstick: coloured side at the top

Caution!

- **Orientation of the disptick : Coloured side on the top** (orange for antigen detection).
- Keep the packet containig the dipstick at 37°C or at RT before opening. Do not use cold dipsticks for testing.
- Remove the dipstick using a pair of forceps, at the coloured end only.
- Keep any unused dipstick at +4°C.
- Take precautions when handling infectious material : wear gloves, decontaminatie used materials.

Step 4: Interpretation



Step 5: Archiving the dipstick

Dry the dipstick in a blotting paper

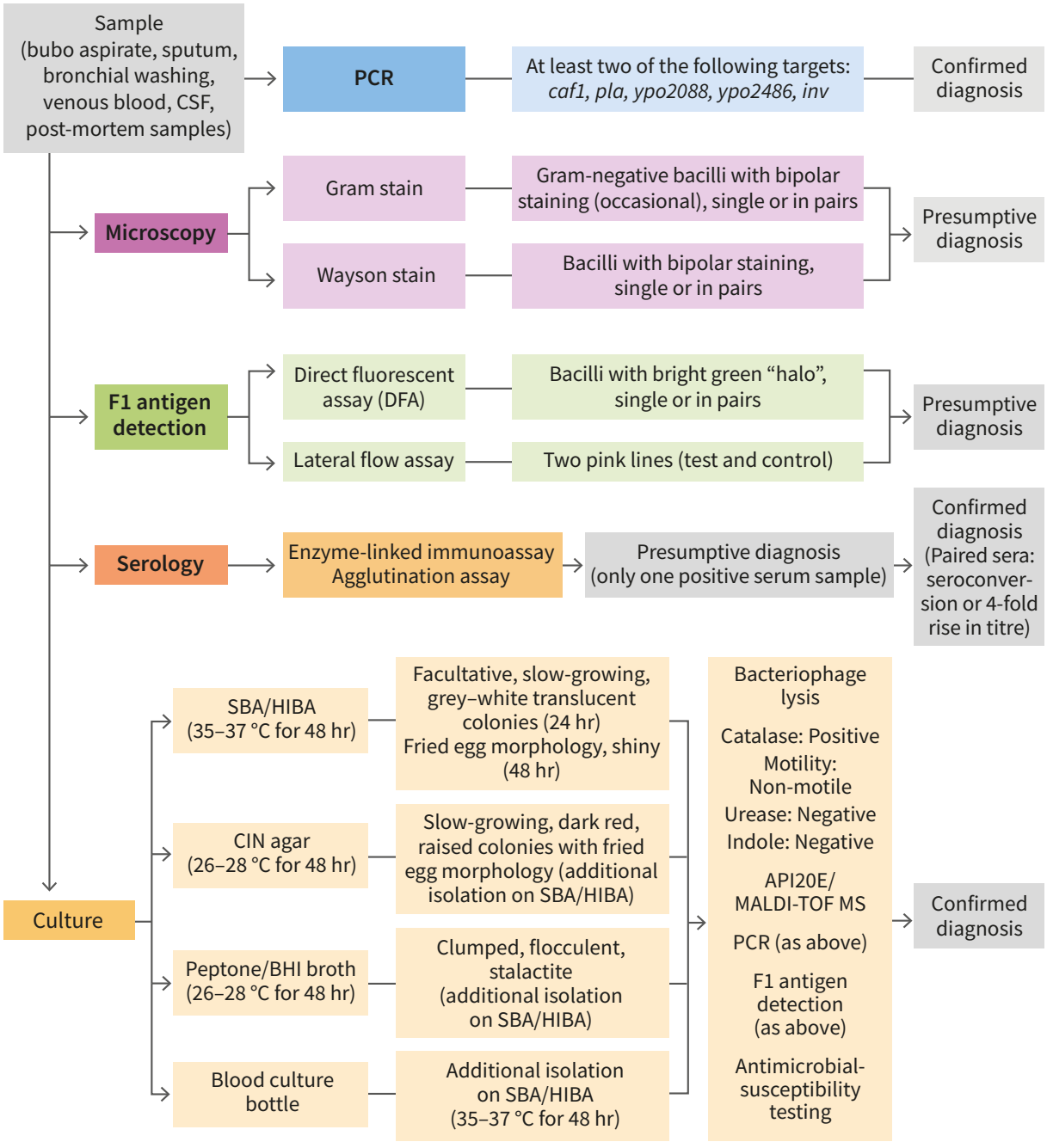
- Stick the dipstick by covering it completely with transparent scotch (in archiving book)
- Note test result and relating information about the test:
Sample identification
Batch of dipstick
Date of test
Operator's Name
Results - Comments

Source: Pasteur Institute Madagascar

Annex 7. Plague diagnostic assays

Fig. A7.1 provides information about the assays and samples used to diagnose plague.

Fig A7.1. Assays and samples used to diagnose plague



API20E: analytical profile index 20E; BHI: brain heart infusion; CIN agar: cefsulodin–irgasan–novobiocin; CSF: cerebrospinal fluid; F1: fraction 1; HIBA: heart infusion blood agar; MALDI-TOF MS: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PCR: polymerase chain reaction; SBA: sheep blood agar.

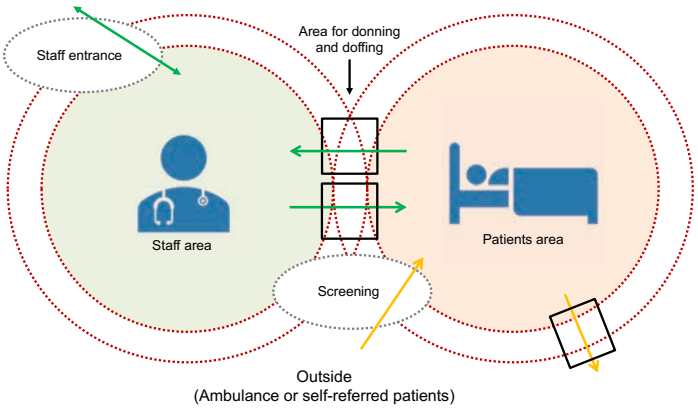
Annex 8. Isolation arrangements for patients with pneumonic plague

Environmental and engineering measures play key roles in reducing the risk of nosocomial infections and also enable the highest level of care to be provided, as well as person-centred care; such measures include considering the facility's layout and the flow of staff and patients, ensuring appropriate ventilation and using appropriate construction materials and finishings. A treatment area can be created by repurposing existing facilities or if the caseload is high, installing a new structure. In both cases, standard precautions and transmission-based precautions for infection prevention and control must be adhered to.

The area where patients with plague are hospitalized should be separated from other wards, clearly signposted and delimited, but it must remain safely accessible to families and communities.

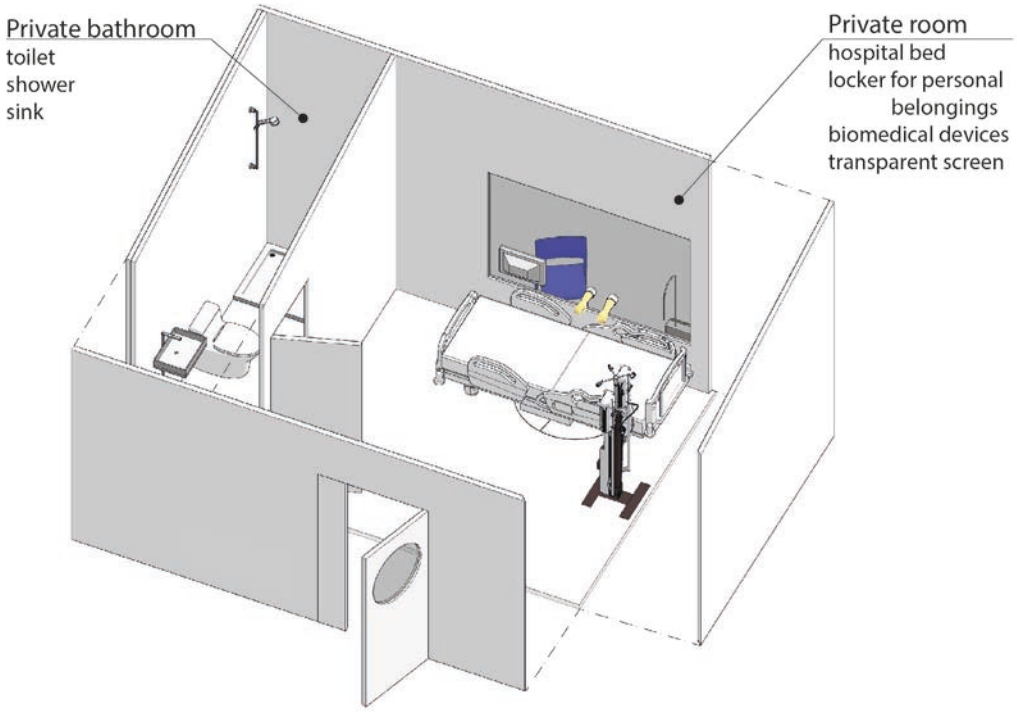
Dedicated areas for patients and staff should be clearly separated, while still enabling a rational flow of people and goods, and there should be specific spaces for donning and doffing personal protective equipment. (Fig. A8.1)

Fig A8.1. Areas for patients with plague and staff should be clearly separated



For infection prevention and control and for privacy, patients should be treated in individual, self-contained and well-ventilated rooms, with vector control measures in place, such as screens on windows and other openings (Fig. A8.2).

Fig A8.2. Patients' rooms should be self-contained, but a transparent screen may be used to allow for constant observation by staff



Patients' rooms

When designing rooms for patients**,** the minimum dimensions should be able to include:

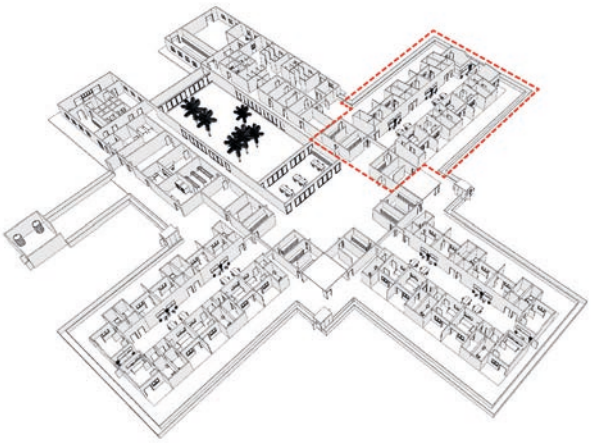
- a hospital bed or stretcher, considering the need for 360° movement of the bed or stretcher;
- any biomedical devices that will be needed;
- a locker, shelves or bedside table for the patient's personal belongings.

A transparent screen can be installed in each patient's room to facilitate constant patient observation directly from the staff area.

If individual rooms cannot be provided for patients, then other alternatives should be considered in the following order of preference.

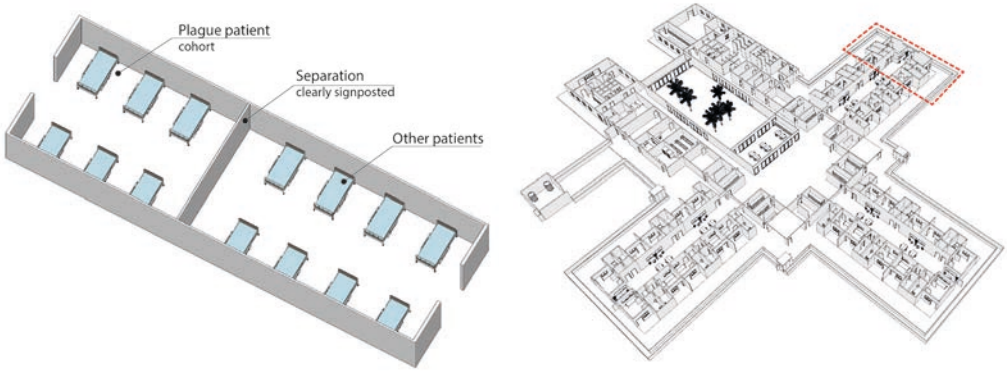
1. Identify an existing building or ward that can be repurposed for cohorts of cases of pneumonic plague (Fig. A8.3).

Fig A8.3. If individual rooms for patients are not available, consider repurposing a building or ward to isolate patients with pneumonic plague



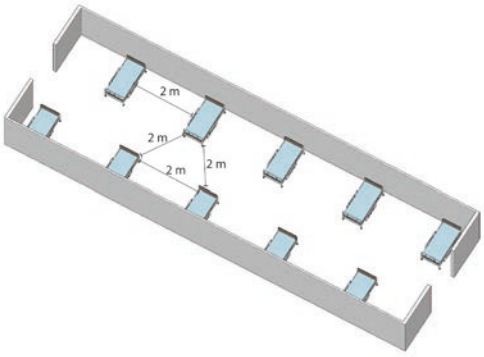
2. Within an existing large ward, identify and delimit a dedicated area as far away from other patients as possible (Fig. A8.4).

Fig A8.4. Part of a larger ward can be defined as an area for isolating patients with plague



3. If a dedicated space cannot be provided for isolation, ensure that there is at least 2 m between patients with plague and other patients and visitors (Fig. A8.5).

Fig A8.5. If patients with plague cannot be easily isolated, ensure there is at least 2 m between patients with plague and other patients



Ventilation

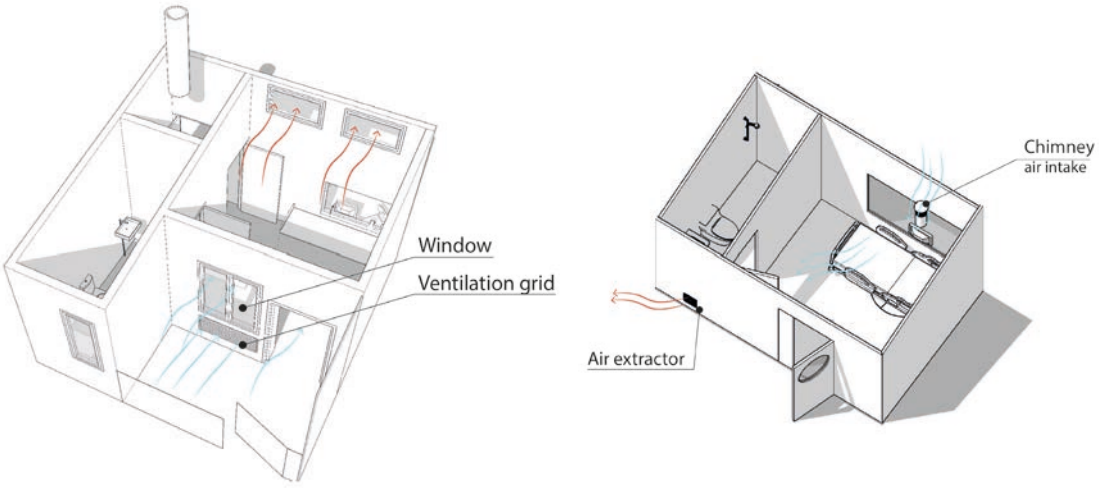
Plague is transmitted between animals and humans by the bite of infected fleas, through direct contact with infected tissue and through inhalation of infected respiratory droplets. Ensuring that there is adequate ventilation may reduce the risk of infection while also removing chlorine vapours that may be produced during disinfection of surfaces.

Isolation areas require not only infection prevention and control measures but also appropriate ventilation to enable a safe working environment and reduce the risk of health care–associated infections among health care workers, patients and visitors. The decision about whether to use mechanical or natural ventilation for infection control should be based on the isolation area's needs, the availability of resources and the cost of the system.

Natural ventilation

Natural ventilation uses only natural forces, such as wind pressure or differences in air density, to ensure that air flows through doors, windows or other openings in the building envelope (Fig. A8.6).

Fig A8.6. Schematic of (left) natural and (right) mechanical ventilation systems that can be used to reduce infection from airborne contaminants, such as plague



Mechanical ventilation

Mechanical ventilation is the active process of supplying air to or removing air from an indoor space by powered air movement components. A fully mechanical ventilation system can partially recirculate indoor air or rely fully on bringing outdoor air inside the building. The air supply is always controlled in terms of filtration, temperature and humidity (Fig. A8.6).

A hybrid ventilation system uses passive mechanical means to ensure that a constant supply of outdoor air is brought indoors through intentional openings in the building, such as windows, grids and chimneys.

Annex 9. Cleaning and disinfection

Cleaning should occur before disinfection. Ordinary household bleach, soap and clean water are useful disinfectants against plague.

Preparation

In a central place in the health facility, prepare two solutions of ordinary household bleach: a 1:10 solution and a 1:100 solution (Table A9.1). Prepare the bleach solutions in a well-ventilated area.

Storage

Bleach solutions must be prepared daily because they lose their strength after 24 hours. Prepared solutions must be kept away from heat and light. If the odour of chlorine is not present, then the solution has lost its strength and must be discarded.

Contact times for bleach solution

The solution must be in contact with the surface for an adequate amount of time for disinfection to occur:

- for disinfecting nonporous surfaces – contact time is at least 10 minutes and up to 30 minutes;
- for disinfecting other surfaces – contact time is 30 minutes;
- for disinfecting spills – contact time is at least 20 minutes and up to 30 minutes.

Caution: Spraying the solution is NEVER recommended because it generates harmful aerosols.

TABLE A9.1. Preparing disinfection solutions for different uses

Chlorine product	Dilution	
	1 : 10[a]	1 : 100
For disinfecting	Excreta Cadavers Spills	Floors Clothing Equipment Bedding
Household bleach (5% active chlorine)	1 L bleach per 10 L water	100 ml bleach per 10 L water or 1 L 1:10 bleach solution per 9 L water
Calcium hypochlorite (70% powder or granules)	7 g or ½ tablespoon per 1 L water	7 g or ½ tablespoon per 10 L water
Household bleach (30% active chlorine)	16 g or 1 tablespoon bleach per 1 L water	16 g or 1 tablespoon bleach per 10 L water

[a] The 1:10 bleach solution is caustic. Avoid direct contact with skin and eyes. Prepare the bleach solutions in a well-ventilated area.

Annex 10. Plague surveillance strategies

The main strategies for animal-based plague surveillance are:

- visual surveillance of rodent populations, with reporting of findings and testing of rat falls (Box A10.1);
- trapping and sampling rodents and other small mammals (Box A10.2);
- collecting fleas from captured animals (on-host populations) or from human homes, rodent burrows and nests, or other likely sites (off-host populations) (Box A10.3);
- serosurveillance of carnivores or other potential medium- to large-sized mammal hosts (Box A10.4).

The plague programme staff conducting these surveillance strategies must follow safety precautions that include:

- knowing about plague transmission and when to seek medical assistance in case of acute febrile illness and other symptoms that may be associated with plague or other zoonotic diseases;
- using personal protective equipment appropriate for the activities being undertaken and the findings of the risk assessment. In situations in which aerosols are likely to be generated, such as while performing an autopsy, respiratory protection (including face masks that meet N95 or FFP2 standards) is required during animal processing;
- applying insect repellent to human skin and clothing, such as benzyl benzoate, diethyltoluamide (DEET) or dimethyl phthalate (DMP), or wearing insecticide-impregnated clothing to avoid flea bites.

Box A10.1 Visual surveillance of rodent populations

This strategy is relatively inexpensive and requires less technical training than the other surveillance techniques mentioned in this annex. Another advantage is that useful data can be gathered by:

- local health care officials who are not primarily employed by the plague programme;
- residents of villages or urban neighbourhoods under surveillance.

The most basic form of visual surveillance is to monitor local rodent populations through visual inspection and to report any sudden increases in numbers or changes in activity of the local rat or field rodent population. An increase in numbers could signal an impending rat fall, and changes in activity could signal an epizootic among field rodents. Such observations can be hard to quantify and standardize among observers, but they can provide potentially useful warnings about areas that need heightened surveillance.

These enhanced activities may include collecting and testing carcasses or fleas for *Yersinia pestis* using direct detection methods, using targeted rodent trapping and flea collection for *Y. pestis* testing, implementing flea control activities or having trained workers use other plague control measures. One means for improving and better standardizing the quality of these observations is to develop an observation reporting form that can be used by local health officials or residents of villages and urban neighbourhoods that aims at describing local levels of rodent activity and other relevant observations. The quality of these reports is likely to improve when local health officials and residents are trained to notice the relevant signs of rodent activity, including:

- increased visibility of animals, including observations made during periods of the day when a nocturnal animal species normally would be inactive;
- evidence of increased rodent damage to crops, stored food products or other items;

- the appearance of dead rodents or sudden declines in diurnally active rodents, which could indicate a plague-associated rat fall or die-off among field rodents.

If rodents are not usually the main reservoir, then other local species associated with human plague may be more appropriate for surveillance.

The identification of dead rodents is particularly helpful when local health officials are able to quickly go to the relevant sites and safely collect carcasses for analysis at a dedicated laboratory. Identification of *Y. pestis* in the tissue of a dead animal means that emergency flea control and other plague control measures are needed. When available at the peripheral level, the carcass can be tested with a rapid diagnostic test.

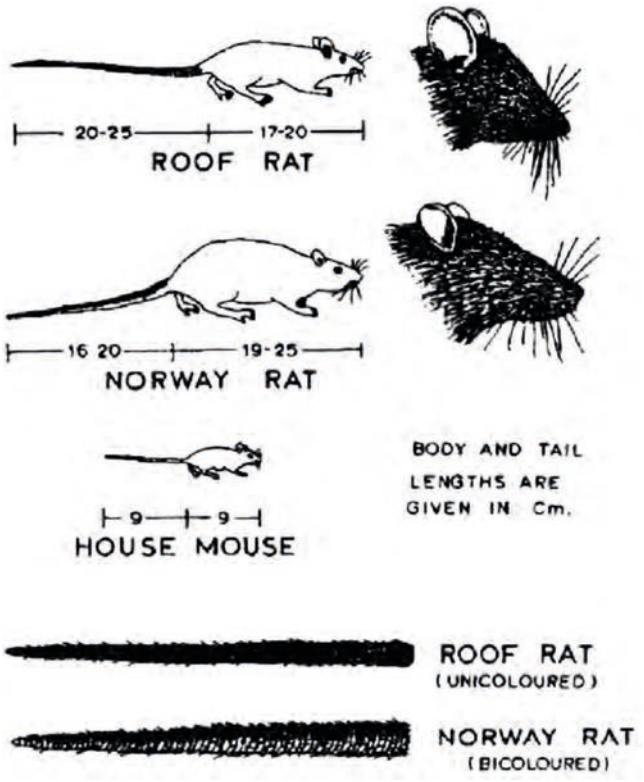
Rodent identification is important. Tips for morphological identification of the roof rat, Norway rat and house mouse are presented for adult rodents in Fig. A10.1 and phenotypic characteristics are summarized in Table A10.1.

Ideally, diagnostic criteria for all small mammal species that may be involved in plague ecology should be made available to local health officers and, if feasible, to local representatives involved in community-based surveillance.

TABLE A10.1. Morphological characteristics of adult Norway rats, roof rats and house mouse

Characteristic	Type of rodent		
	Norway rat (brown rat) *Rattus norvegicus*	**Roof rat (black rat)** *Rattus rattus*	**House mouse** *Mus musculus*
Weight	150–600 g	80–300 g	10–21 g
Head and body	Blunt nose; heavy, stocky body; 18–25 cm	Pointed nose; slender body; 16–21 cm	Pointed nose; slender body; 6–10 cm
Tail	Shorter than head plus body; uniformly dark loured; hairless; 19–25 cm	Longer than head plus body; uniformly dark coloured; hairless; 19–25 cm	Equal to or a little longer than head plus body; uniformly dark coloured; hairless; 7–11 cm
Ears	Relatively small and close-set; appear half-buried in fur; rarely >20–23 mm	Large, prominent, thin and hairless; stand well out from fur; 25–28 mm	Prominent; large for size of animal; ≤15 mm
Hindfoot length (without claw)	35–47 mm	30–39 mm	14–19 mm
Fur	Brownish-grey on back; greyish on belly	Brownish-grey to blackish on back; belly may be white, grey or greyish-black	One subspecies brownish-grey on back and greyish on belly; another is greyish on back and greyish-white on belly
Mammary glands	6 pairs	5 pairs	5 pairs
Habits	Burrows, swims and dives easily; gnaws; lives indoors and in sewers and drains	Agile climber; gnaws; often lives off the ground in trees and vines; lives indoors and outdoors	Climbs, some-times burrows; gnaws; lives indoors and outdoors

Fig A10.1 Morphological identification of adult rodents



Box A10.2 Trapping and sampling rodents and other small mammals

Rodent trapping is often used secondary to visual monitoring of rodent populations (i) as a means of ensuring early detection of plague epizootics (surveillance), (ii) as follow up to reported cases of plague in humans and a means of confirming exposure sites or (iii) in areas considered to pose an elevated risk to humans, particularly where visual monitoring is deemed inadequate.

Trapping rodents can be particularly useful because it allows:

- samples (blood, serum or tissue) to be obtained for laboratory testing from rodents that are likely to belong to species that are local plague reservoirs. These specimens can be used for bacterial culture and isolation of *Yersinia pestis* strains that can be compared with those isolated from human cases and from different plague-endemic regions. This enables infection sources to be identified and the spread of plague to be tracked using genotyping and molecular epidemiology techniques. It can also be useful for monitoring antibiotic resistance among circulating bacteria in reservoir host populations;
- fleas to be collected directly from likely reservoirs for plague testing and species identification; it also allows the numbers of fleas per rodent to be estimated for different rodent hosts (flea index) and flea species.

If live rodent trapping is conducted in conjunction with investigations into human cases, it can provide useful information on the types of rodents and fleas present at suspected sites of case exposure, help to identify likely sources of human infection and suggest which rodent and flea species should be targeted by control measures. If rodent surveillance is performed in areas where plague has not been previously identified but is suspected to occur, it can result in confirmation of *Y. pestis* in the area and provide useful information on the likely reservoir and vector species.

When trapping rodents for plague surveillance, care must be taken to:

- consider the type of trap to be used (e.g. typically live or non-lethal traps of different sizes and shapes) to capture all types of locally present species, and ensure that traps are set in a standardized fashion using locally available bait that is appropriate for the rodents of interest;
- place the traps according to the standardized trapping protocol, which should include specification for the number of traps to be placed in homes, near homes and in field environments;
- check traps frequently because fleas will quickly leave dead hosts, a situation that can lead to an underestimation of flea abundance and also to spill over of fleas to humans; this should also be done in the interest of animal welfare;
- collect blood, tissue samples and fleas using standardized safety protocols so that results can be compared between different time points and locations.

Unfortunately, rodent trapping also presents certain challenges to plague surveillance programmes:

- the infection rates in rodent reservoirs are often very low (<1%) during the periods between epizootics; thus a large number of animals must be captured to identify only a few positive specimens;
- traps and other equipment required for rodent trapping can be expensive;
- transportation costs for field teams can be expensive;
- personnel require a considerable amount of training to perform the tasks correctly and safely, including choosing, placing and setting the traps; processing captured animals for fleas and other samples; storing and transporting specimens; and recording data while following standardized and safe procedures;
- biosafety is a concern. Given that rodents serve as reservoirs of numerous infections, proper personal protective equipment is required to prevent exposures in the field;
- trained laboratory staff are needed to analyse large numbers of rodent and flea samples;
- trapping large numbers of rodents at relatively frequent intervals (monthly to quarterly) is labour- and resource-intensive and might require more workers, equipment, vehicles and fuel than can be funded on the generally limited budgets available to many plague surveillance programmes, thus making this type of surveillance activity difficult to sustain for long periods. Consequently, rodent trapping should be limited to small spatial areas.

Box A10.3 Collecting fleas from on-host and off-host locations

- **On-host fleas**: Knowledge of the expected host–flea associations during interepizootic periods is required. The identification of *Y. pestis*–infected fleas strongly suggests ongoing epizootic activity.
- **Off-host fleas**: Infected fleas that are competent plague vectors, willing to feed on people and found off their normal hosts represent one of the greatest risks for human exposure to plague. It is important to know how prevalent infected off-host fleas are in homes or other environments where they are likely to come into contact with and bite humans.
- As described in Boxes A10.1 and A10.2, collecting and analysing fleas during investigations of human cases can provide useful information on the sources of human infection, the local flea species that are likely to serve as vectors among rodents and to humans, and those flea species and their rodent hosts that should be targeted for control. Regular evaluation of flea resistance to insecticide, carried out by trained experts, may be useful to guide vector control campaigns.

Box A10.4 Serosurveillance of carnivores or other potential medium- to large-sized mammal hosts

- One of the most powerful techniques for detecting evidence of past plague activity is to collect serum samples from carnivores that consume rodents or are likely to scavenge fresh rodent carcasses. Although some carnivore species (e.g. those belonging to the cat family) often die of *Y. pestis* infection, others apparently suffer little, if any, illness. Dogs typically survive plague infection and develop antibodies that can be detected for as long as 12 months. Dogs are more likely to seroconvert (i.e. develop antibodies to *Y. pestis*) than rodents due to their higher exposure to *Y. pestis* (i.e. through consumption of large numbers of live rodents or rodent carcasses, with the consequently higher exposure to infected rodents). These carnivores could also acquire the disease from the bites of infectious rodent fleas.
- Blood can be obtained from a dog or other medium- to large-sized sentinel mammal species by trained personnel for serological testing.
- The drawbacks of using dogs as serosurveillance hosts include the lack of baseline data for seropositivity in local dog populations, that positivity indicates prior exposure to *Y. pestis* and may not be indicative of current epizootics and, more importantly, the risk of being bitten by a dog that has rabies. Thus, plague programme staff who handle dogs and take blood samples for plague surveillance should follow appropriate safety precautions.

Annex 11. Vector control

Table A11.1 summarizes vector control measures and describes how to use them.

TABLE A11.1. Vector control measures to prevent plague in humans

Measures	Instructions for use
Personal prophylactic	• To avoid flea bites, apply repellents to skin and clothing, including benzyl benzoate, DEET or DMP, or wear insecticide-impregnated clothing. • Wear high shoes and knee socks; if possible socks should be impregnated with insecticide. • Sleep on beds that are at least 0.5 m above the floor. • Use insecticide-treated bed nets. • Clean floors, carpets, mats, mattresses and beddings regularly with detergent.
Insufflation	• Treat rodent burrows and rat runs with 10% DDT or 5% malathion wettable powder.[a] • Insecticidal dust should be blown with a rotary plunger-type duster or cyanogas pump: – into the mouth of rodent burrows, with a patch of dusting powder about 0.5 to 1.0 cm thick and 20 to 25 cm wide left around the mouth of the burrow; – into rat runs (i.e. on floors and into walls up to a height of 1 m).
Residual insecticidal spray	• Products should be used according to the label directions and according to the warnings and precautions necessary for safe and appropriate use. • Spraying may be undertaken annually or more frequently, as per local requirements. • Hand-compression pumps should be used, following the techniques for controlling adult mosquitoes. • WHO prequalified residual insecticidal sprays include:[b] – malathion 25% wettable powder (for outdoor use only); – deltamethrin 2.5% wettable powder; – lambda-cyhalothrin 10% wettable powder.
Non-residual insecticidal spray (contact insecticides)	• Directly spray areas with pyrethroid space sprays. • The following methods limit the risks associated with spraying insecticides inside homes and in the environment: – incorporating systemic insecticides into rat bait; – using Kartman bait boxes, which combine a fast-acting insecticide with a delayed-action rodenticide (i.e. an anticoagulant).
Mechanical means	• Products that function by physical means include liquid sticky traps.

DDT: dichlorodiphenyltrichloroethane; DEET: diethyltoluamide; DMP: dimethyl phthalate.

[a] These products have not been prequalified for these applications by WHO.

[b] These are WHO prequalified products for specific vectors; however, these insecticides are not yet prequalified for plague vectors. See Prequalified vector controls [online database]. Geneva: World Health Organization 2022 (https://extranet.who.int/pqweb/vector-control-products/prequalified-product-list, accessed 14 April 2021). Alternative measures for treating home-dwelling rodents include fipronil, a compound not used for indoor residual spraying but that should control rodent-associated fleas without compromising resistance to the insecticides used in public health emergencies.

Annex 12. Rodent control as a plague prevention strategy

Lethal trapping may lead to the release of a massive number of potentially infected fleas that may seek new hosts, including humans. Therefore, lethal trapping should not be conducted during active plague epizootics unless rodents are first treated with insecticide to rid them of fleas (Table A12.1).

TABLE A12.1. Strategies for controlling rodents to prevent plague in humans

Measures for rodent control	Considerations
Environmental sanitation	
In houses	• Grains and other foods should be stored in rat-proof containers, such as glass or earthenware jars, rat-proof bags or metal cans or bins with lids. Consideration should be given to ensuring adequate air flow to prevent aflatoxin exposure. • Water storage containers should be covered to prevent rodent access and contamination by their urine or faeces, and leaking taps should be repaired. • Food waste must not be left where rats can get it. Tables and floors should be swept to remove leftover food. Kitchen refuse should be stored in rat-proof containers with tight-fitting lids. Proper garbage disposal services should be organized. • Remove potential sources of food within the building's premises, for example, seeds kept for birds or ripe fruit that has fallen to the ground. Ensure that food for domestic animals is not accessible to rodents. • Houses should be searched for holes in the walls and floors. All openings should be sealed with rat-proof material (e.g. mortar, concrete, metal sheeting, wire mesh or other materials). Special attention should be paid to spaces under doors and where pipes pass through walls, to windows and other openings, ventilation grills and gaps between the tops of walls and the eaves. Metal guards can be placed along overhead cables and external pipes to prevent rodents gaining access to open windows and under the eaves. • If the exterior wall has a rough surface, a smooth band of paint 15 cm wide can be applied below windows but preferably more than 1 m above ground to prevent rats from climbing up the walls. • Promote the use of rodent-proof housing design and building construction. Thatched roofs should be replaced with rodent-proof material (e.g. sheet metal).
Around houses	• Prevent the accumulation of garbage and food waste. • All premises, including yards and vacant plots, should be kept clean and free of junk and other debris. • All plant growth likely to harbour rats or conceal their activities should be cut down. • Tree branches growing close to the house should be cut back to prevent easy access to roofs. • Ensure that food for humans and domestic animals is not accessible to rodents.

In the community	• Solid waste should be collected and disposed of properly, and as far as possible from the household. Particular attention should be given to piles of industrial refuse, including damaged packing cases and building materials, as they attract rats for nesting and provide safe spaces for them to move around. • It is essential to completely seal drains and sewage systems or other sanitation systems. Rat-proof covers should be placed over access points. The ends of ventilator shafts and disused drains should be sealed at the points of entry into the main sewer. Other underground structures, such as drains for surface water and conduits for electrical cables, should also be rat-proofed as fully as possible. • Buildings where food is stored, such as warehouses, should be made rat-proof. • Favour the presence of rodent predators (e.g. cats and owls).
Physical methods	
Trap barrier system	• This eco-friendly system is implemented by erecting fencing around trap crops sown before the main crop. The trap crop attracts rodents from the surrounding areas, and these rodents are trapped in large numbers before they attack the later regular crop. This is known to work well in systems irrigated for rice, but evidence is lacking on its efficacy for other cropping systems.
Trapping, community hunting and bounty campaigns	• There are many kinds of traps. Kill traps should not be used in areas at-risk of plague. The efficacy of live-capture traps depends on the rodent species, the type of trap and bait used, and where trapping occurs, so it is important to assess the options. If done well, intensive trapping can be a highly effective means of reducing rat numbers, particularly when it is coordinated over a large area. However, the effects are short-lived, with numbers rebounding to pre-trap abundance shortly after trapping ceases. • Community hunting campaigns that target areas of high rodent density, such as roadsides or river or pond banks, can also be effective, particularly if the hunting is timed to occur before rodent populations expand, but it involves risks to community participants (i.e. exposure to rodent-borne zoonoses). • Coordinated community-based action is essential because individual ad hoc action to kill rodents (i.e. with traps or poisons) will not be effective at the population level. • Importantly, during plague epizootics, lethal rodent control should not be implemented without flea vector control, especially in areas where fleas leaving dead rats to seek a new host have elevated chances of biting humans, for example, in indoor and peridomestic areas, or collective rice threshing areas. • If a community-based trapping campaign is implemented, residents should be well informed about procedures for safely handling live rodents as well as rodent carcasses because some rats may die inside the traps.

Chemical methods

First-generation anticoagulant rodenticides

- First-generation anticoagulant rodenticides are slow acting, and rodents must eat several poison-containing meals over a few days before being sufficiently affected by the poison. These poisons interfere with blood clotting, which is considered an advantage as it overcomes rodent neophobia: rodents do not feel immediately ill and continue to eat the poison until they have eaten a lethal dose.
- Anticoagulants are the main rodent control method and are widely used. The table below shows four commonly used first-generation compounds and the recommended doses for mice and rats.

Compound [a]	Dose in parts per million [b]		
	House mouse	Roof rat	Norway rat
Warfarin	250–500	250–500	50–250
Diphacinone	125–250	50–100	50–100
Coumatetralyl	250–500	250–500	250
Pindone	250–500	250–500	250

[a] These compounds may be used in dry or liquid bait.

[b] The dilution factors are for:
500 parts per million (ppm) (0.05%) = 1 part 0.5% concentrate to 9 parts bait;
250 ppm (0.025%) = 1 part 0.5% concentrate to 19 parts of bait;
100 ppm (0.01%) = 1 part 0.5% concentrate to 49 parts bait;
50 ppm (0.005%) = 1 part 0.5% concentrate to 99 parts bait

- Many ready-to-use preparations of anticoagulants are available.
- Children are particularly at risk of accidental exposure to anticoagulants, and the antidote is based on vitamin K.
- Bait can be prepared with locally available materials. The simplest preparation is a dry medium-grind or crushed cereal to which the concentrate is added. The addition of vegetable oil may make the bait more attractive for a time, but only until the oil turns rancid. Sugar at a concentration of about 5% is also a useful additive.
- Permanent bait stations may be constructed near garbage disposal areas or other places where rodent activity is noticed. In such instances, special attention should be paid to trapping schedules to counteract rodents' learning about the traps.

Second-generation anticoagulant rodenticides

- These poisons also work by preventing blood clotting but are usually effective after only a single feeding. There are often restrictions on using them outdoors as the active molecules may accumulate in the environment, potentially negatively impacting many non-target species. These poisons should be used only indoors and placed securely in bait box stations by qualified pest control officers. Local health officers should be made aware of the use of such anticoagulants, and vitamin K should be available in case of human poisoning.
- Examples of second-generation compounds are brodifacoum, bromadiolone, difenacoum and difethialone, which come in many ready-to-use baits, normally at doses of 0.005%.

	• In some situations, anticoagulants are formulated as a liquid or powder, but considerable expertise and safety are required to use such formulations. • The intensive use of anticoagulant poisons has led to the development of resistant rodent populations in some regions. Thus, care should be taken to avoid long-term baiting with these or to plan for an adequate alternative rodent control method to limit selective pressure towards anticoagulant resistance at the population level.
Acute poisons	• This is a large and varied group of poisons that are often highly toxic and have rapid action, with death occurring within 30 minutes. Acute poisons make animals feel ill almost immediately, which often results in the cessation of feeding: if many individuals fail to eat a lethal dose, they may then recover from the poison and avoid the poison in the future. • Acute poisons are general toxins that often do not have an antidote. They are extremely hazardous to humans and domestic animals. Products containing these acute toxic chemicals should be avoided whenever alternative approaches or molecules exist, and their directions for use should always be strictly followed, taking into consideration all warnings and precautions. Usually, they are used only by experts and in restricted circumstances, as detailed by national and international legislation and guidelines. • To overcome neophobia towards acute poisons and to encourage rapid feeding, it is often necessary to pre-bait for several days with food that is not poisoned (known as pre-baiting exposure) so that the animals become accustomed to feeding at the same sites on the same foodstuff. The bait must be highly attractive, and efforts should be made to limit rodents' access to other food sources. • Acute poisons are chosen when speedy action is desired to kill a small portion of the population, and their use must be followed up with other methods (e.g. anticoagulant rodenticides or trapping) to reduce the population substantially. • Often there are many locally available, illegal acute rodenticides made using compounds that are not licensed for rodent control. These poisons are usually extremely dangerous due either to their molecular content or their dose, and they should not be used. It is recommended that the only products used are those that are authorized or licensed as rodenticides and have their active molecules clearly identified on the product labelling. • Examples of common licensed acute poisons are zinc phosphide and cholecalciferol. – **Zinc phosphide** has been widely used for several decades to control rodents. It is a fast-acting, fine greyish-black powder with a garlic-like odour and strong taste. It is fairly stable in air, but reacts with water to release phosphine gas. It is toxic to all animals including humans. – **Cholecalciferol** is sometimes called a subacute poison because death is not immediate. The poison works by increasing calcium levels in the body, resulting in kidney failure, cardiovascular abnormalities and tissue mineralization. It is often used in areas where rodent populations have developed physiological resistance to anticoagulant rodenticides.

Other chemical methods	• **Cellulose** rodenticides are registered for use in some countries. They are often made from powdered corncobs and are marketed as being more "green" than anticoagulant or neurotoxic rodenticides. They have less risk of causing toxicity in non-target species, such as pets, livestock and children, and they are biodegradable. Cellulose rodenticides require exclusive feeding for 3 to 7 days to cause toxicity. The cellulose absorbs water and causes death in rodents from hypovolaemic shock. There is limited evidence that cellulose rodenticides are effective where rodents have access to many alternative food sources. Thus, some caution is advised as conditions in plague areas will often mean that rodents can forage for other food sources beyond the cellulose bait. • **Anti-fertility compounds** are under development by several research groups, and it is likely that new products will enter the commercial market soon. Currently, there is a commercial product available in the United States that has been found to be effective under some local conditions. It is not yet widely available. Such products could play an important role in limiting rodent density in plague-endemic areas. • **Fumigants, such as aluminium phosphide**, usually target rodent burrows. A toxic gas is pumped or released into a burrow system. Fumigants must be deployed by professionals trained to use the specialized equipment necessary to apply the fumigant (i.e. the gas) and who have knowledge of the risks associated with the use of such products and the necessary risk-mitigation measures. All entrances and exits to the burrow system must be identified and blocked to avoid the gas escaping. Operators are at risk of poisoning and must be provided with full personal protective equipment, and humans and domestic animals must be excluded from the treatment area. After fumigation, burrows should be inspected during the next few days to identify any that have been reopened, which indicates rodents are still active. One or two tablets of aluminium phosphide should be inserted into each reopened or new hole at a depth of 25 to 30 cm using a long-handled wooden spoon or aluminium pipe, and the burrow should then be sealed. In sandy soil, 1 L of water should be poured into each burrow before placing a tablet. The process should be repeated the next day to determine whether any burrows have been reopened, and it must continue until the area is cleared of rats.

Biological methods	
	• Pathogenic bacteria, viruses, protozoans, helminths and nematodes have the potential to act as biocontrols. However, not many data are available about their use. Commercial products containing *Salmonella* have been banned in most countries and should not be used even if they are available due to the risks they pose to the health of humans and domestic animals. • Repellents, both natural and synthetic, are available in some areas. The presence of predators – such as cats, dogs, wild carnivores, birds of prey – can have repellent effects on rodents. Such animals are unlikely to have much impact when rodent densities are high, but there is some evidence of positive impact in limited situations. Repellents derived from cat urine or the faeces and fur of predators can have a temporary impact on rodents. Some plant species are also known to have physical or chemical repellent or rodenticidal properties, but generally these are not commercially available. There is no evidence that ultrasonic emitters, which produce sounds that are unpleasant to rodents and that humans cannot hear, are effective. Such ultrasonic devices should be considered a gimmick and should not be used.

Annex 13. Declaration of interests

Name	Region	Country	Institution	Declaration of interests	Participation restriction
Belmain Steve	EURO	OK	Natural Resources institute, University of Greewich	Yes, Government research council award on rodent control in plague endemic areas of Madagascar and Tanzania	No
Cabanillas Angulo Jose	AMRO	Peru	Centro Nacional de Epidemiología, Prevención y Control de Enfermedades MINSA	No	No
Dixit Devika	AMRO	Canada	Department of Pediatrics, Division of infectious Diseases, University of Calgary	No	No
Dobigny Gauthier	EURO	France	Institut de Recherche pour le Développement and Centre de Biologie et Gestion des Populations	No	No
Dubyanskiy Vladimir	EURO	Russian Federation	Stavropol Plague Control Research Institute	No	No
Eisen Rebecca	AMRO	USA	United States Centers for Disease Control and Prevention	No	No
Girod Romain	AFRO	Madagascat	Pasteur Institute	No	No
Heim Katrin Moira	EURO	Germany	Department of Infectious Diseases and Respiratory Medicine, Charité-Universitätsmedizin Berlin	No	No
Le Guern Anne Sophie	EURO	France	Pasteur Institute	No	No
Lucey Daniel	AMRO	USA	Georgetown University Medical Center	No	No
Mead Paul	AMRO	USA	United States Centers for Disease Control and Prevention	No	No
Nelson Christina	AMRO	USA	United States Centers for Disease Control and Prevention	No	No
Petersen Jeannine	AMRO	USA	United States Centers for Disease Control and Prevention	No	No
Pizarro-Cerda Javier	EURO	France	Pasteur Institute	No	No

Name	Region	Country	Institution	Declaration of interests	Participation restriction
Raberahona Mihaja	AFRO	Madagascar	University Hospital Joseph Raseta Befelatanana	Yes, trial comparing ciprofloxacine to ciprofloxacine + aminoglycoside in the treatment of plague. Funded by the Wellcome Trust and the department of international development UK	No
Rajerison Minoarisoa	AFRO	Madagascar	Pasteur Institute	No	No
Renaud Bertrand	EURO	France	aculté de Médecine, Université Paris Descartes	No	No
Rossouw Jennifer	AFRO	South Africa	National Institute for Communicable Diseases	No	No
Shako Lomami Jean Christophe	AFRO	Democratic Republic of the Congo	Ministry of health	No	No
Terriquez Joel	AMRO	USA	Northern Arizona University	No	No
Wang Xin	WPRO	China	National Institute for Communicable Disease Control and Prevention	No	No

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