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# WHO EXPERT COMMITTEE ON PLAGUE

**Fourth Report**

WORLD HEALTH ORGANIZATION

GENEVA

1970

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**CORRIGENDUM**

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## WHO EXPERT COMMITTEE ON PLAGUE

Geneva, 21-29 October 1969

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# WHO EXPERT COMMITTEE ON PLAGUE

## Fourth Report

The WHO Expert Committee on Plague met in Geneva from 21 to 29 October 1969. The meeting was opened by Dr P. Dorolle, Deputy Director-General, who welcomed the members on behalf of the Director-General. He extended a particular welcome to Dr K. F. Meyer, a member of the two first meetings and chairman of the third, who was attending in an honorary capacity.

In his introductory remarks, Dr Dorolle referred to the previous reports of the WHO Expert Committee on Plague<sup>1</sup> and to other WHO publications in the field of plague, in particular the monumental monograph prepared by the late Dr R. Pollitzer.<sup>2</sup> He also emphasized the current importance of investigation, control, and research in view of the existence of natural foci of plague in many parts of the world.

### 1. INTRODUCTION

Although there is no longer a danger of extensive pandemics or large epidemics of plague except in the event of war or other calamities, the Committee emphasized that the permanent character of wild rodent plague in numerous natural foci in various parts of the world still required the constant attention of the health authorities of the countries concerned and, in view of the international aspects of this infection, of WHO.

### 2. DISTRIBUTION OF PLAGUE IN THE WORLD

The Committee discussed the main principles and methods of drawing up maps showing the distribution and characteristics of natural foci in various parts of the world. The need for such maps for plague control is emphasized.

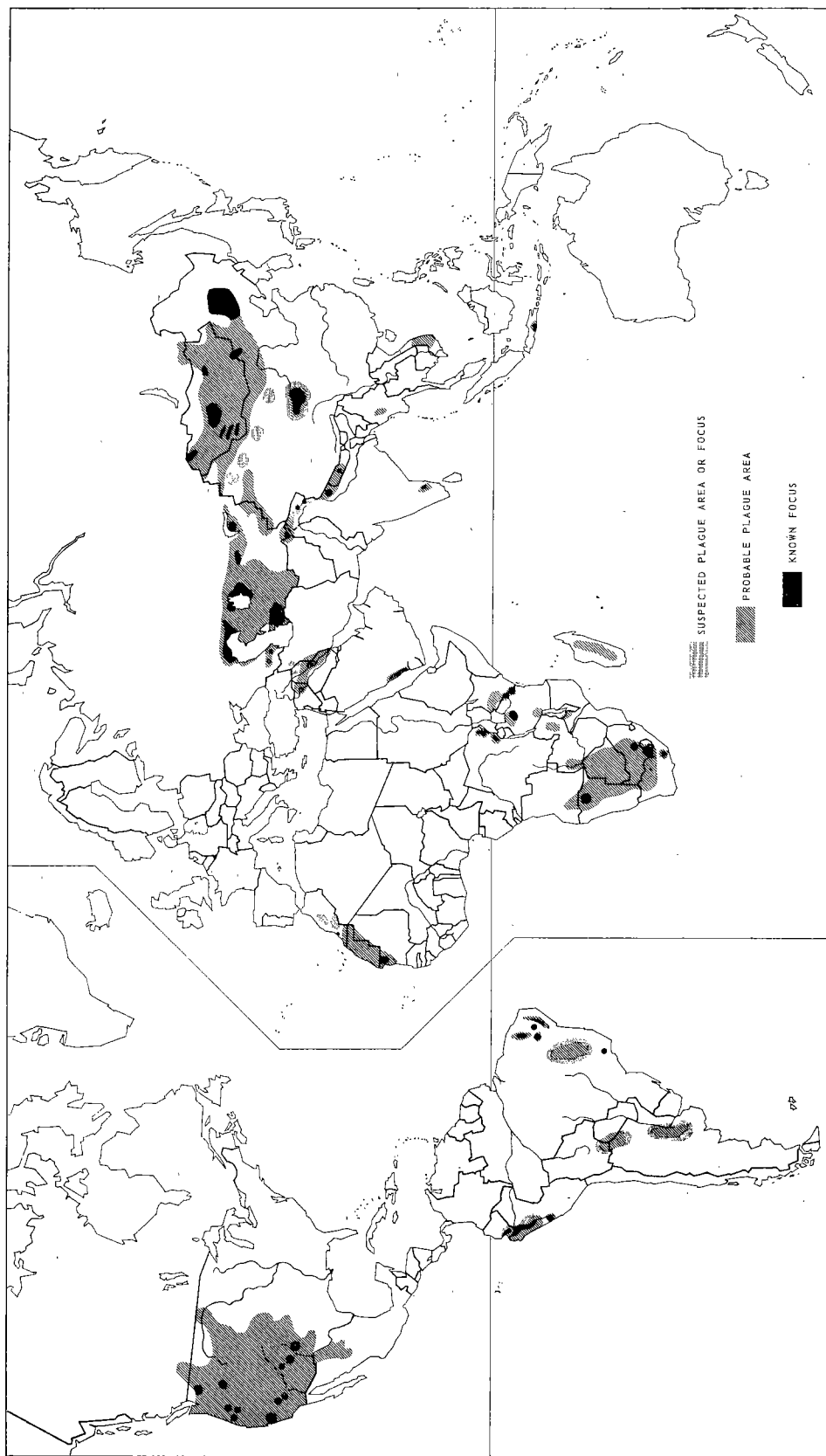
Since the transmission of plague by sea is unlikely to result in massive worldwide invasion and the creation of new natural foci, it is considered

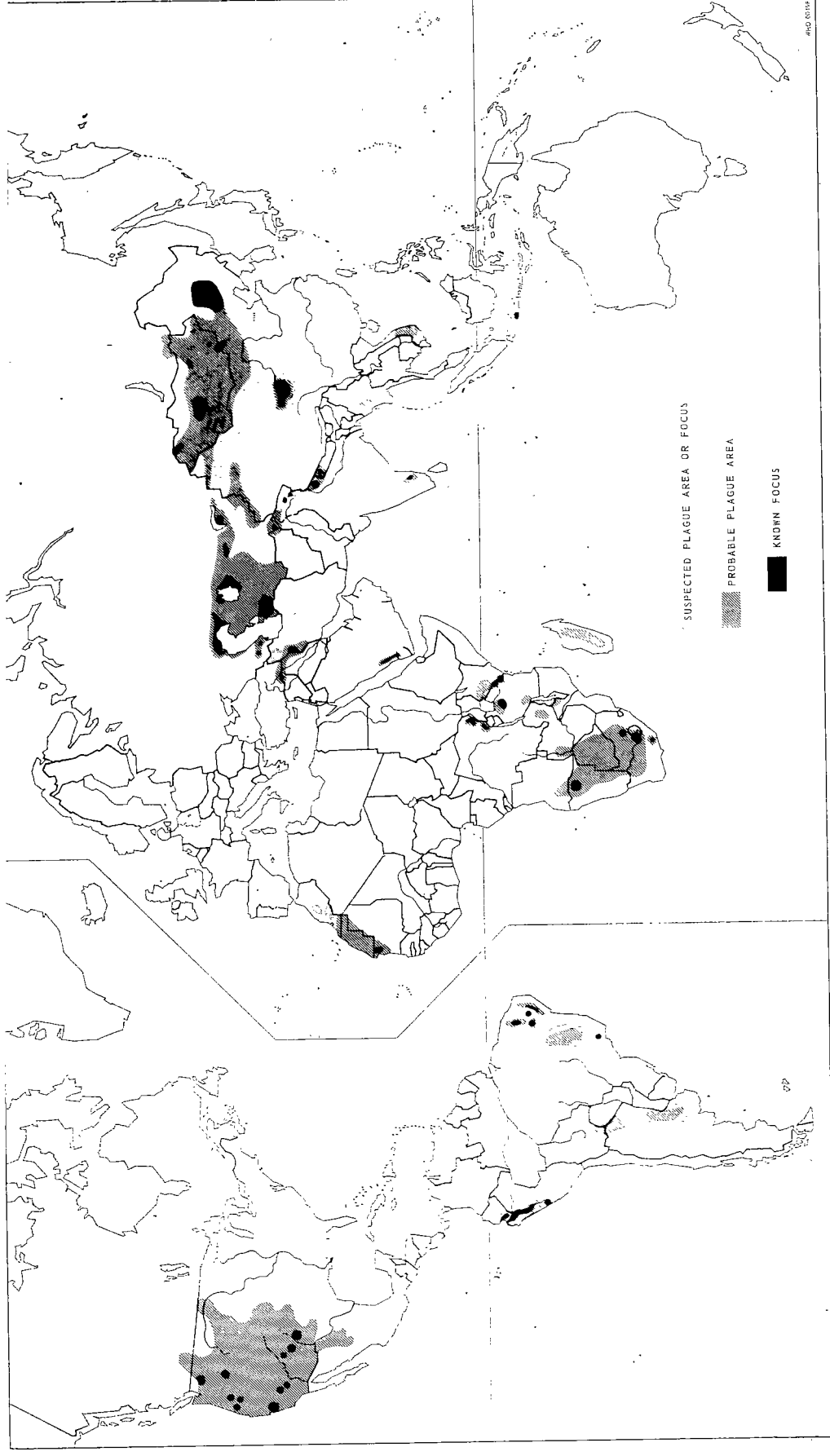
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<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1950, No. 11; 1953, No. 74; 1959, No. 165.

<sup>2</sup> Pollitzer, R. (1954) *Plague*, Geneva (*World Health Organization: Monograph Series*, No. 22).

KNOWN AND PROBABLE FOCI AND AREAS OF PLAGUE, 1969







advisable to replace the distribution maps that show past temporary manifestations by a map indicating only those territories where plague is known or seems to be entrenched and areas to which it might spread. Such a map has been prepared by the Committee and is reproduced opposite. It is emphasized that this map presents current information only, and is likely to require frequent revision as new data become available.

It would be helpful if each country concerned could prepare detailed, large-scale maps of individual affected areas. These maps should provide information on landscape, vectors, mammalian reservoirs, and the status of local vector susceptibility to insecticides. The Committee feels that these data are vital as the basis for any effective plague control programme, whether emergency or long-term.

### 3. THE CONCEPT OF NATURAL FOCI

#### Terminology

In the past, descriptions of plague foci were based upon the character of the rodent reservoir : sylvatic, urban, commensal, peridomestic, wild rodent, etc. This terminology seems ambiguous and misleading, and the Committee recommends that the foci be described in future in terms of the situation as it affects man, i.e., wild or domestic plague. Wild plague is defined as plague existing in nature, independent of human populations and their activities. Domestic plague is defined as plague that is intimately associated with man and rodents living with man, and has a definite potential for producing epidemics.

The term "natural" focus should be adopted in place of the terms "permanent" or "inveterate" focus. A natural focus is defined as the area, strictly delimited, where ecological conditions ensure the persistence of the etiological agent for considerable periods of time, and where epizootics and periods of quiescence alternate without the introduction of infection from outside.

Temporary plague areas are susceptible territories that are occasionally seeded from natural foci. Such invasions are of limited duration.

#### Aspects of the epizootic process

The epizootic process in each natural plague focus has its specific cyclic and periodic pattern. Diverse biotic and abiotic factors condition the ecology of local rodent reservoirs and flea vectors and cause distinct seasonal appearances of epizootic plague, with characteristic peaks of activity reflecting favourable local conditions.

This phenomenon has been observed in all natural plague foci studied to date, but there is insufficient evidence to link it to any single factor.

Both periodicity of plague epizootics and the duration of the quiescent phase are variable.

#### **Present possibilities of invasion or expansion**

There is nothing in the evidence available to the Committee to indicate that new natural foci have been created in the recent past. On the other hand, there is also no definite evidence that known natural foci have spontaneously become inactive or smaller in size, except for a few foci where extensive antiplague measures have been employed.

In the meantime, rapid population growth and new development schemes are likely to bring more human beings into close contact with natural foci and create fresh problems in plague control.

Although the rat-proofing of transoceanic liners has almost eliminated the risk of infected rats and fleas being transported to distant seaports, the new technique of freighting in containers presents a definite threat.<sup>1</sup> Under this system, cargo packed in infected areas cannot be inspected or treated *en route*, and there may be no facilities for inspection and treatment at its destination. If plague-infected rodents and fleas are present in the containers, they may well survive and cause human or rodent plague. The transport of such containers by air may present a particular hazard. With the growing use of rapid passenger aircraft, moreover, there is an ever-increasing possibility that travellers in the incubation phase of plague will disembark in a hitherto plague-free area.

#### **4. CONSERVATION OF PLAGUE IN NATURAL FOCI**

Since the long persistence of plague in some foci may not be achieved through the usual chain of transmission, the Committee examined other mechanisms that may be responsible for the carry-over of plague infection in natural foci.

##### **The role of rodents**

###### *Hibernating rodents*

There is experimental evidence of latent infection in hibernating animals. Although this mechanism of carry-over has not been demonstrated under natural conditions, infection has been detected in animals soon after they

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<sup>1</sup> See: WHO Committee on International Quarantine (1969) *Fifteenth Report (Off. Rec. Wld Hlth Org., 176, 129)*.

awaken from hibernation. Presumably this mechanism of carry-over can be attained only where hibernating (or aestivo-hibernating) animals are present in a natural focus.

#### *Non-hibernating rodents*

Latent infection is known to exist in nature among certain rodents, and it is reported that this can also be reproduced under experimental conditions. On occasions, such animals may relapse and become bacteraemic, and so initiate an epizootic. It is felt that this mechanism needs further confirmation.

It has repeatedly been demonstrated that some moderately resistant species of rodent may develop a generalized non-fatal infection, with subsequent localization of the plague bacillus<sup>1</sup> in some organ. Such an animal harbouring virulent plague bacillus may become a source of infection if eaten by another susceptible rodent, and could start an epizootic.

#### **Role of ectoparasites**

It has repeatedly been shown that infected fleas can survive for long periods in burrows under optimum conditions of microclimate, etc. The life-span of infected fleas is variable, but there is abundant evidence to suggest that they live long enough to maintain the natural focus. Infected fleas may live for at least one year, and certain species may survive in the burrow microclimate for as long as four years. Where local conditions favour a long life-span for fleas, this is considered to be the most likely mechanism of carry-over.

#### **Variation in the susceptibility of rodents**

During epizootics some rodents survive the infection. The mechanism of survival is inadequately understood, but there is evidence that ecological conditions, immunity, and genetic factors are involved. Such rodents serve as hosts for infected fleas, and thus assist carry-over.

#### **Variations in the pathogenicity of the plague bacillus**

Plague bacilli deficient in certain virulence factors have been isolated in both natural and temporary plague foci. These organisms can be transmitted from rodent to rodent by fleas. Reversion of these strains to high

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<sup>1</sup> The International Committee on Bacteriological Nomenclature has decided that the plague bacillus, long considered to belong to the genus *Pasteurella*, should be placed in the genus *Yersinia*, but this change has not yet been published in the International Code of Nomenclature. To avoid confusion, the term "plague bacillus" is used in this report.

pathogenicity has not so far been observed, but the available evidence suggests that this is another mechanism of the conservation of the plague bacillus in nature.

### **Silent periods**

Silences of long duration (10 years or more) followed by sudden explosions of rodent or human plague have been repeatedly confirmed in some natural foci. Possible explanations of the reappearance of plague in the same territory after long periods of quiescence have been examined.

### *Itinerant or wandering plague*

Plague epizootics, by virtue of their explosive nature and the involvement of large rodent populations, are readily detectable. However, the infection may enter a quiescent and hardly detectable phase in territories where its circulation is reduced. Under such circumstances, plague may continue undetected until it comes into contact with new and dense susceptible rodent populations.

### *Reimportation of plague*

It has been claimed that in certain foci plague disappears completely for long periods, and that its reappearance is due to the introduction from other areas of infected rodents or even fleas by migrating birds or other means.

### *Conservation in the soil of burrows*

In a series of detailed studies, it has been shown that the plague bacillus can survive, and indeed multiply, in the soil layers of rodent burrows where microclimatic and other conditions are suitable. The bacillus has even been isolated from the soil layer of burrows where the rodents had died of plague at least 11 months previously. It has also been demonstrated that healthy rodents reoccupying and excavating such burrows may become infected through contact with the contaminated soil layers. Since the conditions required for the conservation of the plague bacillus in the soil of burrows are not fully understood and may not be present in all natural foci, further studies are needed to define the exact mechanisms of conservation in known natural foci.

## 5. EPIDEMIOLOGICAL INVESTIGATIONS

### Serological surveys

The passive haemagglutination test is the only serological method that is widely used at present in epidemiological studies of plague. In regions where plague infection is widespread, this quick and inexpensive test may provide highly valuable epidemiological data. It is especially useful for detecting plague foci in rodent populations.

A routine programme of testing commensal rodent sera for plague antibody should be incorporated into any epidemiological survey, as such tests permit a more rapid and complete evaluation of the epidemiology of plague in the area under study. The serological tests of commensal rodent sera are also highly valuable in assessing the success of a plague control programme. Effective control of rodent plague should be reflected by the prompt disappearance of plague haemagglutinating antibody from the sera of the rodent population.

Further study on the standardization of serological tests for use in plague surveys is definitely needed. Impure Fraction 1 antigen, for example, may be the cause of non-specific reactions. It is recommended that the haemagglutination test be used for reconnaissance only in quiescent plague areas or in areas with an unknown past history. In such areas, it is imperative to confirm serological evidence by actual isolation of the plague bacillus.

Further observations are needed so that criteria for interpretation of the titres found in various animals can be established. This will make the test even more useful in plague investigations.

Since the passive haemagglutination test is extremely delicate, the procedure is described in detail in an annex (see p. 23). This will enable workers in different countries to use the same procedures and so obtain comparable results.

### Bacteriological surveys

Bacteriological confirmation is regarded as essential for determining the presence and distribution of plague infection. While bearing in mind that an occasional variant of the plague bacillus may occur, the Committee puts forward the following criteria for the identification of the bacillus.

The plague bacillus is a Gram-negative non-motile bacterium. There is marked bipolar staining in preparations from tissues, cultures, etc. when stained with Giemsa, thionine, or Wayson's stain. The organism is a facultative anaerobe. Spores are not produced. It grows well on ordinary laboratory media. It ferments glucose, but not sucrose, lactose, or rhamnose (within 24 hours). It is aesculin-positive and urease-negative. The organism

is sensitive to specific antiplague bacteriophage at 22°C and is pathogenic for laboratory animals (white rats, mice, and guinea-pigs) by any route in moderate doses.

## 6. PATHOGENESIS OF PLAGUE

### Microbiology of the plague bacillus and related organisms

During the past few years much information has come to light concerning variations in strains of plague bacilli. These variations include biochemical reactions, antigenic structure, virulence, and newly described virulence factors. Susceptibility to lysis with conventional antiplague bacteriophage, variations in pathogenicity, and "transformations" of the organisms have also been described. In view of new concepts of the variability of the plague bacillus, based on scattered findings in the field and in the laboratory, and since no reference strain has yet been designated, the Committee stressed the need for investigators to exchange as many strains as possible of plague bacillus isolates from all natural foci and all other sources. An international reference centre is needed to provide facilities for the free international exchange of plague strains. The Committee emphasized that studies of the characteristics of the plague bacillus are delicate, and standardization of techniques among the investigators is therefore essential. It recommends :

- (1) the establishment of standard worldwide methods for biochemical and antigenic study ;
- (2) the standardization of tests for pathogenicity ;
- (3) the study of freshly isolated strains from different animal sources in as many geographical areas as possible ;
- (4) the establishment of a definition of the plague bacillus ;
- (5) the conservation, for the use of investigators, of reference strains for studies on the cultural, biochemical, antigenic, and pathogenic variants of the plague bacillus ;
- (6) rejection of the denomination of the different varieties of plague bacillus according to the animal origin of strains. The Committee requests the WHO Secretariat to transmit this recommendation to the *Pasteurella*, *Yersinia* and *Francisella* Subcommittee of the International Committee on Bacteriological Nomenclature.

### Host-parasite relationship

Direct and indirect evidence indicates that the virulence of the plague bacillus is not determined by its ability to survive and grow within phago-

cytes, particularly within fixed macrophages of the reticuloendothelial system.

Recent studies suggest that the four plague bacillus determinants, VW<sup>+</sup>, F1<sup>+</sup>, P<sup>+</sup>, and PCF<sup>+</sup> (Pg<sup>+</sup>)—which determine the production of VW antigens, Fraction 1 antigen, pigmentation on haemin agar, and pesticin-coagulase-fibrinolytic activities respectively—are related either to infectivity or to virulence. It appears that the ability of the plague bacillus to establish an infection in animals inoculated with small numbers of bacteria is dependent on VW<sup>+</sup>. Pathogenicity, however, seems in most cases to depend on the other three determinants, F1<sup>+</sup>, P<sup>+</sup>, and PCF<sup>+</sup> (Pg<sup>+</sup>). It is also known that the pathogenicity varies not only with the strains of the plague bacillus but with the animal species used to test for pathogenicity. Variations may even occur between different populations within each animal species.

It is accepted that definite selection for certain genetic factors of the plague bacillus may occur both in warm-blooded animals and in fleas. Multiplication of the plague bacillus in the flea seems to cause selection for one factor, while multiplication in the vertebrate host may cause selection for another. Continued studies along these lines may provide an explanation of one of the mechanisms responsible for the peaks and declines of epizootic intensity.

## 7. SURVEILLANCE OF PLAGUE

In areas where natural plague foci exist, or where there is a history of past infection, surveillance is indispensable. Modern trends in public health emphasize the integration and co-ordination of all aspects of communicable diseases, including plague. It is essential that specially trained personnel be available, within the public health services of each country concerned, to carry out emergency control of plague episodes.

While many natural plague foci in temperate climates have been adequately studied, there are scarcely any data at all for tropical areas. Since the results of such studies provide a sound basis for plague control programmes, there is a particular need for long-term ecological studies in crucial areas, and the Committee urges the initiation of surveillance programmes utilizing proven methodology. Advice and assistance should be given, whenever possible, to countries that express interest in surveillance.

The Committee considers that all countries where plague is endemic or has occurred in recent times would benefit from participation in the WHO programme for the detection of resistance to insecticides and rodenticides.

## 8. CONTROL OF RODENT PLAGUE

Programmes for the control of rodent plague may be classified according to the purpose for which they are undertaken. They should be concerned with both wild and domestic plague. The main tools for the control of rodent plague are insecticides, rodenticides, and fumigants.

Control programmes undertaken to suppress plague in potentially hazardous natural foci should be planned on a long-term basis. They should be initiated only after a thorough study of the area involved and when the local ecological and epidemiological features are fully understood. Control measures need to be adjusted to the particular conditions found in each focus.

Measures may be aimed at the suppression or even elimination of plague in certain well-studied, well-defined, isolated natural foci. These long-term projects, although costly, have been successful in some countries. One of the main approaches in such programmes has been the transformation of entire landscapes by the introduction of intensive and extensive agriculture, etc., thus profoundly changing the ecology of uncultivated territories. The initial stages of cultivation, however, may result in an activation of natural foci.

## 9. PREVENTION AND CONTROL OF HUMAN PLAGUE

Isolated or sporadic cases of flea-borne bubonic plague are the signals of the possible onset of epidemics. In some outbreaks of bubonic plague, secondary cases of plague pneumonia are seen. These are the index cases that may lead to explosive epidemics of primary pneumonic plague. Control varies according to the clinical form of the disease.

### **Bubonic plague**

An emergency programme for the control of bubonic plague in populated areas requires the rapid suppression of epizootic outbreaks that are a direct threat to man. Both insecticides and rodenticides are used as local conditions require. In all situations, it is essential that rodent poisoning be preceded or accompanied by flea control.

### **Pneumonic plague**

Epidemics of pneumonic plague are brought under control by prompt isolation and on-the-spot treatment. Quarantine and observation of all known contacts is essential. In cases where infection may be due to the



hunting or handling of certain rodents, a temporary ban must be placed on hunting and skinning practices.

### **Disinsectization of human dwellings**

Flea control measures are still considered to be the most effective means of control if instituted early during outbreaks of flea-borne plague. DDT has been recommended and effectively used for this purpose.

Instances of DDT resistance in fleas, particularly *Xenopsylla cheopis* and *Pulex irritans*, have been encountered in several areas in the past few years. This resistance has been confirmed by extensive testing of wild-caught or colonized fleas collected in these foci, using the standard technique recommended by the WHO Expert Committee on Insecticides.<sup>1</sup> In these foci, therefore, transmission could not be controlled by the use of DDT, but the expected prompt control was achieved with alternative insecticides.

The Committee recommends that fleas in potential plague areas be tested regularly for insecticide resistance, using the easy and reliable technique mentioned above, so that effective insecticides for use in anti-plague measures can be selected.

### **Health education**

Health education is an essential part of any plague control programme. Properly related to local circumstances, it is highly effective in securing the understanding and assistance of local populations when carrying out necessary and occasionally unpopular measures. Education should aim at providing the public with the facts about plague and at enlisting their co-operation. It must include details of the role of rodents, fleas, and human beings in the maintenance and spread of the infection. Emphasis must be put on the need for prompt reporting of dead rodents and suspected human cases, so that preventive measures can be taken. Enlightened programmes of health education can contribute substantially to the success of control measures.

The need for awareness among medical practitioners is also stressed. Wherever plague is endemic or has occurred in recent times, the disease should be kept in mind in the differential diagnosis of any case of fever with lymphadenopathy, or when multiple cases of pneumonia occur.

### **Chemoprophylaxis**

Chemoprophylaxis is recommended for persons in contact with plague and for individuals contaminated in laboratory accidents. In selected

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<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 443, Annex 7.

populations it may also be used as a short-term emergency measure in small explosive outbreaks until other measures can be instituted. It is suggested that tetracycline be used whenever possible, or, failing this, sulfonamides.

### **Vaccination**

Both live and inactivated vaccines are available, but their effectiveness is not well established. Field observations indicate that the use of vaccines may reduce both morbidity and mortality to a certain extent in bubonic plague, but not in pneumonic plague. The immunity conferred is of short duration, and for practical purposes should not be considered to last longer than 6 months. Revaccination is needed to maintain immunity. Mass vaccination cannot at present be recommended as a general plague control measure, but under highly endemic conditions or for high risk groups vaccination may be applied for individual protection.

Since live vaccines are difficult to prepare and distribute in large quantities and may provoke undesirable reactions, inactivated vaccines may be more practicable and more acceptable to the population. Under all circumstances, vaccination should be used only for the prevention, not the control, of human plague.

There are wide variations throughout the world in the preparation and testing of plague vaccines. On the basis of available experience, minimum requirements for inactivated vaccine could be laid down and an international reference preparation of plague vaccine could be established. WHO has agreed to draft the minimum requirements for inactivated plague vaccine and to submit this draft to the WHO Expert Committee on Biological Standardization.

As regards the live vaccine, no stable reference vaccine can at present be made available. The Committee recommends that countries desiring to use live plague vaccine should produce it from the original EV strain,<sup>1</sup> strictly preserved. This strain is considered to be the most suitable for the preparation of live vaccine, provided it is maintained under the original conditions of preservation.

## **10. TREATMENT OF HUMAN PLAGUE**

Early treatment is essential, and should be started without waiting for confirmation of the diagnosis. When specific therapy is instituted within 15 hours of overt illness in pneumonic plague, antimicrobial therapy usually has a favourable effect on the outcome of the disease.

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<sup>1</sup> This strain is available at the Pasteur Institute, Paris.

**Sulfonamides**

Bubonic plague can be cured by the administration of sulfonamides alone. In view of the known complications and untoward reactions that may occur, however, the Committee feels that sulfa drugs should be used for the treatment of plague only when effective and safe antibiotics are not available.

Sulfonamides are not recommended for the treatment of pneumonic plague.

**Antibiotics**

The Committee stresses the total inefficacy of penicillin in the treatment of plague. Streptomycin is very effective, but severe intoxication can occur because of its highly bactericidal effect and the massive destruction of the plague organism in advanced cases. Streptomycin should not therefore be administered in excessive doses, but should be combined with other antibiotics. Tetracycline is the preferred antibiotic for both bubonic and pneumonic plague.

**Combined treatment**

Several antibiotics have been successfully used in combination with sulfonamides in the treatment of plague. Such combined treatment is useful when supplies of streptomycin, chloramphenicol, and tetracycline are inadequate for the number of patients requiring treatment.

**Suggested dosages of drugs**

The administration of 12 g of sulfadiazine daily for 4-7 days appreciably reduces mortality from bubonic plague. Recent experiences have shown that in less severe cases, after an initial oral priming dose of 4 g, 2 g may be given every 4 hours until the temperature is down to normal. Thereafter, 0.5 g is given every 4 hours until 7-10 days after the initial dose. The usual precaution of alkalinizing the urine should be taken, using 2-4 g of sodium bicarbonate with each dose of sulfadiazine. This form of treatment has proved ineffective with plague pneumonia.

***Tetracycline***

This is the preferred drug, and should be administered in large doses (4-6 g daily) during the first 48 hours. Intravenous therapy during the first 24 hours is essential in severely ill patients, but should be supplemented by oral administration if the patient tolerates this.

*Streptomycin*

This should be given intramuscularly in the dosage of 0.5 g every 4 hours for 2 days, then 0.5 g every 6 hours until clinical improvement occurs. Under field conditions, repeated injections may be impracticable and the total daily dose may be administered in two equal injections.

*Chloramphenicol*

A total dosage of 20-25 g should be given orally at the rate of 50-75 mg/kg daily.

**Serotherapy**

Before other methods were developed, the administration of antiplague serum was considered useful in the treatment of severe cases. The Committee, after examining the available data, feels that this method has no significant value and therefore does not recommend it. However, it encourages experimental research in the production of antitoxic sera that might be useful as a complementary treatment for severely toxic cases.

**11. PUBLIC HEALTH ASPECTS OF PLAGUE CONTROL****National action**

Plague is firmly entrenched in its natural foci in many countries. Wherever the disease is present, or has occurred in recent times, it needs to be recognized that plague often recurs after long periods of quiescence. Complacency must be avoided, and adequate provision should be made within the health structure for :

- (1) emergency plague control ;
- (2) continuous surveillance of rodent and human plague ;
- (3) the organization of an adequate team of experienced staff who will train and direct local health officials to undertake (1) and (2) and to perform studies so as to obtain accurate information on the plague situation and plague foci within the country. This information constitutes the basis for selecting appropriate short-term and long-term preventive measures.

The Committee draws attention to the possibility of extension of plague within a country or to neighbouring countries by waterway and road traffic, particularly when there is no rat-proofing on small vessels and no rat control at ports and on roads and railways.

**International co-operation**

In view of the need for trained personnel in many countries where plague is a problem, and the inability of some of these countries to provide effective training for their staff without international assistance, the Committee welcomes the valuable inter-regional and regional training courses organized by WHO. Such training is essential to ensure the success of national programmes.

Where emergency situations develop that cannot be dealt with and controlled by local agencies, the Committee recommends that WHO should take immediate action to provide an expert consultant who will establish the diagnosis, ascertain the extent of the problem, determine the most effective control measures that can be applied under the circumstances, and recommend follow-up activities by national health services and, if necessary, by WHO.

Once the epidemic has been brought under control, a long-term study of the epidemiology will provide information that is essential for continuous surveillance and control of plague. WHO will often be able to co-operate in this, especially in developing countries, by providing specialists in the individual disciplines involved in the epidemiological investigation.

Freighting in containers presents a special problem, which should also be approached through international co-operation. Control activities must be carried out in the country of origin, not at the destination. Both insecticides and rodenticides should be used in the container if the cargo originates from a plague area.

**12. RESEARCH NEEDS**

There is a distinct need for basic research. The subjects of immediate interest are :

- (1) study at international level of the microbiology of the plague bacillus, i.e., taxonomy, antibiotic resistance, antigenic characterization, production and significance of virulence factors ;
- (2) studies on the pathogenesis of the infectious process and toxæmia, i.e., the selection of suitable animal models for studies of genetic and conditioned resistances ; the kinetics of cutaneous as opposed to pulmonary infection ; and the role of iron in increasing the pathogenicity of attenuated strains ; and
- (3) studies on mechanisms of immunity, i.e., standardization of tests to assess immunity ; mechanisms of phagocytosis ; the role of allergic response ; the significance of granulomas ; induced immunity and its duration ; prophylaxis of plague pneumonia ; and the efficacy of vaccines.

The Committee noted the recent work that has proved the occurrence of the plague bacillus in the throats of individuals residing in infected areas. This deserves more detailed study.

Research on ecological parameters is needed. The Committee noted the guidelines for ecological studies proposed by WHO in working paper BD/PL/WP/69.11, and recommended that they be made widely available to plague workers. The collection and analysis of data from natural foci need to be promoted, for there are many areas where data have been accumulated but not analysed, and many more where no investigations have been made. Such analysis would make it possible to determine the presumptive plague area. A free interchange of available data between research workers is highly desirable.

The development of research workers is very difficult because of the complexity of training required and the lack of opportunity for practical field experience. It is therefore strongly recommended that WHO facilitate the training of young research workers so that, in the future, plague experts will be available for national and international action.

#### **International reference activities**

The Committee recommends the creation of one or more reference centres for plague. In view of the complexity of the natural history and ecology of plague, it feels that the functions of the reference centre should be as follows :

- (1) to identify and classify the plague bacillus ;
- (2) to collect and maintain representative strains of the plague bacillus of historical, geographical, and epidemiological interest ;
- (3) to provide reference strains for national reference laboratories ;
- (4) to produce and make available important reference materials such as specific bacteriophages, typing antisera, and various specific, purified antigens, e.g., Fraction 1 ;
- (5) to identify insect vectors and vertebrate hosts ;
- (6) to provide national laboratories with advice and assistance on request ;
- (7) to provide training in various aspects, related to plague, of microbiology, entomology, mammalogy, epidemiology, and ecology ;
- (8) to collect and distribute information on plague investigations and research, and thus serve as a clearing-house for research workers.

### 13. CONCLUSIONS AND RECOMMENDATIONS

The third plague pandemic, which began at the end of the nineteenth century and, aided by the development of steam navigation, invaded the entire world, has now come to an end. Plague nowadays causes low fatality, mainly because of prophylaxis and new, effective methods of treatment. Modern insecticides and rodenticides permit the epizootic-epidemic chain to be broken; sulfonamides and antibiotics prevent the occurrence of rare but dreaded episodes of pulmonary plague. However, the natural foci scattered over almost the whole world provide plague with ample opportunities to re-emerge. Recent episodes have demonstrated that plague can, at any instant, break out from these foci and cause widespread invasions. Moreover, the appearance of "resistance" phenomena gives rise to the fear that present methods of combating plague may eventually lose their effectiveness.

The Committee has attempted to draw an up-to-date map of recognized or suspected foci (see page 6), and to outline investigations at both national and international level that would provide confirmatory and supplementary information for the map and would also lead to definition of the bio-ecological characteristics and hence the potential of each of these foci. This information is necessary for the organization of effective control measures in the foci, and for the establishment of precise preventive measures in other vulnerable areas.

Modern means and techniques of transport, such as the aeroplane and the freight container, provide new opportunities for the dissemination of plague. It should be remembered that plague emerging from its strongholds in the hinterland may spread and reach national or international ports. Especial attention should be paid to avoiding the introduction of the infection into ports and large cities. Surveillance of infection in its natural foci should therefore replace quarantine measures, which have been shown to be ineffective.

The Committee makes the following recommendations :

- (1) Countries with plague foci within their territory should constantly endeavour to maintain up-to-date and detailed information on the geographical demarcation of such foci.
- (2) Serological surveys (using the passive haemagglutination test) should be instituted as a dependable means of mapping natural plague foci. Bacteriological confirmation is indispensable.
- (3) Further study of the mechanisms involved in the persistence and conservation of plague infection should be given high priority.
- (4) Strains of the plague bacillus, freshly isolated from different sources,

should be exchanged among workers to ensure comprehensive study; this will help in designating reference strains.

(5) Information should be sought on local rodents and fleas and on their susceptibility to rodenticides and insecticides; the data collected should be widely and continuously distributed.

(6) Mass vaccination should not, at present, be used as a general plague control measure; vaccines should be used only for the protection of high-risk groups. Inactivated vaccine is considered to be more practicable.

(7) Suitable health education programmes are essential, both at the professional level and for the general public.

(8) Antibiotics of proven efficacy should be preferred for chemoprophylaxis and chemotherapy. Sulfonamides may be used under certain circumstances in the treatment of bubonic plague and in chemoprophylaxis.

(9) National organizations should be established, within the public health structure, for the effective implementation of the recommended antiplague measures and for the training of professional and technical staff.

(10) WHO should consider providing support and assistance for (a) research, (b) the establishment of reference centres, and (c) the organization of training courses.

(11) For practical purposes, emphasis in the field of plague should be put on applied research. However, basic research is needed to close certain pronounced gaps in present knowledge.

(12) Principles and methodology for the study of plague should be established at international level.

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### Annex

## PASSIVE HAEMAGGLUTINATION TEST

The following procedure is recommended by the Committee for testing serum for haemagglutinating antibody to the Fraction 1 antigen of the plague bacillus. Formalized cells have also been used, and have proved effective and practicable.

### Reagents

All reagents used in the tests must be of the highest quality. Sterile, non-pyrogenic water for injection<sup>1</sup> and sodium chloride injection<sup>1</sup> are used throughout as saline diluent or as diluent for the preparation of any reagent. Tannic acid used in tanning sheep erythrocytes must be of reagent grade and protected from oxidation.

Normal rabbit sera used as diluent in the tests must be obtained from rabbits free from the risk of exposure to any of the *Pasteurella*. The sera are adsorbed with sheep erythrocytes, as described by Chen & Meyer,<sup>2</sup> prior to the haemagglutination test.

### Equipment

All centrifuging is carried out at 5°C in a refrigerated centrifuge. For all haemagglutination tests suitable equipment is required, such as the "Microtiter" equipment<sup>3</sup> described by Sever.<sup>4</sup>

### Collection and tanning of sheep erythrocytes

Due precautions must be taken to ensure that the erythrocytes used in the tests are obtained from suitable sheep. The cells are stored in Alsever's solution at 5°C. For use, the cells in Alsever's solution are washed (1500 rev/min) 3 times in normal saline. They are then suspended in normal saline to give a 2.5% concentration and an equal volume of freshly-prepared tannic acid solution (1 : 20 000 w/v) is added. The resulting suspension is

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<sup>1</sup> Suitable preparations are described in *Specifications for the quality control of pharmaceutical preparations* — second edition of the *International Pharmacopoeia*, Geneva, WHO, 1967.

<sup>2</sup> Chen, T. H. & Meyer, K. F. (1954) *J. Immunol.*, **72**, 282-298.

<sup>3</sup> Manufactured by the Cooke Engineering Co., Alexandria, Va., USA.

<sup>4</sup> Sever, J. L. (1962) *J. Immunol.*, **88**, 320-329.

thoroughly mixed and allowed to stand for 15 minutes in a water-bath at 37°C, with frequent shaking. The tanned erythrocytes are then washed (1500 rev/min for 3 minutes) once in saline (pH 6.5) and resuspended to a 2.5% concentration in saline (pH 6.5). This suspension is divided into two aliquots.

#### **Sensitization of tanned erythrocytes with Fraction 1 antigen of the plague bacillus**

A 200-µg/ml solution of Fraction 1 is prepared in normal saline (pH 7.0). An equal volume of the Fraction 1 solution is added to one aliquot of the tanned erythrocytes and the mixture is kept at room temperature (about 25°C) for 15 minutes, with frequent mixing. This suspension is centrifuged (1500 rev/min) and the supernatant is discarded. The cells are washed twice (1500 rev/min for 3 minutes) with 1 : 100 normal rabbit serum in normal saline (pH 6.5). The Fraction 1-sensitized erythrocytes are then resuspended in a 1% concentration in 1 : 100 normal rabbit serum in saline (pH 6.5).

#### **Preparation of tanned erythrocytes for control**

An equal amount of 1 : 100 normal rabbit serum in normal saline (pH 6.5) is added to the second aliquot of tanned erythrocytes, which is then treated in exactly the same way as the preparation sensitized with Fraction 1. This antigen is a control for nonspecific agglutination.

#### **Diluent for the antibody inhibition test**

This diluent consists of a 25-µg/ml solution of Fraction 1 in 1 : 100 normal rabbit serum in normal saline (pH 6.5).

#### **Test procedure**

(1) All test sera are inactivated at 56°C for 30 minutes and adsorbed with the sheep erythrocytes used to prepare the sensitized and control antigens. Each series of tests must include 2 known positive control sera and 2 known negative control sera.

(2) Each serum is tested in triplicate.

(3) Diluents are added to 3 rows of the microtitre "U" plate as follows, for each serum that is to be tested :

(a) The volume of each diluent to be added is determined by the dilution scheme that is selected. A two-fold dilution scheme is recommended.

- (b) A 1 : 100 dilution of normal rabbit serum in normal saline (pH 6.5) is added to each well of a microtitre "U" plate in rows 1 and 2.
- (c) The diluent for the antibody inhibition test is added to each well of the "U" plate in row 3.
- (4) Identical volumes of the test serum are added to the first well of the "U" plate in rows 1, 2, and 3. The sera are then progressively diluted in the following wells, using microtitre loops.
- (5) Tanned control cells are added to the serum dilutions in row 1.
- (6) Tanned erythrocytes sensitized with Fraction 1 antigen are added to rows 2 and 3.
- (7) Plates are sealed with plastic tape, left overnight at room temperature on a level surface, and read, using a microtitre mirror, the next morning.

### Interpretation of results

A compact button with smooth edges in the centre of the well represents a negative reaction. Positive reactions are characterized by uneven distribution of erythrocytes over the bottoms of the wells, and the margins of the cell patterns are very uneven and rough. An acceptable test is controlled by the reactions observed in the known positive and negative sera. The known negative sera must be entirely negative, and the titre of the known positive sera must agree within one dilution with the titres observed with the same sera in previous tests. There must be no agglutination in the dilutions of the unknown sera tested with the control cells, or the reaction with tanned erythrocytes sensitized with Fraction 1 must be at least 4 dilutions greater before the reaction can be considered at all specific. Specific reactions are confirmed by the Fraction 1 antibody inhibition test: sera that are positive in the dilution series tested against tanned erythrocytes sensitized with Fraction 1 are negative when diluted in diluent that incorporates a 25- $\mu$ g/ml concentration of Fraction 1.

### Endpoint of titration

The endpoint of titration is considered to be the last clear-cut 4+ reaction.

### Collection of blood

Blood for the tests is collected in tubes. The practicability of collecting blood samples on filter paper is being investigated.

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